APPROVAL SHEET

Title of Dissertation: Identification of Genetic Variants Influencing Efficacy of Lisinopril Treatment on Age-specific Physical Performance: A Genome-wide Analysis in *Drosophila melanogaster*

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ABSTRACT

Title of Document: IDENTIFICATION OF GENETIC VARIANTS INFLUENCING EFFICACY OF LISINOPRIL TREATMENT ON AGE-SPECIFIC PHYSICAL PERFORMANCE: A GENOME-WIDE ANALYSIS IN DROSOPHILA MELANOGASTER

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Age-related decline in physical performance is a general phenomenon in most organisms and in humans confers high risk for disability and mortality. Despite the near ubiquity of senescence and extensive variation among individuals in age-related decline in physical performance, we know little about the genes responsible for this variation. In humans, alterations in the Renin-Angiotensin System (RAS) have been implicated in the pathogenesis of late life physical decline. Pharmacological blockade of RAS, such as that by angiotensin-converting enzyme inhibitor Lisinopril, has been proposed as a treatment to attenuate such age-related declines. Some studies have shown effectiveness of these drugs for treatment of late-age declines while others have failed to show any effect. Conflicting results between studies can potentially be explained by genetic differences among individuals. The primary goal of this research was to develop methods to measure physical

performance with age and identify, via genome-wide association (GWA) and follow-up functional genetic studies, genes associated with physical ability at late age and those that contribute to differences among genotypes in the phenotypic response (climbing speed and endurance) to Lisinopril. I used *Drosophila melanogaster* as a model system and the *Drosophila* Genetic Reference Panel (DGRP) for GWA mapping. The second goal was to map climbing speed and endurance in untreated and Lisinopril-treated flies. This revealed genetic pathways that are acted on by this drug and polymorphisms that altered individual responses to the drug. My results have contributed to our understanding of the genetic bases of natural variation in physical performance at older ages. Many of the genes identified this study have human orthologs. As a result, my findings have laid the groundwork for designing personalized medical applications to treat age-related declines in physical performance and provide novel genetic targets for pharmaceutical development to extend health span in older adults.

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By

Mariann M. Gabrawy

Dissertation submitted to the Faculty of the Graduate School of the
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Dedication

This dissertation is dedicated to Gido Amozis, who is watching from the Paradise of Joy, and Teta Anjel for being my role models. To my nephews Michael, Luke, David, and my nieces Ana and Ava for showing me what it means to have love, peace, and joy in their purest form. To my entire family – I know you will read this in its entirety; thank you in advance.

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Chapter 1

Introduction

Introduction

A long-standing goal in biology is to understand the underlying causes of age-related decline in physical performance. Some studies propose the loss of homeostasis, chronic inflammatory pathway activation (Walston et al. 2002; Singh and Newman 2011; Capettini et al. 2012), and dysregulation in several biological systems (Fried et al. 2001; Boehm and Nabel 2002; Crackower et al. 2002; Oudit et al. 2003; Espinoza and Walston 2005; Der Sarkissian et al. 2006) as part of the pathophysiologic events culminating in the development of physical weakness. One of the key systems that has recently been suggested as a potential culprit in the precipitation of physical weakness is the renin-angiotensin system (RAS) (Abadir et al. 2012). Angiotensin-converting enzyme (ACE) is the main enzyme which controls generation of Angiotensin II (Ang II), the effector hormone of RAS.

ACE is a commonly investigated gene in the study of the genetics of human physical performance (Wang et al. 2008) and also functions in human longevity (Petranovic et al. 2012). Medical treatments commonly used in older individuals, such as angiotensin-converting enzyme (ACE) inhibitors such as Lisinopril, may attenuate age-related decline in physical performance. However, in patients, there are many studies in which results are contradictory or conclusions unclear (Gray et al. 2009; Buford et al. 2012; Tinetti et al. 2014a; Tinetti et al. 2014b). In clinical studies, treatments with ACE inhibitors are not always effective (Gray et al. 2009) and determinants of inter-individual variation in response to ACE inhibitors are unknown.

Given the aforementioned inconsistencies, methods to enhance drug efficacy, such as personalized medicine that accounts for genetic differences between individuals (Giocomoni et al. 2007; Wang et al. 2008; Wang et al. 2011), are needed. Lack of personalized treatments, is due, in part, to gaps in our knowledge of the genetic basis of aging. This lack of our understanding necessitates evaluation of evidence of ACE function and its roles in physical performance, in models. Further, determination of the genetic basis of drug response in humans or vertebrate models is cost prohibitive and often inconclusive. Thus, studies of the genetic basis of variation in response to drug treatment on non-mammalian models offers a practical solution.

Drosophila melanogaster, the fruit fly, is an established model system for studies of personalized medicine (Kasai and Cagan 2010) and human genetic diseases (Ocorr et al. 2007; Cammarato et al. 2008), skeletal muscle aging (Demontis et al. 2013), and genetic basis of age-related decline in physical performance (Rhodenizer et al. 2008). Fly homologs of ACE, angiotensin-converting enzyme (Ance) and angiotensin-converting enzyme related (Acer), have been described (Corvol et al. 1995; Taylor et al. 1996) and the genes that encode these proteins have been implicated as contributors to natural variation in life span (Lai et al. 2007; Wilson et al. 2013; Durham et al. 2014). Therefore, *Drosophila* is of use in studies of genetic basis of diseases and in determining response of individuals to medications such as ACE inhibitors.

In this chapter, I compare and contrast the structure and function of human ACE to that of the *Drosophila* angiotensin-converting enzyme (Ance) and related genes, evaluate recent literature describing use of Lisinopril in humans, and integrate studies of genetic variation underlying decline of physical performance into the context of senescence. I will primarily focus on critical evaluation of contributions of ACE-like genes, such as *ACE2* and its *Drosophila* homolog *angiotensin-converting enzyme related* (*Acer*), to age-related decline in physical performance. I will also assess roles of the enzymes in skeletal muscle and assess genetic tools in flies to identify and validate effects of genes.

i. Comparison of human angiotensin-converting enzyme (ACE) and fly angiotensin-converting enzyme (Ance)

Structure and molecular function: evolutionary conservation of isoforms, protein domains, and active sites

The renin-angiotensin system (RAS) is a hormonal system which is particularly important in regulation of blood pressure and fluid homeostasis in vertebrates. It is also known to have connections with senescence (Ferder et al. 2007; Cassis et al. 2010; Capettini et al. 2012; Conti et al. 2012; Benigni et al. 2013), decline in physical weakness (Wang et al. 2008), and decline in muscle function with age (Wei et al. 2007). Molecular components and

pathway of the RAS have been well-described and characterized, respectively (Berstein and Berk 1993; Donoghue et al. 2000; Brede and Hein 2001; Turner and Hooper 2002; Abadir et al. 2003; Coates 2003; Abadir et al. 2012). Of note, renin converts angiotensinogen to angiotensin I (Ang I) which, in turn, is converted by ACE to angiotensin II (Ang II), a peptide that binds to endothelial receptors, causing vasoconstriction, and regulates salt and water balance via the aldosterone pathway.

ACE is an evolutionarily conserved zinc-metallopeptidase with orthologs in various organisms such as *Drosophila* (Cornell et al. 1995; Tatei et al. 1995; Taylor et al. 1996; Coates et al. 2000), *C. elegans* (Brooks et al. 2003; Macours et al. 2004), and bacteria (Riviere et al. 2007). The molecular cloning of *Drosophila Ance* cDNA, its functional expression, and protein characterization demonstrated that this enzyme is a secreted single-domain homolog of mammalian ACE (Cornell et al. 1995). Also, cDNA encoding a second and distinct ACE-like enzyme, Acer, from *Drosophila* was cloned and sequenced; it has been determined to have a sequence that is 65 percent similar and 40 percent identical to *Ance* (Taylor et al. 1996). This is discussed in more detail later in the next section. Both *ACE* and *Ance* have been evolving in organisms since they diverged from a common ancestral gene; this is estimated to have occurred approximately 270 million years ago (Cornell et al. 1995; Fournier et al. 2013) (Table 1.1).

To gain further insights into the evolution of *ACE*, Fournier et al. (2012) conducted a phylogenetic analysis of RAS components. They found that

many genes that are important parts of the system predated the emergence of primitive chordates and tunicates and that most of the major components were present at the divergence of bony fish. There is also evidence that angiotensinogen made its first appearance in cartilaginous fish (Table 1.1).

Table 1.1. Summary of evolution of components of current day mammalian RAS. The "*" denotes presence of complete mammalian Renin-angiotensin system as currently known; based on Fournier et al. 2012.

Time (millions of years ago)	Organism	Component of RAS
750	Invertebrates	Ance, Acer, P(RR)
550	Lampreys, sharks	ACE, ACE2
500 – 480	Lampreys, sharks	AT1, AT2
400	Bony fish	AGT
390	Bony fish	Renin
300	Amphibians	*Mas
200	Rodents	---

The presence of several RAS genes in organisms that lack some of the RAS components suggests that these genes may have other functions in vertebrates in addition to their role in RAS. For example, another function of RAS genes may be salt regulation via another pathway or mechanism. Additional functions could be explained by the levels of circulating enzymes and their isoforms.

In mammals, ACE can be both membrane-bound (tissue) and circulating (ACE_{plasma}). While there is little intra-individual variation in circulating ACE levels, there is substantial inter-individual variation in circulating levels (Danser et al. 2007); this difference in ACE_{plasma} has a genetic basis (Cambien et al. 1988). Although the tissue and circulating levels of most components of RAS appear to be linked by dependence of plasma renin levels, the synthesis of the final product, Ang II, is not coupled (Van Kats et al. 2000); levels in tissues may not reflect those in plasma. This suggests that there may be some degree of independence between circulating and tissue systems.

Within the vertebrate tissue system, ACE exists as two isoforms with distinct distributions: germinal ACE (gACE), also referred to as testis ACE (tACE) and somatic ACE (sACE). tACE is composed of one catalytic C-domain only, is transcribed from a distinct promoter, and is confined in its expression to male germ cells in the testes where it plays an essential role in fertility (Houard et al. 1998; Corvol et al. 2004). Inactivation of tACE in mice results in fertile females while homozygous males have reduced fertility (Krege et al. 1995). Nevertheless, the physiological role of tACE, including the basis for its role in fertility, has yet to be established. It has been suggested that it is not the dipeptidase activity of ACE that is responsible for its role in fertility but rather its ability to hydrolyze and release from membrane glycosylphosphatidylinositol (GPI)-anchored proteins (Woodman et al. 2006).

To delineate regions of tACE that are important in catalytic activity, intracellular processing, and regulated ectodomain shedding, Woodman et al. (2006) replaced regions of the tACE sequence with the corresponding N-domain sequence. The resultant chimeras were cleaved at the identical site as that of tACE. They also showed acquisition of N-domain-like catalytic properties. Homology modeling of the chimeric proteins showed structural changes in regions required for tACE-specific catalytic activity. In contrast, other chimeras demonstrated defective intracellular processing and were neither enzymatically active nor shed. Therefore, there are critical elements required for the processing, cell-surface targeting, and enzyme activity of tACE specifically.

The second isoform of ACE is sACE. In contrast to tACE, sACE is expressed on the surface of endothelial and epithelial cells in a wide variety of tissues and is composed of two homologous N and C catalytic domains, each containing the His-Glu-Met-Gly-His (HEMGH) zinc-dependent active site motif. The N- and C-domains are both active and have a 55 percent (Fernandez et al. 2003) to 60 percent (Akif et al. 2010) sequence similarity. However, they have different substrate and inhibitor profiles and can be distinguished by selective inhibitors. This indicates that they have different functions. In addition, genes coding for two-domain ACEs have arisen several times during the course of evolution suggesting a common selective advantage to having an ACE with two active-sites in tandem in a single protein (Burnham et al. 2005). The N-domain, composed of 612 amino acids

is implicated in heart function; the C-domain, composed of 650 amino acids, is implicated in blood pressure regulation (Corradi et al. 2006). Inhibitor specificity of each domain is discussed later in this paper.

Both isoforms of ACE are Type-I transmembrane glycoproteins with an extracellular amino-terminal ectodomain and short intracellular cytoplasmic tail. ACE is able to hydrolyze a wide variety of peptides, acting either as a peptidyl dipeptidase (carboxydipeptidase) in the case of substrates such as Ang I, or as an endopeptidase in the case of luteinizing hormone-releasing hormone (Erdos and Skidgel 1985; Erdos 1990). Other physiologically relevant substrates of ACE are bradykinin and the hemoregulatory peptide N-acetyl-SDKP (Rieger et al. 1993).

In flies, *Ance*, the homolog of human ACE, has been characterized (Houard et al. 1998; Bingham et al. 2007; Akif et al. 2010; Akif et al. 2012; Akif et al. 2014) and has several biochemical similarities to each of the aforementioned isoforms of the mammalian enzyme (Coates et al. 2000; Akif et al. 2010; Akif et al. 2012; Akif et al. 2014) (Fig. 1.1). For example, Ance displays greater than 60 percent amino acid sequence similarity to tACE and to the C-domain of sACE; the function and several active-site interactions are conserved (Coates et al. 2000; Akif 2010; Akif 2014) (Table 1.2). Ance lacks a hydrophobic C terminal; it is not bound to any type of membrane and is therefore soluble in most body fluids. This suggests that Ance may have other physiological functions that have not been identified.

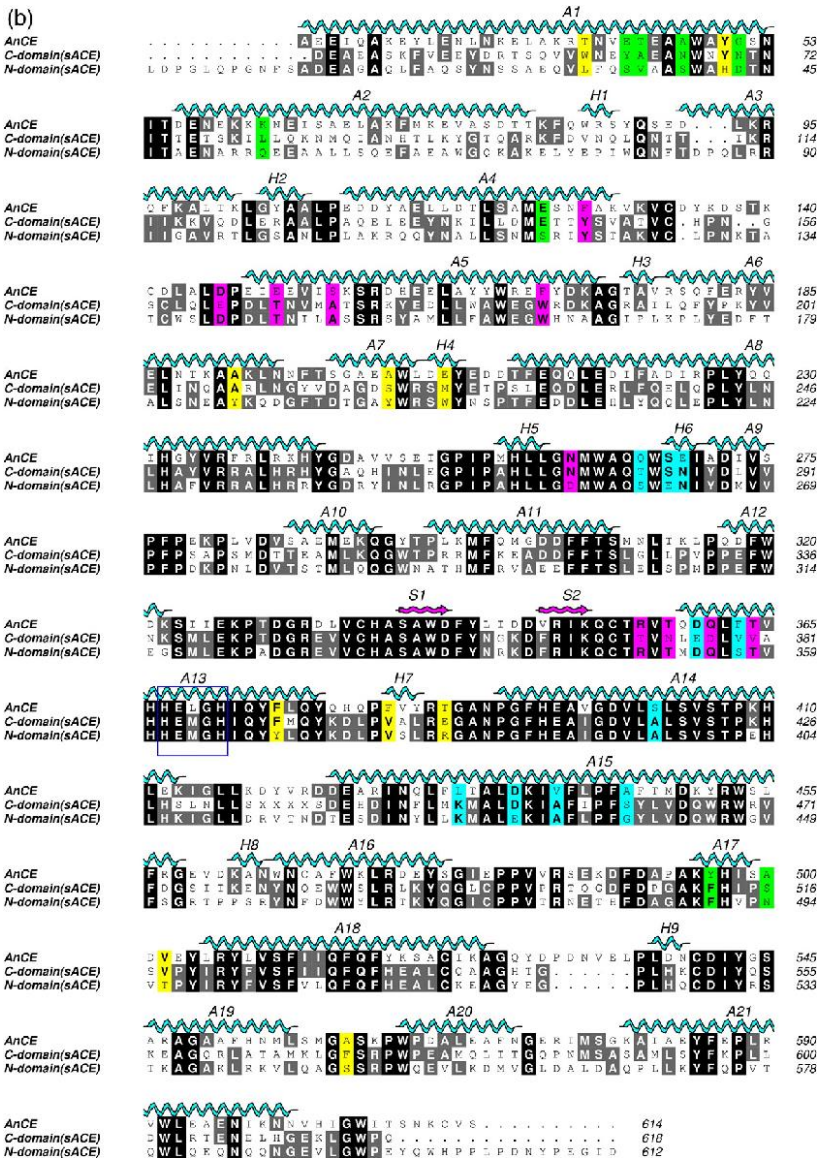
(b)
AnCE
C-domain(sACE)
N-domain(sACE)

Figure 1.1. Structure-based sequence comparison of *Ance* with C-domain and N-domain of sACE. Helices and strands for Ance are represented above the amino acid sequence with respective symbols. The secondary-structure elements are represented with the following codes: “A” for α-helices, “H” for 310 helices and “S” for β-strands. Identical residues are colored in black and the zinc binding motif is shown in a box. The residues at different binding pockets, S2, S1, S1 ′ and S2 ′, are colored yellow, green, magenta and cyan, respectively. Figure taken from Akif et al. 2010 with permission; license number 4359390247277 provided by Elsevier and Copyright Clearance Center.

Table 1.2. Comparison of structural similarities between human and *Drosophila* proteins.

Human Protein	***Drosophila* Protein**	**Amino Acid Sequence Similarity (percent)**	**References**
C-domain of sACE	Ance	60	Coates et al. 2000; Donoghue et al. 2000; Houard et al. 1998; Siviter et al. 2000; Riordan, 2003
N-domain of sACE	Acer	60	Coates et al. 2000; Donoghue et al. 2000; Houard et al. 1998; Siviter et al. 2000; Riordan, 2003
tACE	Ance	>60	Coates et al. 2000; Donoghue et al. 2000; Houard et al. 1998; Siviter et al. 2000; Riordan, 2003

Additionally, by X-ray crystallography and enzymatic study, Ance has been shown to bind to and cleave human Ang1 and Bradykinin, respectively (Akif et al. 2012). Furthermore, fly Ance has broad substrate specificity and its presence in the hemolymph of insects raises the possibility that, like mammalian sACE, it is required for extracellular metabolism of peptide hormones (Isaac et al. 1999; Macours and Hens 2004). It is important to note that *Drosophila* has six ACE-like genes (*Ance, Acer*, *Ance-2, Ance-3, Ance-4* and *Ance-5*) which all code for single domain proteins (Table 1.3). It is unlikely that Ance-2, Ance-4, and Ance-5 function as peptidases because they lack one or more of the residues that are essential for peptidase activity (Coates et al. 2000).

Table 1.3. **Comparison of molecular function and localization similarities among *Drosophila* Ance and Acer.** A “?” denotes no experimental evidence of molecular function has been demonstrated.

Gene	Molecular Function	Tissue	References
Ance	Dipeptidase	Testis	Houard et al. 1998; Isaac et al. 1999; Coates et al. 2000; Siviter et al. 2002; Hurst et al. 2003; Rylett et al. 2007; Fisher et. al. 2012; dos Santos et al. 2015
Ance-2	?	Testis	Coates et al. 2000; dos Santos et al. 2015
Ance-3	Dipeptidase	Head, Testis	Coates et al. 2000; dos Santos et al. 2015
Ance-4	?	Head	Coates et al. 2000; dos Santos et al. 2015
Ance-5	?	Head, Testis	Coates et al. 2000; dos Santos et al. 2015
Acer	Dipeptidase	Heart, Nervous	Houard et al. 1998; Siviter et al. 2002; Isaac et al. 2010; Carhan et al. 2011; Fisher et. al. 2012; dos Santos et al. 2015

Biological function of human ACE and fly Ance

Drosophila is an established model for studying muscle physiology and function in humans (Demontis et al. 2013). Expression of *Ance* in cardiac cells suggests that this enzyme has a role in heart development. Although expression levels of Ance in adult fly muscle has not been studied explicitly, expression in the carcass, which contains muscle, is moderate (modENCODE).

In humans, polymorphisms in *ACE* have been linked to muscle atrophy and to functional decline in muscle in the young (Wang et. al. 2008) and the aged (Carter and Groban 2008). Also, Mitsuishi et al. (2009), determined that reduced mitochondrial content in skeletal muscle was associated with down-regulation of the genes involved in mitochondrial biogenesis. Polymorphisms in *ACE* have also been implicated in producing variation in locomotor ability (reviewed in Hagberg et al. 2011).

In addition to muscle, Ance activity has been found in the gonads and accessory glands of several insect species in addition to *Drosophila*, suggesting a conserved role for this enzyme in reproduction (Isaac et al. 1999; Macours and Hens 2004; Rylett et al. 2007). One function of Ance is as a peptidase for peptides responsible for sperminal differentiation (Hurst et al. 2003). This is seen through infertility in flies carrying hypomorphic alleles of *Ance* which reduce the function, but does not completely eliminate it (Tatei et al. 1995; Rylett et al. 2007).

In humans, the precise function of tACE is not fully understood. Many speculate that human prostate tACE functions in spermatid differentiation and the processing of peptides in seminal fluids (Hurst et al. 2003; Chapman and Davies, 2004; Isaac et al. 2007; Rylett et al. 2007; Ram and Wolfner 2007). Others demonstrate that mice that are deficient in tACE are infertile (Hagaman et al. 1998), but infertility of male mice lacking tACE appears to be independent of the RAS and likely results from failure to cleave a peptide distinct from Ang I (Fuchs et al. 2005). Nevertheless, other studies have suggested that Ang II affects fertility in human males because of its ability to stimulate sperm mobility, induce the acrosome reaction, and increase oocyte penetration (Vinson et al. 1995; Vinson et al. 1996; Kohn et al. 1997; Kohn et al. 1998).

ii. Comparison of human Angiotensin-converting enzyme 2 (ACE2) and fly Acer

In vertebrates, Ang II is a key component of an endocrine signaling system that increases vasoconstriction, blood pressure, and inflammation. Its blockade has a role in slowing of aging (Benigni et al. 2010) perhaps by protecting mitochondria (De Cavanagh et al. 2011). Angiotensin II functionally interacts with two forms of G protein-coupled receptor (GPCR), the angiotensin II receptor type 1 (AT_1R) or angiotensin II receptor type 2 (AT_2R). Variations of the AT_1R gene are associated with extreme human longevity (Benigni et al. 2012). The A subtype of the Ang II receptor ($AT_{1A}R$) is located on the surface of vascular smooth muscle cells and its activation by Ang II

results in elevated levels of intracellular calcium, generation of reactive oxygen species (ROS), and contraction of the cells. Ang II therefore acts to increase vascular pressure, and accordingly ACE inhibitors and $AT_{1A}R$ antagonists have proven to be highly effective for treatment of hypertension (Werner et al. 2008). Ang II is further converted to Angiotensin (1, 7) by angiotensin-converting enzyme 2 (ACE2) via hydrolysis.

Structure and molecular function of angiotensin-converting enzyme 2 (ACE2) and Drosophila Acer

Human ACE2, like ACE, is a zinc-metallopeptidase that displays approximately 42 percent amino-acid identity with ACE in its catalytic domain (Rice et al. 2004). ACE2 catalyzes the conversion of angiotensin II to angiotensin 1-7, thereby counterbalancing ACE activity. ACE2 is a multifunctional enzyme and thus potentially acts on other vasoactive peptides, such as Apelin, a vital regulator of blood pressure and myocardium contractility. In addition, ACE2 is structurally a chimeric protein that has emerged from the duplication of 2 genes: homology with ACE at the carboxypeptidase domain and homology with Collectrin in the transmembrane C-terminal domain.

However, unlike somatic ACE, ACE2 only contains a single catalytic site and functions as a carboxymonopeptidase, cleaving a single C-terminal residue from peptide substrates. In keeping with its distinct catalytic properties, ACE2 displays distinct substrate specificities and inhibitor profiles

from those of ACE. Although both enzymes are able to cleave Ang I, the kinetics of this with respect to ACE2 (which would hydrolyze it to Ang 1-9) are not favorable, making this an unlikely physiological substrate (Rice et al. 2004). Unlike ACE, ACE2 is able to cleave Ang II to Ang 1-7, suggesting ACE2 may oppose the effects of ACE in the local RAS. *ACE2*, like *ACE*, is widely expressed (Gembardt et al. 2005) but in humans is only found at high levels in the heart, kidney, and testes (Tipnis et al. 2000).

Of the other *Drosophila ACE*s, only the *Acer* gene product has been studied biochemically (Burnham et al. 2005). Acer, like Ance and human ACE, is a peptidyl dipeptidase, but is generally less efficient than Ance at cleaving dipeptides from many oligopeptide substrates (Siviter et al. 2002). *Acer* is expressed in the embryonic heart (Taylor et al. 1996) and in both the male and female gonads and brain of adult flies (Burnham et al. 2005; Carhan et al. 2011) where it is assumed to have a role in the metabolism of, as yet unidentified, biologically active peptides involved in neuroendocrine signaling and reproduction (personal communication).

Biological function of ACE2 and fly Acer

Evidence indicates that the enzymatic activity of ACE2 has a protective role in cardiovascular diseases and its loss leads to functional deterioration of the heart and progression of cardiac, renal, and vascular pathologies (Corvol et al. 1995; Boehm and Nabel, 2002; Der Sarkissian et al. 2006). However, in the heart, mechanisms by which ACE2 mediates its cardio-protective

functions have yet to be fully elucidated (Clark et al. 2012) and little is known of its regulation (Lambert et al. 2014).

Lambert et al. (2014) examined the potential role of miRNAs in the regulation of ACE2 expression in primary human cardiac myofibroblasts. Putative miRNA-binding sites were identified in the 3'-UTR of the ACE2 transcript using online prediction algorithms. Two of these, miR-200b and miR-421, were selected for further analysis. A reporter system using the 3'-UTR of *ACE2* fused to the coding region of firefly luciferase was used to determine the functionality of the identified binding sites *in vitro*. This identified miR-421, but not miR-200b, as a potential regulator of *ACE2*. The ability of miR-421, a miRNA implicated in the development of thrombosis, to down-regulate *ACE2* expression was subsequently confirmed by Western blot analysis of both primary cardiac myofibroblasts and transformed cells transfected with a synthetic miR-421 precursor. Real-time PCR analysis of miR-421 revealed widespread expression in human tissues. The miR-421 levels in cardiac myofibroblasts showed significant inter-patient variability, in keeping with the variability of *ACE2* expression observed previously.

Although it is not an exact replica of a mammalian heart, the *Drosophila* cardiac tube has been used as a model for understanding heart disease in humans (Sohal, 1970; Bodmer, 1998; Paternostro et al. 2001; Walker and Benzer, 2004; Wolf et al. 2006; Ocorr et al. 2007; Taghli-Lamallem et al. 2008; Cammarato et al. 2008; Piazza and Wessells 2011; Spindler et al. 2012). In spite of more than 500 million years of evolutionary

divergence, the basic cellular and molecular mechanisms of cardiac fibers are conserved between vertebrates and flies (Taghli-Lamallem et al. 2008). Vertebrates and flies also have significant similarities in the embryonic origin and embryonic structure of the heart (Bodmer 1995; Bodmer and Frasch 1999). The cardiac proteomics of *Drosophila*, described by Cammarato et al. (2011), provide further evidence of its usefulness in studies on cardiomyopathies. More specifically, Cammarato et al. (2011) mapped 5,169 distinct heart–associated peptides. The study resulted in identification of excitation-contraction protein landmarks, orthologues of proteins associated with cardiovascular defects, and conservation of protein ontologies.

Like human ACE2, fly Acer is also involved in cardiac morphogenesis and function (Crackower et al. 2002; Coates et al. 2003). During embryogenesis, both *Ance* and *Acer* mRNA are found in developing heart cells (Tatei et al. 1995; Taylor et al. 1996). To determine the role *Acer* has on heart development, Crackower et al. (2002) studied mutant flies that carry a P-element insertion in the *Acer* locus (Spradling et al. 1999). Mutation of *Acer* resulted in early embryonic lethality. As such, they studied morphogenesis of the heart tube using the even-skipped (Eve) and Tinman (Tin) proteins as markers for heart progenitor cells. *Acer* mutant flies displayed reduced numbers and disorganization of Eve positive progenitor cells and of the Tin positive dorsal mesoderm. Therefore, *Acer* mutant flies have defective heart morphogenesis. *Acer* may also have a role in the specification of heart progenitors in *Drosophila* embryos (Crackower et al. 2002).

Interestingly, in addition to disruption of heart morphogenesis, loss of Acer also disrupts night-time sleep in adult *Drosophila.* To study the role of *Acer* in circadian activity, Carhan et al. (2011) generated *Acer* null mutant flies by imprecise excision of a P-element. It was determined that night sleep, which is clock-regulated, is disrupted in flies lacking Acer. *Acer* null adults have reduced night-time sleep and higher sleep fragmentation, but no disruption of daytime sleep. Wild-type flies treated with Fosinopril, an inhibitor of Acer, influences the quality of night sleep in a similar manner (Carhan et al. 2011). As Acer is secreted from the adult fat body of the head and abdomen into the hemolymph, it may therefore cleave peptides involved in metabolism and activity behavior. Human ACE peptidases are likewise expressed in adipose tissue and are thought to be involved in a signaling system that links metabolism with sleep.

iii. Human ACE and ACE2 have non-catalytic functions

Although RAS has not yet been identified in invertebrates, investigating other functions of angiotensinases may elucidate additional functions in vertebrates and in insects. Several non-catalytic functions have been determined. One function is that of signaling. For example, ACE and ACE2 can bind integrin subunits and act as cell adhesion substrates; cellular expression of ACE2 enhances cell adhesion (Clarke et al. 2012). Soluble

ACE2 (sACE2) is capable of suppressing integrin signaling mediated by Focal Adhesion Kinase (FAK). In addition, sACE2 increases the expression of *Akt* and subsequently lowers the proportion of the signaling molecule phosphorylated Akt. These results suggest that ACE2 plays a role in cell-cell interactions, possibly acting to fine-tune integrin signaling. Therefore, expression and cleavage of ACE2 at the plasma membrane may influence cell-extracellular matrix interactions and the signaling that mediates cell survival and proliferation (Clarke et al. 2012).

Another non-catalytic function is that of molecular chaperoning. For example, in vertebrates, *ACE2* and *collectrin*, a homolog of *ACE2*, act as molecular chaperones (Lambert et al. 2010). Collectrin, like ACE2, is a type 1 transmembrane protein which is subject to ectodomain shedding (Akpinar et al. 2005). However, it has only a short extracellular domain, lacks any catalytic residues, and has no homology to ACE. ACE2, therefore, appears to resemble a chimaera of an ACE-like catalytic domain and collectrin C-terminal domain. Originally thought to be restricted to the kidney (Zhang et al. 2001), *collectrin* expression has subsequently been detected within the pancreas, liver, and brain, and has been implicated in kidney and pancreatic development, where it is expressed during gestation and the neonatal period (Akpinar et al. 2005).

Furthermore, ACE2 also functions as the key SARS coronavirus receptor and stabilizer of neutral amino acid transporters (Hashimoto et al. 2012). ACE2 has been implicated in the pathology of Hartnup's disease, a

disorder of amino acid homeostasis. Via its function in amino acid transport, ACE2 regulates dietary amino acid homeostasis, innate immunity, and the gut microbiome (Hashimoto et al. 2012).

iv. Comparison of other components of RAS in humans and flies

Human Pro-renin receptor (PRR) and fly Pro-renin receptor P(RR)

In humans, the (Pro) Renin Receptor (PRR) binds renin and prorenin which induces the conversion of Angiotensinogen to Angiotensin I. This is essential for its conversion into Angiotensin II, III or IV, which all have critical functions in the body. Renin is an aspartyl protease that cleaves angiotensinogen into angiotensin I. The existence of a receptor for renin and for its inactive precursor, prorenin, was found in 1998, but the receptor binding renin and prorenin, termed the (pro) renin receptor was not cloned until 2002. The PRR is a true receptor that is able to activate intracellular signaling, and surprisingly, PRR-bound prorenin is enzymatically active as a result of the conformational change without cleavage of the prosegment.

Like aforementioned components of the RAS, the *PRR* gene is highly conserved among species including *Drosophila* (Nguyen et al. 2010). However, it has been suggested that the PRR has functions unrelated to the hemodynamic aspects of the RAS since there is no evidence that *Drosophila* has the full RAS system. Some of the newest developments reveal that the

PPR is involved in both the Wnt/β-catenin and non-canonical Wnt/PCP pathways, which are essential for adult and embryonic stem cell biology, embryonic development and disease, including cancer. After the discovery of the PRR, there has been a great deal of excitement as scientists postulated the role it might play in diabetic nephropathy and cardiac fibrosis and that tissue damage might be totally prevented by blocking prorenin binding to the PRR (Nguyen et al. 2010).

(Pro) Renin Receptor in humans

Prorenin, the precursor of renin, is cleaved to its active form by the removal of a 43 amino acid prosegment. Binding of prorenin, the inactive precursor of renin, to the PRR results in a full catalytic activity of prorenin through a nonproteolytic mechanism that likely involves a conformational change by which the prosegment moves out of the catalytic cleft, which then becomes available for angiotensinogen. The binding of renin or prorenin to the PRR also activates intracellular signaling pathways in several cell models independent from angiotensin generation, resulting in increased Angiotensin I production and the subsequent generation of angiotensin II at the tissue level, which leads to increased cellular proliferation, cytoskeletal rearrangements, and the production of pro-fibrotic and pro-inflammatory factors.

The gene encoding the PRR is named *V-ATPase 6 accessory protein 2* (*ATP6ap2*) which is located on the X-chromosome. V-ATPase is a multi-subunit complex responsible for ATP hydrolysis and proton translocation.

Genome-wide association studies have revealed various polymorphisms in *ATP6ap2* associated with increased cardiovascular, neurological, and renal risks in humans. For example, a studies on populations of Japanese and Caucasian cohorts show the 5+169C>T polymorphism is significantly associated with high blood pressure in Japanese men (Hirose et al. 2009) and Caucasian men (Ott et al. 2011). The +1513A>G polymorphism was found to be significantly associated with the risk of lacunar infarction and left ventricular hypertrophy in Japanese women (Hirose et al. 2011). Two additional polymorphisms were significantly associated with hypertension in more populations with vascular diseases. However, these do not provide evidence of an impact of these polymorphisms on PRR expression or function.

Other studies have addressed polymorphism effects. For example, a unique exonic splice enhancer mutation resulted in the deletion of exon 4 (∆e4 isoform) and a reduction in the amount of functional protein. This reduction has been implicated in X-linked mental retardation and epilepsy (Ramser et al. 2005). Also, experiments conducted on the lymphocytes of patients bearing the mutation ∆e4 isoform were unable to activate extracellular signal-regulated kinase ½ (ERK ½) in the presence of renin. These results suggest that the function of renin as a receptor might be involved in neuron physiology (Ramser et al. 2005). In addition, a silent mutation residing in a putative exonic splicing enhancer site resulted in a minor and reproducible impairment of ERK ½ activation. Another recent study

has also identified the same mutation in a patient with another X-linked mental disease (Korvatksa et al. 2013). The authors also described a new mutation, c.345C>T, associated with X-linked Parkinsonism with spasticity (XPDS) which also resulted in exon 4 skipping and overexpressing the minor splice of the ∆e4 isoform. In human embryonic kidney (HEK)-293 cells transfected with siRNA, the PRR impaired autophagy and lysosomal clearance as observed in XPDS brain sections. The most drastic effects were found in the striatum; a region in the brain associated with Parkinson's disease, and involved an excessive accumulation of Tau proteins (Bartscherer et al. 2006). These studies confirmed that PRR mutations are associated with various mental diseases, but the described mechanism was different from that of the initial study (Ramser et al. 2005).

(Pro) Renin Receptor in *Drosophila*

Studies by Beuchling et al. (2010) and Hermle et al. (2010) in *Drosophila* describe the role that PRR plays in the PCP pathway. The dual function of VhaPRR establishes it as a key factor for epithelial morphogenesis. They observed that siRNA against PRR resulted in severe PCP defects, such as abnormal anterior-posterior orientation and hair mispolarization. The phenotype could be rescued by the injection of a full-length human PRR mRNA, but not with an N-terminally truncated form; which suggests an essential role of the extracellular domain to transduce non-canonical Wnt signaling, in a method similar to that of the canonical signaling

pathway. PRR has also been shown to interact with Fz and a lack of PRR impaired targeting of the Fz to the plasma membrane and hence disrupted its localization necessary for normal pupal wings. This suggests that PRR was important for PCP initiation by trafficking Fz specifically to the plasma membrane.

The research in (Beuchling et al, 2010) explains that dPRR is a conserved modulator of Wnt/Fz signaling, that the PRR is required for PCP, and that the dPRR interacts with Fz receptors in the plasma membrane. The research in Hermle et al. (2013) explains that PCP controls the orientation of cells within tissues and the polarized outgrown of cellular appendages, and that there are two roles for VhaPRR, one for the PCP and another in endosomal trafficking.

To better understand the role of PRR within the PCP pathway, mutant flies with clonal deletion of PRR in the pupal wings have been generated (Hermle et al, 2013). In *Drosophila* epithelial cells, an important feature of PCP is to signal the formation of asymmetric PCP domains at apical junctions. Clonal elimination of PRR led to strong PCP defects; this is consistent with previous reports by Beauchling et al. (2010) and Hermle et al. (2010), but also showed new molecular mechanisms for PRR.

For example, PRR was shown to co-localize with PCP core proteins during all stages of pupal wing development while absence of PRR reduced the presence of PCP proteins such as Fz and Fmi at apical junctions of cells and were instead found localized in vesicular compartments. The extracellular

domain of the PRR, known to bind to Fz, was also found to interact with Fmi and is important for normal targeting of both proteins. The cleavage site and the sPRR were not required for normal PCP signaling, as a rescue for mutant flies expressing a non-cleavable PRR restored all aspects of the phenotype. An alternative association revealed an essential role of Fmi in recruiting PRR to the PCP domain for subsequent apical trafficking. Collectively, these results suggest that PRR possesses all of the characteristics of a PCP core protein and may also be considered as such.

Furthermore, by using experiments based on endocytosis quantification and pH monitoring, Hermle et al. (2013) showed that PRR regulated acidification of specific apical vesicles but did not interfere with other vesicles. Defective apical vesicles led to mistrafficking of the transmembrane protein E-cadherin, which could not travel through the endolysosomal pathway and undergo lysosomal degradation. Instead, E-cadherin was recycled back into the apical membrane and thus appeared that PRR had a specific role for recycling apical vesicles in the epithelial cells. It was also discovered that V-ATPase mediated acidification of certain compartments did not require PRR. In comparison, mutant *Vha6-2* flies showed impaired endocytosis and subsequent acidification at the apical and basal areas. Overall PRR and V-ATPase share an overlapping role in the endolysosomal pathway but also exhibit distinct functions in the PCP pathway (Hermle et al. 2013).

v. Renin-Angiotensin System (RAS) blockade

The RAS pathway has roles in cardiovascular function, renal function, and physiological senescence, all of which impact longevity and physical weakness in humans and thus, disruption of the pathway has been studied in mammals. First, the role of RAS and its blockade in cardiovascular function has been well-studied. For example, Benigni et al. (2009) examined the consequences of disruption of the $AT_{1A}R$ gene on aging of the cardiovascular and renal organ systems in mice. Both the average and maximum lifespans of $AT_{1A}R$-deficient mice were increased by approximately 26 percent. Also, another study was completed in aged rats and humans that had age-related increases in the left-ventricular weight characterized by heart fibrosis and hypertrophy; chronic treatment with ACE inhibitors and AT_1R antagonists reduced left-ventricular weight (Capettini et al. 2012). Second, the role of RAS and its blockade in renal function has also been studied. In the kidney, Hostetter et al. (1981) demonstrated that use of ACE inhibitors or AT_1R antagonists attenuated glomerulosclerosis and improved the intra-glomerular pressure in rats. Other groups showed a significant reduction in kidney focal sclerosis by the chronic use of Losartan or Enalapril, an AT_1R antagonist and an ACE inhibitor, respectively (Liern et al. 2004; Inserra et al. 2009).

Third, the RAS plays a role in physiological senescence. Physiological senescence was first hypothesized to be related to production of reactive oxygen species (ROS) by Harman (1956). According to his theory, ROS contributes to age-related damage of several organs. Ang II-induced ROS

production and the increase of both circulating Ang II levels and ROS production during aging suggest a direct relationship between AT_1R-mediated ROS increase and physiological senescence (Dal-Ros et al. 2010). Supporting this theory, treatment with AT_1R blockers (ARBs) such as Losartan, prevents age-related disorders such as hypertension and atherosclerosis. This suggests that the vascular senescence is mediated by AT_1R activation which decreases ROS production (Stein et al. 2010).

Inhibition of ACE by Lisinopril

ACE inhibitors (ACE*i*) have been widely used in the clinic as anti-hypertensive agents with great success (Hostetter et al. 1981; Liern et al. 2004; Inserra et al. 2009; Capettini et al. 2012). However, the presence of undesirable side-effects (e.g. dry cough and angioedema) and the increase of mortality from cardiovascular disease necessitates the development of novel therapeutic agents targeting enzymes of the RAS. Efforts to understand the specific inhibition of the catalytic function of ACE have been made on the basis of the X-ray structures of other enzymes with analogous efficacy in the hydrolytic cleavage of peptide substrate terminal fragments (Georgios et al. 2013). ACE has the sequence and topology characteristics of gluzincins, a sub-family of zinc metallopeptidases. Such similarities are used to show common structural elements among these enzymes. Conformational analysis of the zinc-free and zinc-bound peptides through high resolution NMR

spectroscopy also provides insights into the solution structure of ACE catalytic centers.

The structure of ACE and the structure of ACE*i* are important in predicting their binding. ACE has four active sites for inhibitor binding, known as S1, S2, S1`, and S2`. The nature of the binding of an inhibitor to these active sites depends entirely on the structure of its side chains; for example, captopril, one of many ACE*i*, interacts with S1` and S2`, whereas Enalapril and Fosinopril, other ACE*i*, interact with the S1 and S2 sites. Likewise, the structure of Lisinopril is important to its role as an ACE*i*. Lisinopril contains a lysyl group at the P1′ position and in the inhibitor-bound structure, the carboxyl group of Lisinopril coordinates tightly with the Zn^{2+} (Akif et al. 2010). In addition, the inhibitor is bound to the protein through extensive H-bonds. The proline residue of ACE*i* serves to block the enzymatic active site, binding to the zinc (II) center of the ACE. In Lisinopril, this binding takes place via the presence of several binding pockets present on the active site of the ACE (Bhuyan and Mugesh 2011).

Flies are ideal for studies in drug treatment of disease, drug screening, and personalized medicine (Akif et al. 2010; review by Kasai and Cagan 2011) and are frequently used in such studies (Kang et al. 2002; Ge et al. 2004; Tan et al. 2004; Stilwell et al. 2006; Wang et al. 2007; Bahadorani et al. 2008; Jordan et al. 2012). Of relevance for the proposed study, the mechanism of Lisinopril binding to fly Ance is known and is similar to that of human ACE (Akif et al. 2010).

Lisinopril activity

Lisinopril activity is dependent on selectivity of N- versus C-domain in human sACE and method of binding. The N- and C-terminal domains of human sACE-1 demonstrate distinct physiological functions with resulting interest in the development of domain-selective inhibitors for specific therapeutic applications. First, affinity of Lisinopril toward the C-domain has been demonstrated by Watermeyer et al. (2010). In a study by Hocharoen et al. (2013), the activity of Lisinopril-coupled transition metal chelates (M-chelates) was tested for both reversible binding and irreversible catalytic inactivation of each domain of sACE. The C- to N-domain binding selectivity ratios ranged from 1 to 350, while rates of irreversible catalytic inactivation of the N- and C-domains were found to be greater for the N-domain. This suggests a more optimal orientation of M-chelate-Lisinopril complexes within the active site of the N-domain of sACE.

Second, as do all peptide-based inhibitors and enzymes, Lisinopril displays three methods of binding: 1) interactions with the peptide backbone, 2) interactions with the terminal amino or carboxyl group, 3) and interactions of the side chains (Ondetti and Cushman 1984). The peptide backbone interactions include those between hydrogen donors and acceptors. Interactions between Lisinopril and the amino-terminal group of ACE can be either ionic or hydrogen bonding based, whereas the interaction with the carboxyl-terminal group is most likely ionic in nature (Ondetti and Cushman 1984). Side chain interactions vary; they can be hydrophobic, dispersion,

hydrogen bond interactions, or a mixture of all three, depending on the nature of the side chains (Ondetti and Cushman 1984).

Given the aforementioned selectivity of domain and variable methods of binding, it is very possible that potency of these inhibitors can vary from patient to patient based on the degree of success of substrate-enzyme binding. In humans, Lisinopril treatment has resulted in various patient responses from effective (Buford et al. 2012) to detrimental (Tinetti et al. 2014a); there are also many studies in which results are contradictory or conclusions are unclear (Gray et al. 2009; Tinetti et al. 2014a; Tinetti et al. 2014b). In clinical studies, treatments with ACE inhibitors (ACE*i*) are not always effective (Gray et al. 2009) and determinants of inter-individual variation in response to ACE inhibitors are unknown.

Positive effectiveness has been determined in a study by Buford et al. (2012). In that study, they found an association between ACE*i* and improvements in the physical function of older adults in response to chronic exercise training was identified. It was determined that physical activity significantly improved the walking speed of ACE*i* users but had reduced effect on non-users. Physical activity also improved Short Physical Performance Battery (SPPB) score of ACE*i* users and of individuals who used other antihypertensive drugs, but not of those using non-antihypertensive medications (Buford et al. 2012).

Ineffectiveness of treatment has also been determined in studies. For example, Gray et al. (2009) determined that ACE inhibitor use had no

association with incident frailty in women ages 65 years and older. Also, Schjoedt (2011) analyzed the effect of a RAS blockade in the treatment of diabetic neuropathy and cited a large amount of inter-individual variation in response to therapy.

Because Lisinopril is administered in response to several diagnoses and is not the only method of blocking the RAS pathway, potency must be evaluated and genetic differences between individuals must be taken into account (Giocomoni et al. 2007; Wang et al. 2008; Wang et al. 2011). The latter can be elucidated through the use of model organisms.

There are no published *in vivo* studies of the effects of Lisinopril on flies. However, there are crystallography and enzymatic studies that demonstrate that Lisinopril inhibits fly Ance. The orientation of the Lisinopril molecule in Ance is very similar to that in the tACE–Lisinopril complex (Natesh et al. 2003; Natesh et al. 2004). The lysyl group at the P1′ position of Lisinopril is held deep inside the S1′ pocket through ionic interactions with Glu150, Asp360, and Asp146. Similar ionic interactions of the lysyl group have been reported with Glu162 in the tACE–Lisinopril structure (Natesh et al. 2003; Natesh et al. 2004). The C-terminal carboxylate group of the inhibitor binds to residues Gln265, Lys495, and Tyr504 at the S2′ subsite. The amino group which connects the N-terminal phenyl and lysyl groups of Lisinopril makes a strong H-bond with the carbonyl oxygen of Ala338.

In addition, in cell culture, a network of water molecules makes indirect interaction between the inhibitor and the residues at the S1′ and S2′ pockets

of Ance. One example of such an interaction involves the lysyl group of the inhibitor with residue Thr364 of Ance. Even though the orientation of Lisinopril is almost identical in both form I and form II structures, the orientation of the phenyl group at the P1 position of Lisinopril in form II is slightly different due to the presence of two HEPES molecules bound in the S2 pocket (Masuyer et al. 2013).

Although a highly efficient inhibitor of the C-domain of sACE (binds with a Ki of 2.4 nM), Lisinopril has a lower affinity for Ance (Ki of 180 nM) (Williams et al. 1996) and the N-domain (Ki of 44 nM) (Wei et al. 1992). The ionic interactions of the lysyl group at the P1′ position with the charged residues Glu162 and Glu376 at the S1′ subsite of the C-domain might contribute to the enhanced specificity. These ionic interactions are lost due to replacement of the corresponding residues with a shorter charged residue, Asp, in both Ance and the N-domain protein of sACE.

In addition to the fact *D. melanogaster* is a well-established model for studying muscle physiology and function (Jones and Groteweil 2011; Demontis et al. 2013), human diseases (Ocorr et al. 2007; Cammarato et al. 2008), senescence (Durham et al. 2014), drug treatment (Akif et al. 2010), and personalized medicine (Kasai and Cagan 2010), there are several genetic tools that are available in flies that help to identify genes associated with phenotypes of interest. These tools are discussed in the next section.

vi. Genetic tools in flies to identify and validate effects of genes

The Drosophila Genetic Reference Panel (DGRP)

To study the role of genetic natural variation in inter-individual differences in a phenotype, one can use the *Drosophila* Genetic Reference Panel (DGRP). This panel consists of 205 inbred lines derived from one outbred population and the genome of each line is publicly available (Mackay et al. 2012; Mackay et al. 2013). The DGRP was constructed by collecting mated females from a population in Raleigh, North Carolina then inbreeding their progeny for 20 generations prior to sequencing. In the 2012 study, Mackay et al. sequenced 168 lines using a combination of Illumina and 454 sequencing technology. The assay of 113.5 million bases (94.25 percent) resulted in identification of over 4.6 million single nucleotide polymorphisms (SNPs). A rapid decline in linkage disequilibrium (Hill and Robertson 1966) and lack of global population structure make genome-wide association studies (GWAS) a good method for identifying SNPs that have causal effects on the phenotypes in this population.

Genome-wide association studies (GWAS)

Genetic variation in *ACE* may be associated with the wide range of outcomes in cardiovascular disease and response to ACE*i* (Taylor et al. 1996; Chung et al. 2010). In humans, Chung et al. (2010) used GWAS to search for

genes/loci that influence ACE activity, and observed that the *ABO* gene is associated with ACE activity as well as variation in the ACE gene.

Drosophila Ance and *Acer* have been identified as candidate genes influencing lifespan and life history traits through use of GWA. For example, Durham et al. (2014) used the GWA approach to identify SNPs associated with longevity and fecundity. *Ance-3* was a candidate identified in that study; it may also have a role in muscle function (Schnorrer et al. 2010), circadian behavior (Ceriani et al. 2002), stress and immune response (Vermeulen et al. 2013), and mitochondrial disease (Vartiainen et al. 2014).

Other mapping techniques have also identified *Ance* and *Acer* as candidate genes influencing lifespan and life history traits. Lai et al. (2007) used *Drosophila* and previously identified quantitative trait loci (QTL) affecting lifespan to determine 49 candidate genes and four pathways that could potentially be involved in regulating life span in and aging processes in *Drosophila. Ance* and *Acer* were among the 49 candidates identified in that study. More recently, in a similar study, Wilson et al. (2013) determined that expression of *Ance* and *Acer* differed in long-lived versus shorter lived control populations of *Drosophila*.

GAL4/UAS and RNAi to validate the influence of candidate genes on phenotypic traits

The GAL4/UAS (upstream activating sequence) is one of the most valued and widely used systems for targeted gene expression. GAL4 is a

protein identified as a regulator of genes induced by galactose in the yeast *Saccharomyces cerevisiae* (Oshima 1982; Laughton and Gesteland 1984). DNA binding and transcriptional activation functions of GAL4 have been identified, defined, and shown to be separable (Ptashne 1988). The GAL4/UAS system is used for targeted gene expression in organisms including, but not limited to, *Drosophila*, in which GAL4 expression has the ability to stimulate transcription of a gene of interest under UAS control. Development of the system revealed that the expression of GAL4 in *Drosophila* had no deleterious phenotypic effects; this paved the way for future experiments (Brand and Perrimon 1993).

The GAL4/UAS system functions by using a promoter/enhancer to direct expression of *GAL4* in a specific pattern (Duffy et al. 2002). The GAL4 then directs transcription of the GAL4-responsive UAS-target gene. Specifically, in the *GAL4* line the GAL4 activator protein is present with no target gene to activate whereas the UAS-target gene line has the target gene but is silent due to the lack of the activator protein. Thus, by crossing the two lines, the target gene is activated within the progeny. These two separate components, or lines, allow for several applications when studying gene expression.

One application allows researchers to introduce genes that code for potentially deadly products such as those that cause cell death; this is valuable in studies of loss of specific cell types and functions (Duffy et al. 2002). Another application of the two-part system is the ability to target the

expression of any gene given the distinct GAL4 drivers that can be generated and stocked for future use; most of the capabilities of the system stem from the range of such lines available and the diversity of ectopic expression patterns that lie within these lines (Phelps et al. 1998).

Another use of the GAL4/UAS system is the specific elimination of synaptic transmission (Phelps et al. 1998). This targeted disruption of synaptic communication is a useful way of linking a neural circuit to a particular behavioral phenotype such as climbing ability. This is done by using the UAS-tetanus toxin line as the expression of the toxin cleaves synaptic vesicle membranes that are required for neurotransmitter release (Phelps et al. 1998). Since there are many neuronal responses that require the use of these neurotransmitters, this allows researchers to effectively remove these normal responses when certain stimuli are applied (Phelps et al. 1998).

Furthermore, the GAL4/UAS can be used in combination with RNA interference (RNAi) tools. The Vienna *Drosophila* RNAi Center (Table 1.4) provides access to an RNA*i* library consisting of 22,247 transgenic *Drosophila* strains; 12,251 genes, or 88.2 percent, of the *Drosophila* genome are represented in this collection. Stocks are also available from the Transgenic RNAi project at the Harvard medical school (www.flyrnai.org/TRIP-HOME.html).

Table 1.4. Resources of genetic tools. Modified from Mackay et al. 2012.

Resource	Location	Website
DGRP lines	Bloomington *Drosophila* Stock Center	flystocks.bio.indiana.edu/Browse/RAL.php
Sequences	Baylor College of Medicine Human Genome Sequencing Center; National Center for Biotechnology; Mackay Laboratory	hgsc.bcm.tmc.edu/project-species-i-DGRP_lines.hgsc; ncbi.nlm.nih.gov/sra?term; dgrp.gnets.ncsu.edu
Read alignments	Baylor College of Medicine Human Genome Sequencing Center	hgsc.bcm.tmc.edu/projects/dgrp
SNPs	Baylor College of Medicine Human Genome Sequencing Center; National Center for Biotechnology; Mackay Laboratory	hgsc.bcm.tmc.edu/projects/dgrp/freeze1_July_2010/snp_calls; ncbi.nlm.nih.gov/SNP/snp_viewBatch.cgi?sbid=1052186; dgrp.gnets.ncsu.edu
Genome-wide association	Mackay Laboratory	dgrp.gnets.ncsu.edu
GAL4-UAS enhancers	Bloomington *Drosophila* Stock Center	flystocks.bio.indiana.edu/Browse/RAL.php
RNA*i* library	Vienna *Drosophila* RNA*i* Center	

These strains have RNAi downstream of a UAS site for gene-specific knockdown in the pattern determined by the GAL4 line of choice. For example, the GAL4 driver, *dj667* (Bloomington stock number 8171), is commonly used to alter gene expression in adult muscle of flies (Seroude et al., 2002; Azad et al., 2009; Azad et al., 2012; Rinkevich and Scott, 2012).

The Gal4-RNAi-UAS system allows for validation of candidate genes identified through genome-wide association studies (GWAS). Many GWAS have identified candidate genes which influence natural variation in lifespan (Jordan et al., 2012; Mackay et al., 2012; Burke et al., 2014; Durham et al., 2014), in mitochondrial function (Jumbo-Lucioni et al. 2010; Jumbo-Lucioni et al., 2012; Wilson et al. 2013; Zhu et al., 2014). However, we have an incomplete understanding of how genetic effects contribute to age-related declines in phenotypes, such as physical ability. No previous GWAS have assessed natural variation effects on climbing ability and endurance (indicators of aging) nor have any such studies been done in Lisinopril-treated flies. Here, I use the aforementioned genetic tools to identify genes that are associated with the age-related decline in physical performance and with response to Lisinopril treatment.

Research goals and dissertation summary

My doctoral research elucidated the genetic basis of natural variation in physical performance traits and lifespan and the responses of these traits to variation in drug treatment using *Drosophila melanogaster*. My research has contributed to studies in the biology of aging by providing novel methods to measure climbing, endurance, and strength in fruit flies. To my knowledge, this is also the first GWAS testing the effects of Lisinopril on age-related decline in the aforementioned traits in fruit flies. I have also provided a strong foundation for several future research avenues.

There were four main goals of my dissertation project: 1) to evaluate the age- and genotype-specific effects of Lisinopril treatment on physical performance in *Drosophila*, 2) to identify candidate polymorphisms and their associated genes that influence age specific physical performance and to assess the extent to which this genetic variation is treatment-specific, 3) to identify candidate polymorphisms and their associated genes that influence the sensitivity of age specific climbing ability to drug treatment, and 4) to apply genetic information gained from goal 2 and 3 to identify gene networks and validate a subset of identified candidate genes, in muscle tissue, on climbing ability and response to Lisinopril. To accomplish these goals, I used the DGRP (Mackay et al. 2012) to complete a GWAS on physical performance traits in non-mated *Drosophila* males maintained on either standard food or Lisinopril-containing food.

Chapter 2 of this dissertation contains the findings of the physical performance (climbing speed, endurance, and strength), lifespan, and the effects of Lisinopril on these traits. In summary, I found significant variation in age-specific climbing speed and endurance and in lifespan among the three DGRP lines 229, 73, and 304 tested. I then compared changes in physical performance, Fly Physical Performance Index (FPP), and life span in my three fly lines to test the impact of genetic background on the effects of ACE inhibition. Lisinopril treatment influenced age-related decline of climbing speed, endurance, and strength that was dependent on genotype. Treatment of DGRP_229 flies significantly attenuated the decline of all three measures of physical performance: climbing speed, endurance, and strength. In contrast, treated flies of DGRP_73 and DGRP_304 showed no effect on climbing speed nor endurance, but rather only on strength. I then tested the effects of Lisinopril on the composite measure, FPP. I noted a decline in prevalence of LC performance in DGRP_229 and an increase in percentage of HC flies with treatment.

While treatment with Lisinopril significantly extended the average life span of all lines, this reduction in mortality was associated with improvement of all 3 physical function measures only in DGRP_229. To investigate the apparent dissociation between individual measures of physical performance and rate of decline in my DGRP lines, I constructed a composite index analogous to criteria used in humans (Fried et al. 2001) to identify worst performers (lowest quartile) of all three physical measures with age. My

results indicate a strong relationship between genotype and performance capacity. Specifically, I observed the highest prevalence of low capacity performers in DGRP_304, which was associated with medium rate of decline in physical function but long life span. My results differ from previous studies which show that high physical performance ability is directly and positively related to long life span (Roshanravan et al. 2017), while low physical capacity is directly and negatively related to short lifespan (Fried et al. 2001). However, my results are consistent with studies which demonstrate that physical performance can be inversely related to life span (van de Vijver et al. 2016) or not necessarily associated with life span at all.

I tested whether survivorship is affected by the expression of *Ance* in muscles. My results show that knockdown of skeletal muscle-specific *Anc*e was associated with a significant increase in survivorship compared to untreated control males. Treatment of the RNAi knock down flies with Lisinopril had no added effects on survivorship. At a molecular level, aging is associated with changes in muscle fiber type and accumulation of protein aggregates (Stefani and Dobson 2003), potentially leading to defects in physical performance. My data suggest that the differential effect of Lisinopril on climbing speed, endurance, and strength in the three lines is driven by differences in the accumulation of protein aggregates in muscles.

Results from a follow-up RNA-Seq experiment identified several genes that responded to Lisinopril treatment. Many of these have been implicated in some aspect of stress and immune responses. These include genes in the *Turandot* family, CHK kinases and genes involved in the humoral response to infection. This experiment also identified genes whose expression in response to Lisinopril depended on genotype in an age-specific manner. Many of these genes are also involved in stress responses, suggesting that genetically based variation in the phenotypic response to drug treatment may depend on the extent to which stress response pathways are activated in different genotypes.

Chapter 3 of this dissertation contains the characterization of natural variation and identification of candidate polymorphisms and genes involved in age specific physical performance of flies as well the assessment of the extent to which this genetic variation is treatment-specific. I performed the climbing speed and endurance assays on 126 DGRP lines maintained on either control or Lisinopril-treated food. I found that the genetic basis of climbing and endurance differ across ages as there was little overlap in the genes or polymorphisms that were significantly associated with either trait across ages.

For climbing speed, two genes, *mib1* and *klu*, were identified as candidate genes at both ages. *mib1* is a regulator of the Notch signaling pathway which plays a role in stem cell muscle. The gene *klu* has been implicated in stem cell maintenance and cell division. Age-specific effects of

polymorphisms on complex phenotypes are commonly found in other mapping studies in both *Drosophila* (Leips et al. 2006, Felix et al. 2012, Durham et al. 2014, Carbone et al. 2016) and humans (Medina-Gomez et al. 2012, Dumitrescu et al. 2013, Simino et al. 2014, Winkler et al. 2015).

While there was little overlap in the candidate genes for climbing speed identified by GWA across ages, 14 genes were part of the climbing speed networks at each age. These included the two genes identified as candidates at both ages, *Mind Bomb 1* (*mib1*) and *Klumpfuss* (*klu*); the remaining 12 were non-candidates. Interestingly, nine of the 12 are involved in programmed cell death [*Reaper* (*rpr*), *Grim, p53, Delta* (*Dl*), *Decapentaplegic* (*Dpp*), *RAS Oncogene at 85D* (*RAS85D*)*, Notch* (*N*), *Klu*, and *Epidermal Growth Factor Receptor* (*Egfr*)]. The predominant functions for *dpp*, *RAS85D*, *N*, and *Egfr* are regulation of cell growth and developmental patterning and 7 of the 12 are specifically involved in stem cell fate (*p53, Dl, dpp, RAS85D, N, klu* and *Egfr)*.

Similar to my finding that genetic influences on climbing speed and endurance were age-specific, candidate genes contributing to variation in these traits differed between Lisinopril and control conditions in most cases. Of the 114 candidates influencing climbing speed at one week of age, 28 genes contributed to the variation in control and Lisinopril treatments. At five weeks of age, of the 128 candidates identified, only 14 were identified in both conditions. For endurance, of the 79 genes identified as candidates at one week of age, none were identified as candidates in both control and Lisinopril

treatments. At five weeks of age, of the 82 genes identified as candidates, only two genes were candidates in the control and Lisinopril treatment, *Eip78C* and *caps.*

Genes in both the Notch and Wnt signaling pathways appeared in many of the networks affecting both traits, particularly old age climbing speed, and so these pathways should be the focus of future studies. Notch is involved in many developmental process and in adults is important for homeostasis and regulation of stem cell lineages (Liu et al. 2010). Genes in the Notch pathway were also a significant component of the human gene network identified in this study. Likewise, Wnt signaling has also been implicated in development and stem cell maintenance and in particular shown to influence age related deterioration of muscle function (Brack 2007). Many genes in the Wnt signaling pathway were also found in the networks including *Axn*, *Wg, Fz, Ribosomal Protein L35A* (*Rpl35A*), and *Nemo.*

The network analyses also pointed to genes involved in epigenetic regulation as candidates that may influence age-related physical performance. Muscle stem cells exhibit epigenetic changes with age which may be an underlying cause of the loss of skeletal muscle mass or function with age (Schnorrer et al. 2010). Schnorrer et al. (2010), identified the human gene *Hoxa9* as contributing to the regenerative decline in muscle with age. Misexpression of *Hoxa9* with age due to epigenetic changes in muscle stem cells was associated with age-related functional decline of muscle cells. The most similar gene in flies to *Hoxa9* is *Abdominal B* (*Abd-B*). *Abd-B* was part of

the network of genes contributing to the variation in climbing speed at young age and the network of the genes contributing to the variation in climbing speed when young and old ages were combined. These results suggest that some of the genetically based differences in age-specific physical performance could be due to differences in epigenetic regulation in aging organisms.

Chapter 4 of this dissertation contains the validation of a subset of candidate genes, identified in Chapter 3. In brief, I used the GAL4-UAS system in *Drosophila* and eight RNAi lines to reduce the expression of candidate genes to validate the influence of these genes on climbing speed and endurance. I also compared the effects of the ACE-inhibitor, Lisinopril, on these traits when gene expression was reduced to test the hypothesis that the effects of Lisinopril on physical performance traits were mediated through genes in the Wnt signaling pathway. I found that each of the genes tested, *Axn*, *Nemo*, *Wg*, and *Fz* influenced climbing speed and endurance in an age specific manner. I also found that beneficial effects of Lisinopril on these performance traits were abolished when the expression of these genes was reduced. My results support the findings of the GWA reported in Chapter 3, and suggest an important role for the Wnt signaling pathway in maintaining age-specific physical performance traits. The results also suggest that the effects of Lisinopril on physical performance are dependent, at least in part, on Wnt signaling. Overall, my dissertation results contribute to identification of genetic bases of variation in physical performance,

provide a foundation for predictions about treatment response of a patient, and provide novel genetic targets to extend health span in older adults.

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Chapter 2

Lisinopril preserves physical resilience and extends life span in a genotype-specific manner in *Drosophila melanogaster*

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Abstract

Physical resiliency declines with age and comorbid conditions. In humans, Angiotensin Converting Enzyme (ACE) has been associated with attenuation of the decline in physical performance with age. The effects of ACE-inhibitor (ACE*i*) compounds, such as Lisinopril, which are commonly prescribed for hypertension, on physical performance remain controversial. The gene encoding ACE (*Ance*) is evolutionarily conserved in *Drosophila melanogaster*. Here, I tested the effects of Lisinopril on life span and speed, endurance, and strength (physical resiliency) with advancing age using three lines of the *Drosophila* Genetic Reference Panel (DGRP) that exhibit genetically based differences in life span. I define a Fly Physical Performance Phenotype based on climbing speed, endurance, and physical strength tests. I show that age-related decline in physical performance and survivorship varies with genetic background. Lisinopril treatment increased mean life span in all lines, but the effects on lifespan, speed, endurance, and strength depended on genotype. I detected increased protein aggregation area in muscles of flies that were less responsive to Lisinopril, suggesting that protein aggregation is one potential mechanism underlying the age- and genotype-specific measures of physical performance. Knockdown of skeletal muscle-specific *Ance* abolished the beneficial effects of Lisinopril on lifespan, suggesting a role for skeletal muscle Ance in the crosstalk between physical performance and survivorship. Further, using transcriptome profiling, I identified effects of Lisinopril on genes involved in stress responses that were

age- and genotype-specific. My data demonstrate a role for *Ance* on the rate of decline in physical abilities and genetic variation in phenotypic responses to an ACE inhibitor.

Introduction

Advanced age confers high risk for disability and mortality. Approximately 20% of older adults living independently require the aid of another person or a walking device and experience higher incidence of falls, hospitalizations, and subsequent need for long-term care. In contrast, the ability of certain older adults to maintain physical activity and function in later life is an important hallmark of those with longer health spans and life spans (Hogan 2005). Resilient individuals tend to demonstrate hardiness and to optimize physical function in the face of age-related losses or disease (Resnick et al. 2011). What sets apart resilient from frail older adults is currently unclear, but changes in metabolic, inflammatory, and stress responses have been suggested (Whitson et al. 2016).

Previous studies have shown that there is a great deal of variation among individuals in the age at which they begin to exhibit decline in physical ability and that there is a genetic basis for this variation (Montgomery et al. 1998). Notably, the gene encoding angiotensin-converting enzyme (ACE), the essential regulating enzyme of the renin-angiotensin system, has been associated with physical performance (Montgomery et al. 1998) and longevity in humans (Petranovic et al. 2012). ACE inhibitors (ACE*i*), such as Lisinopril,

are commonly prescribed for hypertension and their primary protective benefits are believed to arise from systemic effects on blood pressure (Dietze and Henriksen 2008). There is also a muscle-specific Renin Angiotensin System (RAS) including a muscle-specific ACE (Dietze and Henriksen 2008). Skeletal muscle ACE activity is crucial for the optimal regulation of muscle bioenergetics and glucose homeostasis (Dietze and Henriksen 2008). Furthermore, ACE and ACE*i* influence protein aggregation (Hu et al. 2001; Hemming and Selkoe, 2005). Protein aggregation is a common stress response in many contexts (Ogen-Shtern et al. 2016; Vasconcellos et al. 2016), a common problem age-related diseases (Squier 2001), and is seen as a pathognomonic sign of skeletal muscle aging (Stefani and Dobson 2003). The impact of oral treatment with ACE*i* on age-related buildup of skeletal muscle protein aggregates is unclear and the effects of ACE*i* on maintaining physical resilience as an individual ages remain controversial and inconsistent (Buford et al. 2012; Tinetti et al. 2014). Studying aging related decline and physical strength and its genetic mechanisms in humans is difficult due to the duration of aging and *in vitro* results from cellular models may not be representative of what occurs *in vivo* (de Magalhaes 2004; Mitchell et al. 2015).

In this study, I use the fruit fly, *D. melanogaster,* to test the hypothesis that the inconsistent responses to ACE*i* treatment depend, in part, on age and genotype of the individuals and to identify evolutionarily conserved loci that modulate the response to drug treatment. *D. melanogaster* is a well-

established model for studying muscle physiology and function (Jones and Groteweil 2011; Demontis et al. 2013), aging Durham et al. 2014), and drug treatment (Akif et al. 2010; Kasai and Cagan 2010). In particular, flies exhibit age-related decline in physical performance (Groteweil et al. 2005), which is one of the traits theorized to be enhanced by ACE*i* treatment. The closest *Drosophila* ortholog to mammalian *ACE*, angiotensin-converting enzyme (*Ance*) (Coates et al. 2000), has been identified as a candidate gene contributing to natural variation in lifespan (Durham et al. 2014). In addition, the mechanism by which Lisinopril binds to fly Ance is similar to that of human ACE (Akif et al. 2010).

I tested whether Lisinopril treatment would impact age-specific physical performance and longevity, using three inbred lines from the *Drosophila* Genetic Reference Panel (DGRP) (Mackay et al. 2012) that exhibit genetically based differences in life span (Durham et al. 2014). I evaluated genome-wide changes in gene expression in response to Lisinopril using RNA-sequencing (RNA-Seq) of two of these lines at young and old ages. Finally, to investigate a potential physiological mechanism to explain the observed phenotypes, I tested whether Lisinopril treatment affects protein aggregation in skeletal muscle with age.

Materials and Methods

i. ***Drosophila* stocks and maintenance**

Virgin males of DGRP_229, DGRP_73, and DGRP_304 from the *Drosophila* Genetic Reference Panel (Mackay et al. 2012) were used for all survivorship and physical performance assays. All control groups were fed standard food medium (solid ingredients: 79% cornmeal, 16% yeast, and 5% agar). Flies were maintained in population cages at 25^{o}C and approximately 55% relative humidity under a 12-hour light and dark cycle. All physical performance assays were completed between 8 a.m. and 2 p.m.

ii. **Lisinopril treatment**

Treatment groups were administered Lisinopril (Sandoz Pharmaceuticals. Princeton, NJ), which was added to the fly food in the following serial doses: 0.2, 0.4, 1, 2, 4, and 10 mM. Dosage was determined by using the established human dosing equation, based on body mass, and then estimated for mass of fly (mean = 0.5 mg). Optimal (1 mM) and toxic (10 mM) doses were determined by survivorship assays.

iii. ***Drosophila* life span studies**

I measured life span by placing between 250 – 270 male virgin flies in each of six plexiglass population cages (20l x 21w x 21.5h cm). Forty milliliters of control or drug food was placed in 100 x 15 millimeters BD Falcon

plastic petri dishes and replaced in their respective cages every other day. I then examined the effect of chronic Lisinopril treatment on survivorship of all three DGRP lines (n = 1,560) compared to untreated flies of these genotypes. Flies were monitored every other day and dead flies were removed until all individuals had died.

iv. Physical performance assays

I used three assays, climbing speed, endurance, and strength, to test age-related decline of physical performance. I used 30 flies for each genotype (DRGP_73, DGRP_229, and DGRP_304), at each age (weeks one, three, and five), per assay (three); I used 270 flies for each genotype (n = 810). When I tested effects of Lisinopril, I used 30 flies for each of the three aforementioned genotypes, at each age (weeks one, three, and five), per treatment (two), per assay (three); the number of flies per genotype, age, condition, and treatment is 540 (n = 1,620). This protocol was also used to test 126 DGRP lines (Chapter 3).

Climbing speed was tested by aspirating an individual fly from a population cage and placing it into the bottom of a Costar® 25-mL in 2/10, non-pyrogenic serological pipet, marked at nine and 27 centimeters. Flies were tapped down to the bottom of the inverted pipet on a solid surface and the start time was measured, in seconds, once the body of fly passed the zero mark on the bottom of the pipet. The trial ended when the fly either reached nine centimeters on the pipet, paused for greater than five seconds,

or dropped to the bottom. The distance of nine centimeters was chosen based on pilot studies which demonstrated that more than 90% of all genotypes tested can climb nine centimeters at one week of age. Endurance was measured as a climbing rate using same technique as that for climbing speed but was calculated based on the distance traveled in 15 seconds or the time it took to reach 27cm. This maximum distance, 27 cm, and the 15 second cutoff time was based on pilot studies which demonstrated that more than 90% of all genotypes tested can climb 27 cm in 15 seconds or less at one week of age but less than 10% can either climb continually for 15 seconds or reach 27 cm at five weeks of age. The wet mass of all flies was obtained immediately after each climbing assay.

Strength was tested by measuring the time it took for a fly to escape from a clear, colorless, 1 cm by 3 cm, strip of double-sided Scotch^{TM} tape. For each trial, to slow the flies for transfer and to avoid potential adverse effects of CO_2 use, individual flies were placed at -20^{o} C for 60 seconds. Each fly was then placed dorsal side-down onto the tape and wings were gently tapped into place. Pilot studies indicated that a time limit of three minutes is the maximum length of time taken for one-week old flies to escape. Thus, for this assay the maximum allotted time for each trial was three minutes. If flies were unable to escape from the tape within that time, a maximum score of three minutes was given.

v. Consumption assays

The Capillary Feeder Assay (CAFE) (Ja et al. 2007; Dieglemann et al. 2017) was used, with modifications. In brief, groups of four 5-day old virgin males were placed in each vial, without use of CO_2 anesthesia. Non-fasted flies were allowed to feed on either 1mM sucrose or 1 mM Lisinopril with sucrose for 24 hours. The 1mM sucrose was used as it was determined to be the concentration resulting in optimal rate of consumption in male flies (Dieglemann et al. 2017). Food was loaded into the capillary tubes by capillary action and the initial food level marked on the tube. Capillary size was 100 mm in length and 5 µL capacity (#53432-706, VWR). To exclude the effect of evaporation on food consumption, I calculated mean evaporation in control 1mM sucrose (n = 10) and sucrose plus Lisinopril (n = 10) using vials without flies. Food loss by evaporation or consumption by flies was measured using a digital caliper. Food loss was converted to µL by measuring the distance of food consumed (mm)/20 mm. I used the following formula to determine total consumption: food consumption of flies (µL) = (Food loss [µL] - Evaporative loss [µL])/total mg of flies in the vial. This accounts for differences in body size such as that between genotypes. Flies in each vial were weighed immediately following the end of the assay.

vi. Development of a physical performance index in *Drosophila*

In humans, a frailty index is often used as a tool to identify vulnerable patients at risk of adverse outcomes and as a predictor of lifespan (Fried et al. 2001). For this study, I took a similar approach using a composite of the three physical performance assays (climbing speed, endurance, and strength) to establish a novel FPP in *Drosophila*. These three measures and the ranking methods were chosen and modified based on the human clinical criteria and mouse model data previously described (Liu et al. 2014). Each individual fly was ranked for performance, for each of the three tests, and the ranked data were then divided into quartiles as follows. Individuals were classified as “high performers” and placed in the highest quartile if their score in each of the physical tests did not fall 1.5 SD below the cohort mean for speed and endurance and did not fall 1.5 SD above the cohort mean for strength (larger values indicate poorer performance in the strength assay). Individuals were classified as “medium performers” if their performance score was 1.5 SD away from the cohort mean in the direction of reduced performance (below the mean for climbing speed and endurance or above the mean for strength) for either one or two of three tests; if ranked 1.5 SD away from the mean for one of the tests they were placed in the second quartile while if they ranked 1.5 SD away the mean for two of the tests they are placed in the third quartile. Individuals were classified as “low performers” (fourth quartile) if

performances of speed and endurance were 1.5 SD away from the mean in the direction of poor performance for all three tests.

vii. Validation of muscle-specificity of *dj667*-Gal4

To determine the relative muscle-specificity of two commonly used adult fly muscle drivers, virgin female dj667-Gal4 and mhc-Gal4 were each crossed with male UAS-*GFP*. Male offspring were collected and aged to one week. Flies were dissected along the dorsal midline and fixed. High resolution images were taken using a Leica SP5 confocal microscope using a 10x objective. Images of live, whole flies were taken with a 20x magnification using a Leica M205 fluorescent stereoscope (Buffalo Grove, IL). Images were visually examined for presence and location of GFP fluorescence.

viii. Generation and life span of a skeletal muscle-specific *Ance* knockdown

Male flies with the dj667-Gal4 driver34 (P{w[+mW.hs]=GawB}DJ667; Bloomington *Drosophila* Stock Center; http://flystocks.bio.indiana.edu) were crossed with virgin female UAS-RNAi-*Ance* (Harvard TRiP.GLC01369}attP2; https://fgr.hms.harvard.edu/trip-rnai-fly-stocks) flies to knockdown expression of this gene in skeletal muscle (Seroude et al. 2002); the F1 generation is denoted as RNA*i-Ance*. Flies used to control for the effects of RNAi knockdown were derived from the following crosses: (1) dj667-Gal4 x y1 v1; P{CaryP}attP2 (control stock #36303, Bloomington *Drosophila* Stock Center)

to control for genetic background and (2) *dj667*-Gal4 x y1 sc* v1; P{VALIUM20-mCherry}attP2 (control stock # 35875, Bloomington *Drosophila* Stock Center) to control for activation of RNAi machinery; the F1 generation is denoted as attP2 or mCherry. Life-span of the RNAi-Ance and of the two controls was measured as previously described above.

ix. Validation of RNA knockdown

To determine the levels of *Ance* expression in my fly lines, qRT-PCR was performed. RNA*i* lines were created as described previously. One-week old male flies from each line were flash frozen with liquid nitrogen and stored at -80 ^{o}C prior to RNA extraction. RNA was extracted from homogenized tissue of 10 males per strain using the RNeasy Mini Kit from Qiagen. DNA was removed from the samples using the TURBO DNA-free™ Kit (ThermoFisher Scientific). cDNA was synthesized using a BioRad iScript™ cDNA Synthesis Kit and 0.25 µg of RNA. 1X iTaq™ Universal SYBR® Green Supermix (BioRad) was then mixed with 0.5 µL of the newly synthesized cDNA and 0.5 µM of the appropriate forward and reverse primers. Real-time amplification was performed on a Biorad CFX384 Real-time Detection System. Three biological replicates were run for every reaction, each with three technical replicates. Relative expression values were normalized to Ribosomal Protein L32 (rp49) expression levels. mCherry driven RNAi lines were used as a negative control. Primers for *Ance* and *rp49* were designed according to the fly primer bank (http://www.flyrnai.org/cgi-bin/DRSC_primerbank.pl). Primers for *Ance* expression were: Forward,

GTGATACCACCAAGTTCCAATGG, Reverse, GGCATAGTCGTCTTCAGGTAGAG. Primers for *rp49* expression were: Forward, GTGAAGAAGCGCACCAAGCAC, Reverse, ACGCACTCTGTTGTCGATACCC.

x. Whole-mount immunostaining of *Drosophila* skeletal muscle and protein analysis

Whole-mount immunostaining of *Drosophila* indirect flight skeletal muscle was completed as described previously (Azad et al. 2012). In brief, 32 one and five-week old control and treated flies from each genotype were dissected by separating the thorax from the head and the abdomen. Thoraces were cut longitudinally into two halves and cuticles were removed. Thoraces were transferred, fixed, and stained using anti-ubiquitinylated proteins antibody, clone FK2 (1:100, Millipore, cat. no. 04-263) to mark protein aggregates, Alexa Fluor 488 phalloidin to label actin (1:200, cat #A22284. Molecular Probes, Eugene, Oregon), and Cyanine3 anti-mouse secondary antibody (1:200, ThermoFisher Scientific, cat #A10522). Images were taken using Zeiss LSM 78, 63x oil immersion, and using the same setting for brightness and contrast. Protein aggregate areas (μm^2) were measured for set size regions of tissue within each whole tissue. I analyzed 30 samples from 32 - 40 individuals, from each of three genotypes, for each condition (control and treated), at each age (week one and week five) (n = 360) using Volocity 6.3 Perkin Elmer cellular imaging.

xi. Transcript profiling of control and Lisinopril-treated *Drosophila*

Virgin male flies from lines DGRP_229 and DGRP_73 were maintained on control or Lisinopril treated food for either one or five weeks. I extracted RNA from at least fifty flies for each age and treatment combination. Prior to RNA extraction these flies were separated into two separate groups to yield two biological replicates for each age and treatment combination. Total RNA was extracted with QIAzol lysis reagent (Qiagen) and the Quick-RNA MiniPrep Zymo Research Kit (Zymo Research). Ribosomal RNA (rRNA) was depleted from 5 µg of total RNA using the Ribo-ZeroTM Gold Kit (Illumina, Inc). Depleted mRNA was fragmented and converted to first-strand cDNA using Superscript III reverse transcriptase (Invitrogen). Second strand cDNA was synthesized using dUTP instead of dTTP to label the second strand cDNA. cDNA from each sample was used to produce barcoded cDNA libraries using NEXTflexTM DNA barcodes (Bioo Scientific) with an Illumina TruSeq compatible protocol. Briefly, each sample was subjected to end-repair (Enzymatics), adenylation of 3'-ends (Enzymatics), and ligation of indexed adapters (Enzymatics and Bioo Scientific). Each enzymatic reaction was purified using 1.8X Agencourt AMPure XP beads (Beckman-Coulter). Size selection of each library was performed using Agencourt AMPure XP beads (Beckman Coulter) to an approximate insert size of 130 bp and a total library size of approximately 250 bp. Second strand cDNA was digested with Uracil-DNA Glycosylase prior to PCR-enrichment to produce directional cDNA

libraries. PCR-enrichment of the purified barcoded DNA was carried out with KAPA HiFi Hot Start Mix (Kapa Biosystems) and NEXTflex Primer Mix (Bioo Scientific). Libraries were quantified using the Qubit dsDNA HS kit (Life Technologies) and their sizes determined with the 2100 Bioanalyzer (Agilent Technologies). Each sample was diluted to equal molarity, quantified, multiplexed, denatured, and diluted to 14 pM. Clonal clusters for each pooled library sample were generated on the Illumina cBot and then sequenced on the Illumina HiSeq2500 using 125 bp single-read v4 chemistry (Illumina Inc.). I generated two multiplexed libraries containing eight samples each (one week old or five week old flies). Each multiplexed library was run on one lane of the HiSeq2500.

Barcoded sequence reads were demultiplexed using the Illumina pipeline v1.9. Adapter sequences were trimmed using cutadapt v1.638 and trimmed sequences shorter than 50bp were discarded from further analysis. Trimmed sequences were then filtered for ribosomal RNA sequences by aligning against a database containing the complete 5S, 18S-5p8S-2S-28S, mt:lrRNA, and mt:srRNA sequences using BWA v0.7.10 (MEM algorithm with parameters '-v 2 –t 4') (Durbin 2010). The remaining sequences were aligned to the *Drosophila melanogaster* genome (BDGP5) and known transcriptome (FlyBase v5.57) using STAR v2.4.0e40. Read counts were computed for known gene models using HTSeq-count41 with the 'intersection-nonempty' assignment method. Tabulated read counts were then analyzed for all known genes across all samples using EdgeR (Robinson et al. 2010) as follows.

First, genes with low expression overall (<20 aligned reads in at least one replicate of every sample condition) were excluded from the analysis. Library sizes were recomputed as the sum of reads assigned to the remaining genes, and further normalized using the Trimmed Mean of M-values (TMM) method (Robinson and Oshlack 2010). I then used the generalized linear model (GLM)-based methods44 for estimating tag-wise dispersion and fit model parameters to the following model design: $x = l + d + l*d + b + \varepsilon$, where x = observed $\log_2$(read count), l = line effect (RAL_73 vs RAL_229), d = drug effect (Lisinopril vs Control), $l*d$ = line by drug interaction effects, b = overall batch effects (each line and drug combination was analyzed using two biological replicates, with the first replicates processed in a separate batch from the second replicates), and ε = model error following a negative binomial distribution with estimated gene-wise dispersion (McCarthy et al. 2012). I then selected gene expression levels with significant line effects, drug effects and line by drug interactions passing a 10% FDR threshold (based on Benjamini-Hochberg corrected p-values) from the EdgeR likelihood ratio test on the interaction term coefficient (Huang et al. 2009). This analysis was run separately and independently on one-week old and five-week old flies.

xii. Statistical analyses

Climbing, endurance, and strength data were analyzed using ANCOVA (PROC GLM, SAS V9.3) using wet fly mass as a covariate. I used the following model to assess the influence of genotype, treatment, and age on climbing speed and endurance: $y = c + m + g + t + a$ + all interactions + ε,

where *c* is a constant, *m* is fly mass, *g* tested for differences among DGRP lines, *t* tested the effects of Lisinopril treatment, and *a* is the effect of age. None of the interactions between mass and the main effects in the model were significant so interaction terms involving mass were dropped from the model. Strength was analyzed in the same manner. However, mass was not measured for flies in the strength assay so ANOVA was used to analyze the effects of genotype, treatment, and age. Food consumption data were analyzed using Student's *t*-Test to identify effects of Lisinopril treatment. Whenever necessary in the ANOVA and ANCOVA models above, I used the Tukey test for post hoc pairwise comparisons of differences among genotypes. All data met the assumptions of ANOVA so transformations were not necessary. Survivorship data were analyzed by Cox logistic regression (PROC PHREG, SAS V9.3). I used the sensitivity index of Falconer (1990) to compare the effect of age on performance traits across lines on a week to week basis, and overall decline (from week 1 to week 5, which is the latest age for which I had data for all three lines). To calculate the week to week rate of decline of each line, I took the average phenotypic value of the trait at week one minus the average value of that trait at week three and divided this difference by the average decline of this trait across all lines. To calculate the overall sensitivity to aging for each line, I took the average phenotypic value of the trait at week one minus the average value of week five divided by the average decline of this trait across all lines. I tested for the effects of Lisinopril treatment on protein aggregation area using a *t*-Test.

Results

i. Genetic variation in life- and health-span

I measured lifespan of virgin males from three DGRP lines reared under control conditions and their climbing speed, endurance, and strength at different ages. I found that the lines differed significantly in life span (X^2_1 = 16.8, $P < 0.0001$); the rank order of average life span was DGRP_73 (31 days), DGRP_229 (42 days), and DGRP_304 (61 days). Additionally, I found a significant decline with age in climbing speed ($P < 0.001$; Fig. 2.1A), endurance ($P < 0.0001$; Fig. 2.1B) and strength ($P < 0.0001$; Fig. 2.1C). However, the magnitude of the decline in the performance measures varied across the genotypes, as indicated by significant age by line interaction terms in the analysis of covariance (ANCOVA) (Table 2.1). To determine pairwise differences between the lines at each age, I used the post hoc Tukey test with a $P < 0.05$ threshold (Fig. 2.1).

Table 2.1. Analysis of covariance (ANCOVA) tables for three untreated genotypes. (A) climbing speed, (B) endurance, and (C) strength assay data.

(A) Climbing speed at age 1, 3, and 5 weeks

Age	Source of Variation	df	SS	MS	F	*P*-value
1	Mass	1	0.22	0.22	0.46	0.499
	Genotype	2	7.29	3.64	7.88	0.001
	Error	56	26.17	0.47		
3	Mass	1	0.01	0.01	0.07	0.7951
	Genotype	2	2.27	1.13	5.91	0.0047
	Error	56	10.74	0.19		
5	Mass	1	0.27	0.27	0.89	0.3487
	Genotype	2	3.92	1.96	6.55	0.0028
	Error	56	17.04	0.30		

(B) Endurance at age 1, 3, and 5 weeks

Age	Source of Variation	df	SS	MS	F	*P*-value
1	Mass	1	0.05	0.05	0.22	0.6424
	Genotype	2	22.64	11.32	50.65	<0.0001
	Error	46	10.45	0.23		
3	Mass	1	0.73	0.73	2.94	0.0919
	Genotype	2	7.49	3.74	14.38	<0.0001
	Error	56	14.09	0.25		
5	Mass	1	0.03	0.03	0.10	0.7544
	Genotype	2	2.06	1.03	3.43	0.0394
	Error	56	16.28	0.30		

(C) Strength at age 1, 3, and 5 weeks

Age	Source of Variation	Df	SS	MS	F	*P*-value
1	Genotype	2	60456.23	30228.12	10.92	<0.0001
	Error	177	489835.77	2767.43		
3	Genotype	2	36182.43	18091.22	8.90	0.0002
	Error	177	359614.77	2031.72		
5	Genotype	2	178571.03	89285.52	54.72	<0.0001
	Error	177	288829.92	1631.81		

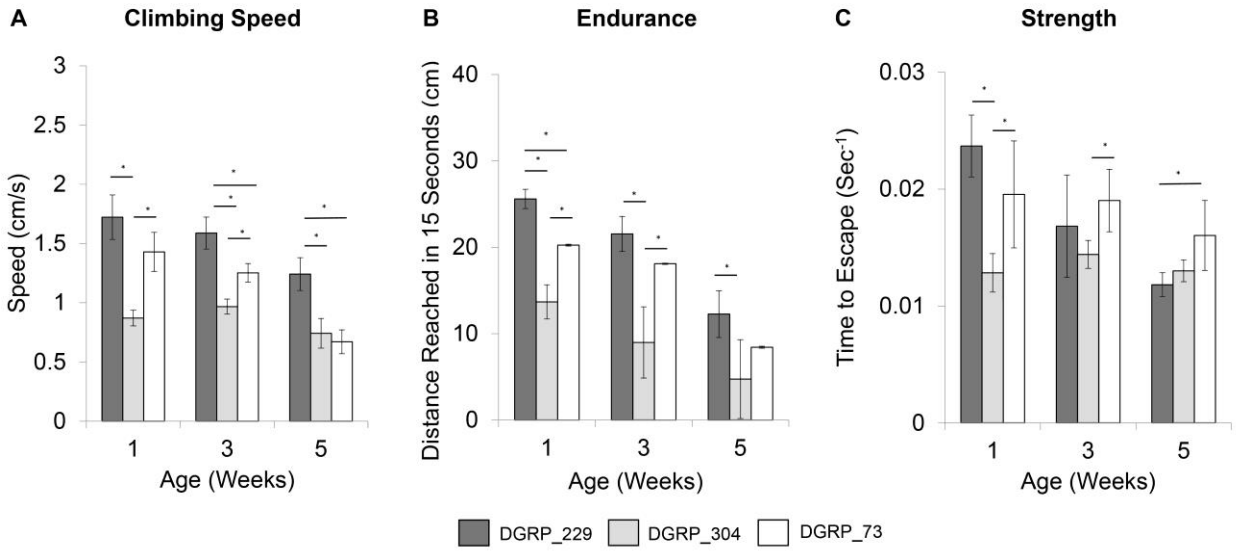

Figure 2.1. Physical performance differs with age and line in three DGRP lines. (**A**) Climbing speed rate of decline (*n* = 270). (**B**) Endurance rate of decline (*n* = 270). (**C**) Strength rate of decline (*n* = 270). Data are means ± SEM. For pairwise analysis, **P* < 0.05. Post hoc Tukey test.

To estimate the relative rate of decline in performance traits in each line, I modified the sensitivity index of Falconer (1990). Comparisons of sensitivities to age shows that DGRP_73 exhibited a greater overall decline in climbing speed and endurance with age than DGRP_229 and DGRP_304 (Table 2.2)

Table 2.2. Sensitivity to effects of aging in three DGRP lines. Higher Sensitivity Index score indicates greater effect of aging on climbing speed (CS), endurance (EN), and/or strength (ST). Overall decline, measured from week one to week five of age, is highest in EN and ST of DGRP_229 and in CS of DGRP_73. Overall decline is minimal in CS, EN, and ST of DGRP_304.

Genotype	Assay	Week 1 to 3 Sensitivity	Week 3 to 5 Sensitivity	Week 1 to 5 Sensitivity
	CS	1.196	0.900	1.110
DGRP_229	EN	0.511	1.201	1.447
	ST	1.051	2.518	1.539
	CS	0.857	0.587	0.300
DGRP_304	EN	0.589	0.549	0.586
	ST	0.642	0.039	0.443
	CS	0.946	1.512	1.590
DGRP_73	EN	1.900	1.250	0.967
	ST	1.307	0.443	1.018

I noted that some lines may perform well in one or more of the measures, but not in all three. Therefore, I constructed a composite measure, the Fly Performance Phenotype (FPP), which considers individual physical performance in all three measures. This index is analogous to that used to measure frailty in humans (Liu et al. 2014) to identify the most vulnerable adults based on their functional performance. Using the FPP, I classified flies from each line as exhibiting high capacity (HC), medium capacity (MC), or low capacity (LC) to perform physical assays. I found that DGRP_73 flies

displayed not only the shortest average life span but also the highest prevalence of LC performers across all ages (49.8%). In contrast, DGRP_229 had the highest prevalence of HC performers (48.9%) and a mean life span that fell between the other two lines. Finally, DGRP_304 had the highest prevalence of MC performers (43.9%) and the longest average life span.

ii. Lisinopril impacts life-and health-span traits

I tested different doses of Lisinopril to determine at which concentrations it had an effect on life span. I used a serially increasing dose of Lisinopril on survival (tested by adding Lisinopril to the fly food in the following doses 0.2, 0.4, 1.0, 2.0, 4.0, and 10.0 mM). The lowest dose of Lisinopril had no effect on life span while the highest dose resulted in a significant reduction in life span (Appendix 2.1). 1 mM Lisinopril produced the greatest gain in mean life span (Appendix 2.1) and was used for all subsequent assays. I found that 1mM Lisinopril treatment increased mean life span for DGRP_229 (Fig. 2.2A), DGRP_73 (Fig. 2.2B) and DGRP_304 (Fig. 2.2C) flies, but did so to different degrees among lines.

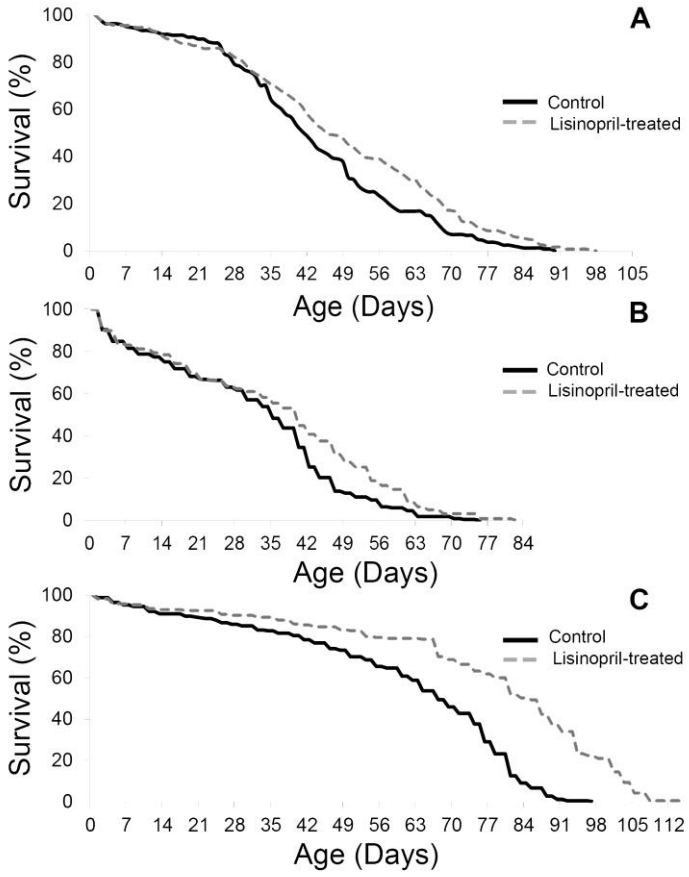

Figure 2.2. Lisinopril treatment increases life span. (**A**) DGRP_229 (P < 0.01; n = 520). (**B**) DGRP_73 (P < 0.01; n = 520). (**C**) DGRP_304 (P < 0.001; n = 520). Solid black lines depict control and dashed lines depict Lisinopril.

Next, I investigated the effects of 1 mM Lisinopril on fly physical performance with age. Lisinopril treatment affected the age-related decline of climbing speed, endurance, and strength, in a genotype-specific manner (Fig. 2.3, Table 2.3). Lisinopril treatment significantly attenuated the decline of all three physical performance measures for DGRP_229 flies (Fig. 2.3A). In contrast, DGRP_73 flies showed no significant effect of treatment on climbing speed or strength (Fig. 2.3B) and a significant decrease in endurance (2.3B).

Lisinopril treatment did not affect climbing speed, endurance, or strength in DGRP_304 flies (Fig. 2.3C).

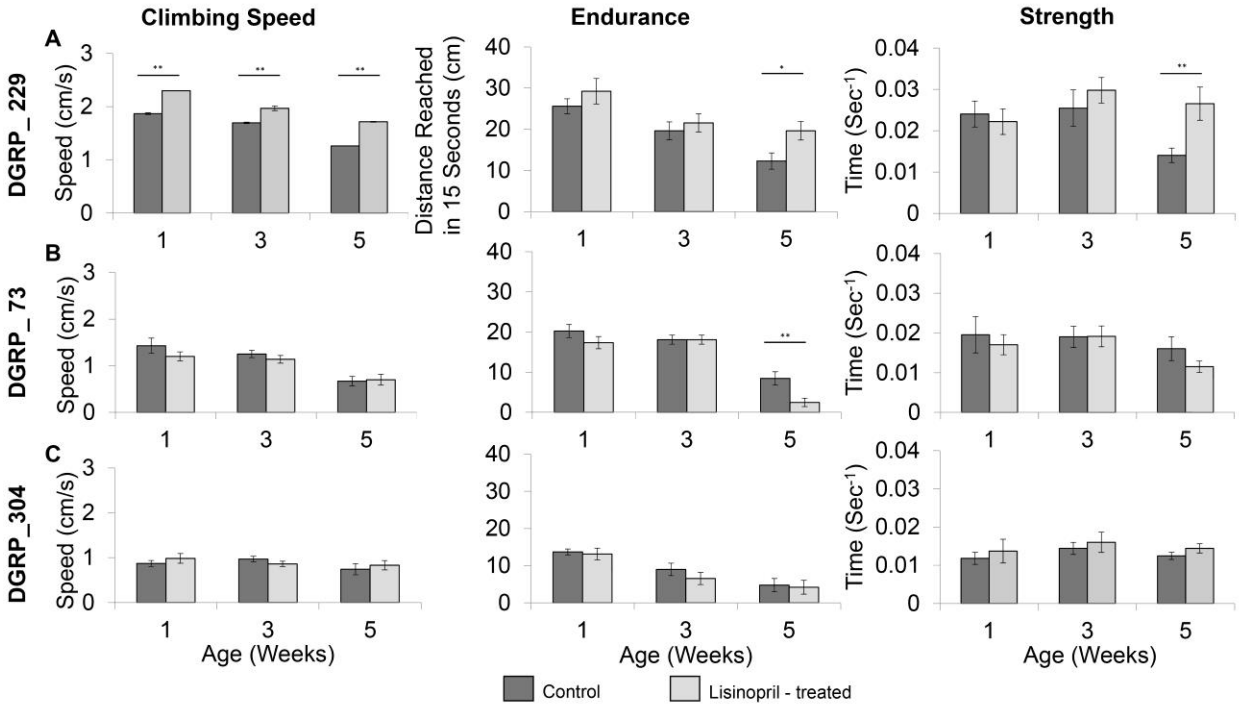

Figure 2.3. Lisinopril treatment improves speed, endurance, and strength in an age- and genotype-specific manner. (A) DGRP_229 (*n* = 540). (**B**) DGRP_73 (*n* = 540). (**C**) DGRP_304 (*n* = 540). Data are means ± SEM. **P* < 0.05; ***P* < 0.01.

Table 2.3. Analysis of covariance (ANCOVA) tables for untreated versus Lisinopril-treated genotypes. (A) climbing speed, (B) endurance, and (C) strength assay data.

(A) Climbing speed

Source of Variation	df	SS	MS	F	*P*-value
Mass	1	0.35	0.35	2.12	0.1468
Genotype	2	23.38	11.69	71.50	<0.0001
Treatment	1	0.10	0.10	0.59	0.4434
Age	2	1.74	0.87	5.31	0.0054
Treatment x Genotype	2	1.15	0.58	3.52	0.0306
Age x Genotype	4	2.71	0.68	4.14	0.0028
Age x Treatment	2	0.39	0.19	1.19	0.3063
Mass x Age	2	0.36	0.18	1.09	0.3369
Age x Treatment x Genotype	4	0.24	0.06	0.37	0.8295
Error	322	52.64	0.16		

(B) Endurance

Source of Variation	df	SS	MS	F	*P*-value
Mass	1	0.01	0.01	0.11	0.7394
Genotype	2	8.65	4.33	40.17	<0.0001
Treatment	1	0.01	0.01	0.10	0.7573
Age	2	1.49	0.75	6.93	0.0012
Treatment x Genotype	2	0.79	0.40	3.69	0.0267
Age x Genotype	4	1.60	0.40	3.71	0.0061
Age x Treatment	2	0.11	0.05	0.50	0.6097
Age x Treatment x Genotype	4	0.45	0.11	1.04	0.3896
Error	209	22.51	0.11		

(C) Strength

Source of Variation	df	SS	MS	F	*P*-value
Genotype	2	11.28	5.64	16.73	<0.0001
Treatment	1	1.27	1.27	3.78	0.0524
Age	2	4.11	2.05	6.09	0.0024
Treatment x Genotype	2	1.65	0.83	2.45	0.0871
Age x Genotype	4	3.89	0.97	2.89	0.0221
Age x Treatment	2	0.40	0.20	0.59	0.5568
Age x Treatment x Genotype	4	0.11	0.03	0.08	0.9879
Error	455	153.36	0.34		

iii. Consumption rate

Genetic differences in the response to Lisinopril may have resulted from differences in Lisinopril treatment due to variation in the amount of food consumed. To test for differences among lines in the consumption of food containing Lisinopril, I performed the CAFE Assay on male flies. Evaporation was accounted for by measuring volume loss in vials containing no flies. Mean evaporation was 0.274 and 0.277 for sucrose control and Lisinopril – sucrose treatment, respectively; evaporation did not vary with content of capillary tubes (Fig. 2.4).

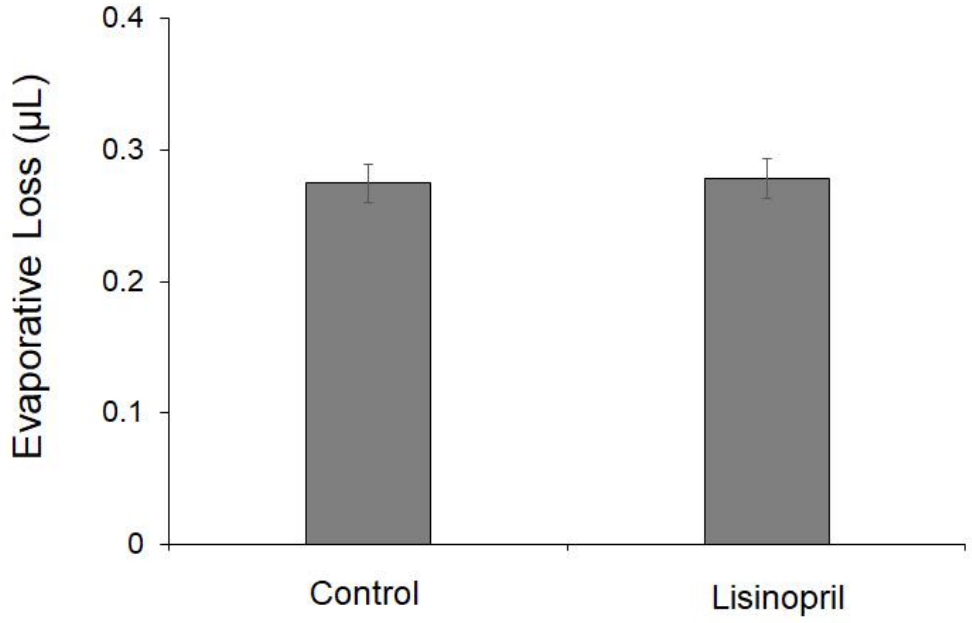

Figure 2.4. Volume loss through evaporation in the Capillary Feeder (CAFE) Assay. Data shown are mean values for evaporation over 24 hours for both solutions tested (N = 20). There is no difference in volatility between Lisinopril-containing and non-containing sucrose solution.

In DGRP_229, the consumption of Lisinopril-containing sucrose was significantly higher than that of sucrose alone (P = 0.0043, n = 49) (Fig 2.5A). Similarly, in DGRP_73, the consumption of Lisinopril-containing sucrose was significantly higher than that of sucrose alone (P < 0.0001, n = 50) (Fig 2.5B). In contrast, DGRP_304 flies showed no significant difference in consumption of sucrose and Lisinopril-containing sucrose (n = 50) (Fig 2.5C).

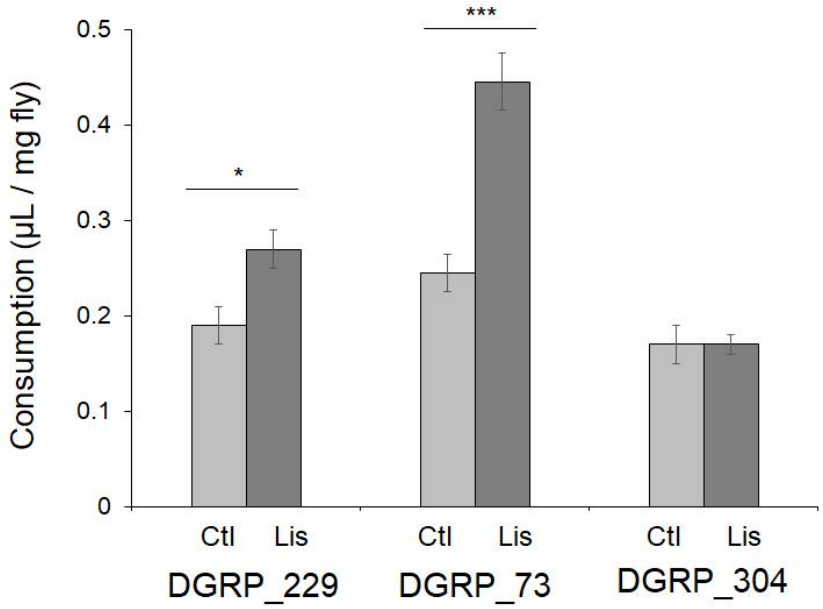

Figure 2.5. Consumption (µL/mg fly) of food varies with genotype and treatment. (**A**, **B**) In DGRP_229 and DGRP_73, the consumption of Lisinopril-containing sucrose is significantly higher than that of sucrose alone. (**C**) In DGRP_304, there is no significant difference between consumption of sucrose and Lisinopril-containing sucrose. *P < 0.01; **P < 0.001; ***P < 0.0001, Student's *t*-Test.

iv. Lisinopril reduces prevalence of low-capacity physical performance in a genotype-specific manner

Since the three genotypes displayed differential responses to treatment with respect to physical performance traits, I compared the effects of Lisinopril on the composite measure, FPP, at three and five weeks of age. Consistent with the results from the individual traits, DGRP_229 exhibited increased incidence of high capacity (HC) flies and a decreased incidence of medium capacity (MC) and low capacity (LC) flies at both ages, as well as an increased overall prevalence of HC flies when treated with Lisinopril (Fig. 2.6A-C). However, Lisinopril treatment had little effect on the incidence or prevalence of HC flies for DGRP_73 at either age. Lisinopril treatment decreased the incidence of MC flies in this genotype and increased the incidence of LC flies (Fig 2.6D-I). In DGRP_304, I observed a slight increase in HC and LC flies and a reduction in MC flies at three weeks of age. At five weeks of age, Lisinopril treatment also caused a slight increase in HC flies but, in contrast to week three of age, I saw a slight decrease in LC flies.

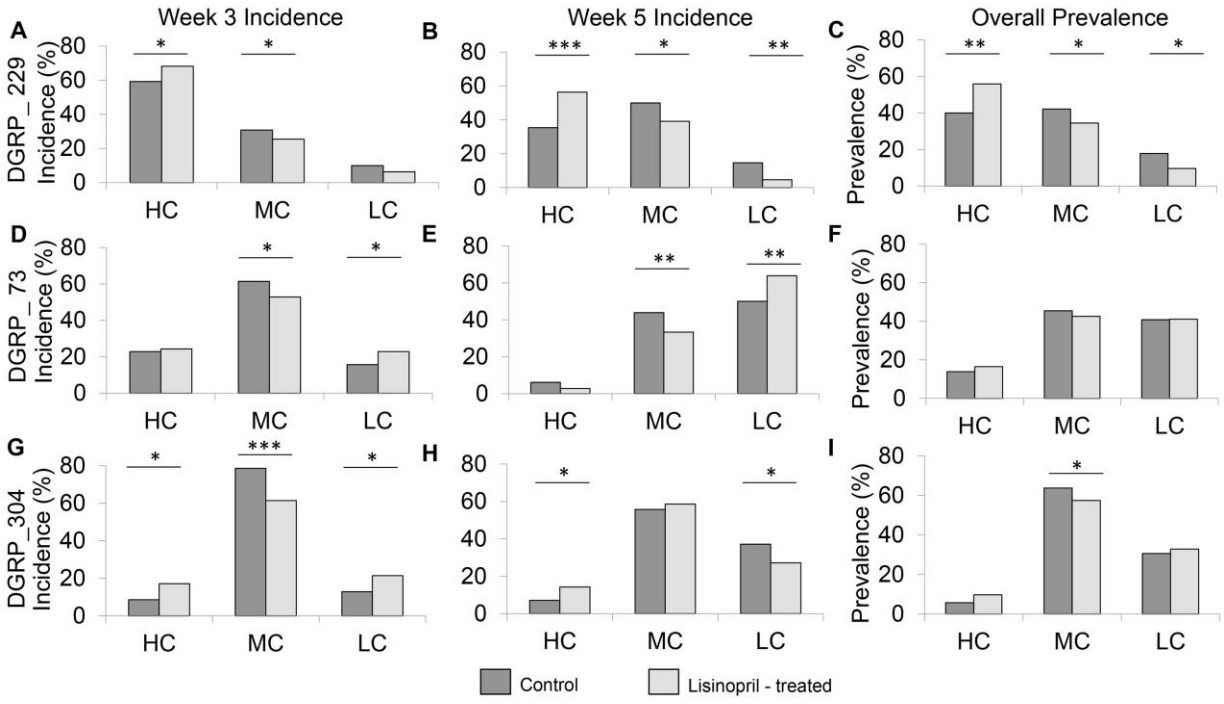

Figure 2.6. Lisinopril decreases incidence and prevalence of low capacity performance in three DGRP lines based on FPP Index. Change in percent incidence among high capacity (HC), medium capacity (MC), and low capacity (LC) performance at week 3 (baseline) and week 5 versus prevalence. (**A – C**) DGRP_229. (**D – F**) DGRP_73. (**G – I**) DGRP_304. * = 5 – 10% change, ** = 11 - 15 % change, *** = 16 - 21% change.

Although treatment with Lisinopril significantly extended the average life span of all lines, this reduction in mortality was associated with improvement of physical function most notably for DGRP_229 flies. The FPP index, as interpreted in the context of the human Frailty Index, indicates that reduction in mortality was associated with the general reduction of the incidence of ‘frailty’ for DGRP_229 flies.

v. Driver validation

I characterized muscle specificity of two commonly used drivers, dj667-Gal4 x UAS-*gfp* (n = 10) and mhc-Gal4 x *gfp* (n =10), using fluorescent images of dissected and whole, live male flies. As previous studies have shown, I found that both dj667-Gal4 (Seroude et al. 2002; Azad et al. 2009; Azad et al. 2012; Jones et al. 2016) and mhc-Gal4 (Osterwalder et al. 2001) are muscle-specific (Fig. 2.7).

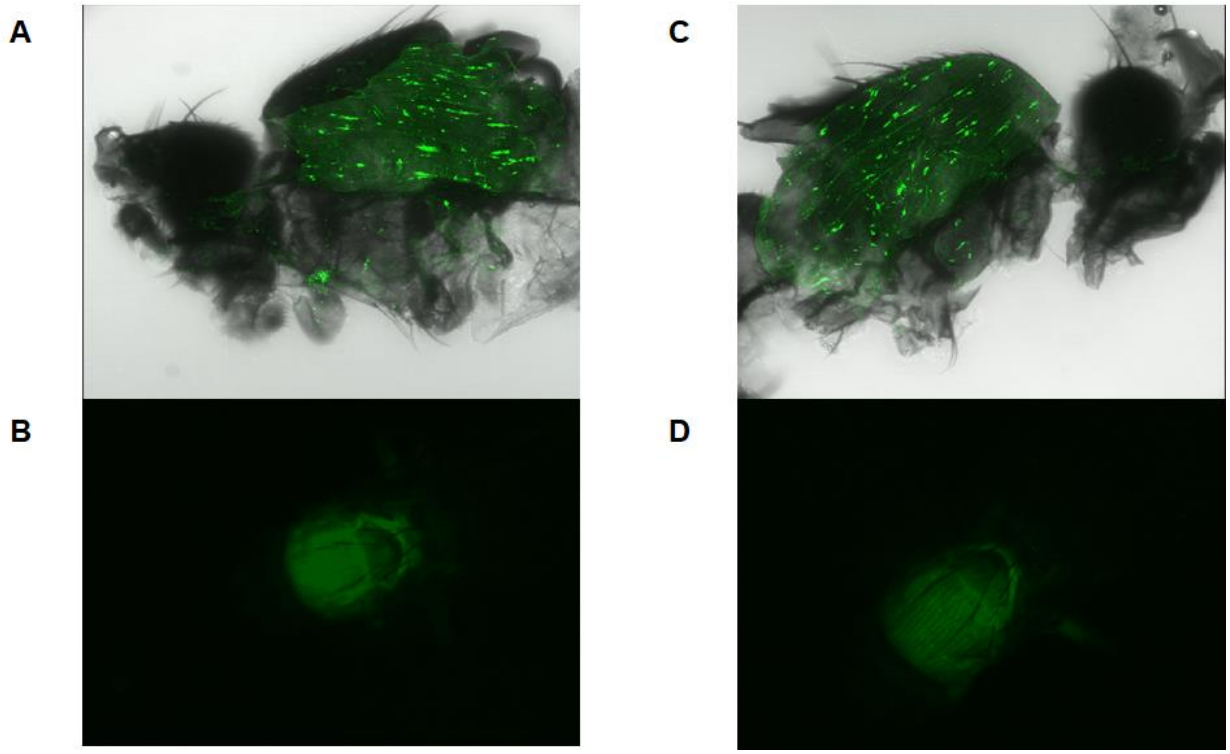

Figure 2.7. Fluorescence in two muscle-specific drivers. Both drivers have equal fluorescence that is concentrated in the thorax of dissected and whole, live flies. (**A**) *dj667*-Gal4 dissected fly (**B**) *dj667*-Gal4 whole, live fly (**C**) *mhc*-Gal4 dissected fly and (**D**) *mhc*-Gal4 whole, live fly.

vi. RNAi against *Ance* in skeletal muscle increases lifespan

Similar to the human ortholog, *Ance* is expressed ubiquitously throughout the body. Dissecting the impact of local, organ-specific enzyme activity on physical performance is difficult in humans. Many of the therapeutic benefits of ACE*i* are thought to be from their effects on blood pressure, but given its widespread expression, there are multiple possibilities. The impact of local skeletal muscle-specific *Ance* on the cross talk between physical function and life span is an uncharted territory. My data suggest that the knockdown of skeletal muscle-specific *Ance* in untreated RNA*i* flies led to a significant increase in survivorship compared to untreated controls ($P < 0.0001$; Fig. 2.8). Interestingly, treatment of RNA*i*-*Ance* flies with Lisinopril had no added benefit on survivorship, suggesting a requirement for skeletal muscle Ance in the survivorship benefits of Lisinopril (Fig. 2.8).

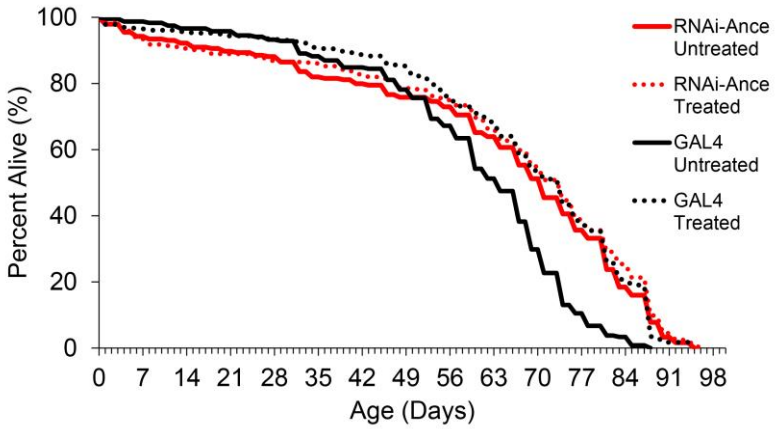

Figure 2.8. RNA*i-Ance* in skeletal muscle mimics positive effect of Lisinopril on life span. Life span of drug-treated Gal4 males is higher than that of untreated Gal4 males. There is no significant difference in life span of untreated RNA*i-Ance* males versus treated Gal4 males. There is no significant difference in lifespan between untreated and treated RNA*i-Ance* males.

vii. RNAi-*Ance* reduces expression in flies

To ensure the effects of Lisinopril treatment were due to a reduction in *Ance* expression, I tested the efficacy of RNA*i* using qRT-PCR. As seen in Figure 2.9, I observed roughly a five-fold reduction in *Ance* mRNA expression in the *dj667GAL4 x RNAi-Ance* fly line compared to the *dj667Gal4 x RNAi mCherry* control. I also observed greater than a two-fold reduction *dj667GAL4 x RNAi-Ance* flies compared to the *dj667GAL4 x attP2* control. Differences in the levels of *Ance* between the mCherry and attP2 controls could possibly be attributed to slight variations in the genetic background of these two lines.

However, it is clear that *dj667GAL4 x RNAi-Ance* flies have reduced expression compared to both controls (Fig. 2.9).

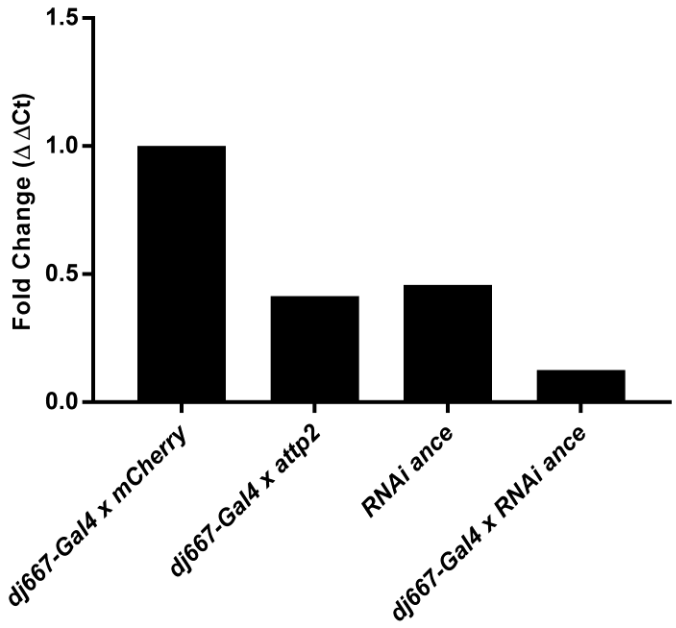

Figure 2.9. Verification of *in vivo* RNAi knockdown of *Ance* mRNA. qRT-PCR analysis demonstrates a reduction in *Ance* mRNA expression upon targeted RNA*i*. Fold change was made relative to the mCherry control.

viii. Protein aggregation in skeletal muscle changes with genotype, age, and treatment

Previous studies suggest that protein aggregation contributes to the decline in muscle function (Demontis et al. 2013) and may be affected by ACE inhibitors (Montgomery et al. 1998). I hypothesized that treatment with Lisinopril slows the damage and increases the turnover of dysfunctional proteins in skeletal muscle, and that the extent of improvement in physical

performance in each line is dependent on the degree of clearance of these proteins. Therefore, I quantified age-related accumulation of protein aggregates in skeletal muscles of the three DGRP lines with and without Lisinopril treatment. The extent of protein aggregation was determined by measuring the area (μm^2) of polyubiquitinated proteins. As shown in Figure 2.10C, there was a significant increase in protein aggregate area with age in the fibrillar muscles of DGRP_229 flies ($P < 0.0001$). At old age, treatment with Lisinopril significantly reduced protein aggregate area in DGRP_229 ($P = 0.0002$) (Fig. 2.10A, B, F). Similarly, protein aggregate area also significantly increases with age in line DGRP_73 ($P = 0.0004$) and treatment with Lisinopril significantly reduces protein aggregate area ($P < 0.0001$) (Fig. 2.10 D, E, F). In contrast, DGRP_304 showed a marginal increase ($P = 0.0680$) of protein aggregate area with age and treatment with Lisinopril has no effect of treatment at old age. (Fig. 2.10 G, H, F).

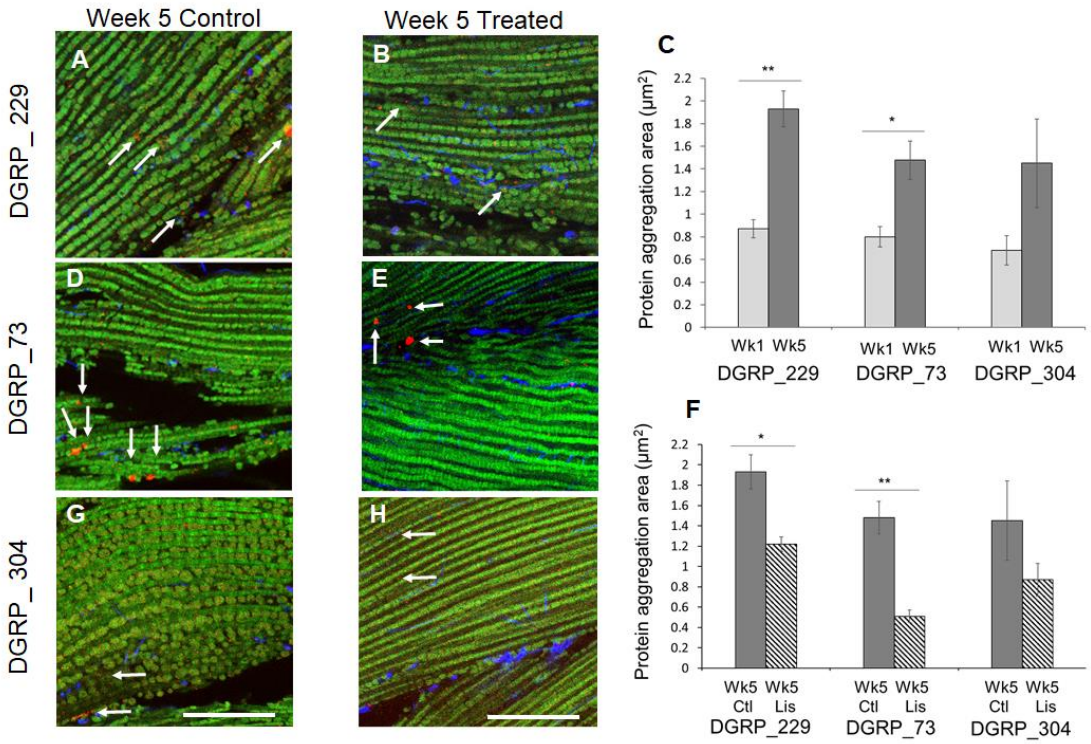

Figure 2.10. Lisinopril reduces protein aggregate area with age and genotype. Immunostaining of indirect flight muscles from control and treated (A-B) DGRP_229, (D-E) DGRP_73, and (G-H) DGRP_304 flies at five weeks of age. Poly-Ubiquitin (Cy3, red) immunoreactivity reveals deposition of aggregates (arrows), phalloidin staining (green) labels F-actin, and DAPI (blue) marks nuclei. (C) Mean area of protein aggregates increases with age in DGRP_229 and DGRP_73. Light gray bar is one week of age, dark gray is five weeks of age (F) At old age, treatment reduces mean area of protein aggregates in DGRP_229 and in DGRP_73. Dark gray bar is control, striped gray is Lisinopril-treated; data are means and SEM bars (n = 8 to 12 flies). Scale bar, 100µm. * $P < 0.001$, ** $P < 0.0001$ unpaired *t*-Test.

ix. Transcriptional response to Lisinopril treatment changes with age.

I next used RNA sequencing (RNA-Seq) to evaluate the effects of Lisinopril treatment on gene expression, comparing two of the lines that showed the most different responses to drug treatment. I first assessed the average effect of drug treatment on transcript levels, when I pooled data from both lines at each age. For all results reported below I used 5% FDR corrected *P*-values. I used gene ontology (GO) cluster analyses (Huang et al. 2009) to identify genes in resultant gene lists with similar molecular or biological function. For one-week old flies, 25 genes were differentially expressed between drug treated and control flies (Appendix 2.2A). With this small number of genes, I did not identify any gene ontology terms that were significantly overrepresented in this gene list. However, four of the genes have been implicated in stress response (*Cytochrome P450-4e3*, *Invadolysin*, *Turandot A* and *Turandot C*), and *Troponin C isoform 4* is involved in muscle activation. In contrast to the results from one-week old flies, 192 genes were differentially expressed between Lisinopril-treated and control flies when they were five weeks old (Appendix 2.2B). Gene ontology analysis identified six distinct clusters of functionally related genes that were overrepresented in this list (Appendix 2.3). Notably these clusters include genes involved in detoxifying xenobiotics (*CYP* genes), immunity, and metabolism.

As the two lines responded differently to Lisinopril, I also tested for genes that responded differently to Lisinopril treatment (those genes that

exhibited significant genotype by drug treatment interaction) at each age. At one week of age, 117 genes exhibited a significant genotype by drug treatment interaction (Appendix 2.4A). Gene ontology analysis revealed three clusters of genes that were functionally overrepresented in this list (Appendix 2.5A). The first cluster was enriched for CHK kinase genes which have been implicated in stress responses (Zhou and Elledge 2000). The second cluster contained genes in the *Turandot* family, a family of genes also associated with stress response (Ekengren and Hultmark 2001), including the immune response (Brun et al. 2006). The last cluster included many genes involved in membrane transport. At five weeks of age I found far fewer genes (29) that exhibited genotype-specific responses to the drug treatment (Appendix 2.4B). This may explain the relatively smaller phenotypic differences between the control and drug treated flies among genotypes at older compared to younger ages. Gene ontology analysis identified one cluster of genes significantly overrepresented in this list and these were primarily involved in proteolysis (Appendix 2.5B).

Conclusions

Medications commonly used in older individuals, such as angiotensin-converting enzyme (ACE) inhibitors, may attenuate age-related decline in physical performance (Hu et al. 2001; Hemming and Selkoe 2005; Ogen-Shtern et al. 2016). However, treatments with ACE inhibitors are not always effective (Montgomery et al. 1998) and determinants of inter-individual variation in response to ACE inhibitors are largely unknown. Conflicting results between studies can potentially be explained by genetic differences among individuals. Although the complete RAS system has only been identified in vertebrates, many genes regulating RAS are also found in *Drosophila* (Coates et al. 2000; Akif et al. 2010; Demontis et al. 2013). This indicates that these genes serve other physiological functions and that amelioration of age-related declines in vertebrates by treatment with Lisinopril may be due to additional physiological effects that are not solely due to blockade of the RAS pathway.

As physical performance and life span are closely linked in humans (van de Vijver et al. 2016), I compared changes in physical performance, FPP, and life span in my three fly lines to test the impact of genetic background on the effects of ACE inhibition. Lisinopril treatment influenced age-related decline of climbing speed, endurance, and strength that was dependent on genotype. Treatment of DGRP_229 flies significantly attenuated the decline of all three measures of physical performance: climbing speed, endurance, and strength. In contrast, treated flies of

DGRP_73 and DGRP_304 showed no effect on climbing speed nor endurance, but rather only on strength. To further segregate responders from non-responders, I tested the effects of Lisinopril on the composite measure, FPP. I noted a decline in prevalence of LC performance in DGRP_229 and an increase in percentage of HC flies with treatment. As decline in physical function is associated with rate of mortality, I tested the relationship between change in physical function and mortality with treatment. While treatment with Lisinopril significantly extended the average life span of all lines (Fig. 2.2), this reduction in mortality was associated with improvement of all 3 physical function measures only in DGRP_229 (Fig. 2.3).

To investigate the apparent dissociation between individual measures of physical performance and rate of decline in my DGRP lines, I constructed a composite index analogous to criteria used in humans (Fried et al. 2001) to identify worst performers (lowest quartile) of all three physical measures with age. My results indicate a strong relationship between genotype and performance capacity. Specifically, I observed the highest prevalence of low capacity performers in DGRP_304, which was associated with medium rate of decline in physical function but long life span. My results differ from previous studies which show that high physical performance ability is directly and positively related to long life span (Roshanravan et al. 2017), while low physical capacity is directly and negatively related to short lifespan (Fried et al. 2001). However, my results are consistent with studies which demonstrate

that physical performance can be inversely related to life span (van de Vijver et al. 2016) or not necessarily associated with life span at all.

I tested whether survivorship is affected by the expression of *Ance* in muscles. My results show that knockdown of skeletal muscle-specific *Anc*e was associated with a significant increase in survivorship compared to untreated control males (Fig. 2.8). Treatment of the RNAi knock down flies with Lisinopril had no added effects on survivorship. At a molecular level, aging is associated with changes in muscle fiber type and accumulation of protein aggregates (Stefani and Dobson 2003), potentially leading to defects in physical performance. My data suggest that the differential effect of Lisinopril on climbing speed, endurance, and strength in the three lines is driven by differences in the accumulation of protein aggregates in muscles. Morphologically, there are two major muscle types in adult *Drosophila*: fibrillar muscles, which are exclusively present as indirect flight muscles and provide power for oscillatory flight, and tubular muscles, such as the jump muscles and leg muscles, which are neurogenic and used for activities including climbing and the initiation of flight (Groteweil et al. 2005). Although I specifically concentrated on the flight muscles, protein aggregation appears to be a general contributor to the decline of adult muscle function. As such, future studies should assess the effects of Lisinopril treatment on protein aggregation in other muscle types. I also suggest assessment of protein aggregation in other locations, such as nervous or cardiac tissue as this might

provide additional insight into the variable effects of Lisinopril on traits such as life span.

Results from the RNA-Seq experiment identified several genes that responded to Lisinopril treatment. Many of these have been implicated in some aspect of stress and immune responses. These include genes in the *Turandot* family, CHK kinases and genes involved in the humoral response to infection. Additional experiments will be required to determine the functional effects of these genes on the phenotypes examined. This experiment also identified genes whose expression in response to Lisinopril depended on genotype in an age-specific manner. Many of these genes are also involved in stress responses, suggesting that genetically based variation in the phenotypic response to drug treatment may depend on the extent to which stress response pathways are activated in different genotypes. Given the fact that stress responses have also been associated with protein aggregation (Squier 2001; Ogen-Shtern et al. 2016; Vasconcellos et al. 2016), additional experiments directed at elucidating the interrelationships between Lisinopril, stress response, and protein aggregation offer a promising line of future research that could have direct application to personalizing medical treatment for patients taking this and related medications.

With the number of humans older than 60 years expected to double over the next 40 years, lack of physical ability is a major public health issue (Roshanravan et al. 2017). A major gap in our knowledge is the role that genetic variation plays in contributing to individual differences in age-related

decline of physical ability and the response to treatment. The biological functions of many of the genes that responded to Lisinopril treatment are unknown. This reflects the broader fact, that for most organisms, the biological roles of many genes in the genome are unknown. In this study, I demonstrate that *Drosophila* are a strategic model to elucidate the functions of particular genes relevant to human health.

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Chapter 3

Genome-wide Analysis of Age-Specific Physical Performance: Genotype-Specific Response to Lisinopril

Content of this chapter, in its entirety, is being submitted for publication.

Networks analyzed by Tatiana Morozova

Abstract

Age-related decline in locomotion and other traits reflecting physical performance is a universal feature of senescence and a major health risk for the elderly. While common, individuals vary in the extent to which age influences physical performance traits and this variation has a genetic component. In this study, I used genome wide association and genetic network analyses to identify genetic variants underlying age-related changes in climbing speed and endurance using the model genetic organism *Drosophila melanogaster*. In addition, I mapped polymorphisms contributing to age-specific variation in these traits when flies were treated with Lisinopril, a drug that has been shown to ameliorate the effect of age on these traits in humans. I identified a number of polymorphisms in genes affecting each trait at two different ages, one and five weeks of age. Of interest, I found that the genetic basis of variation in these traits depended on both age and Lisinopril treatment. Gene ontology and network analyses pointed to genes in the Notch and Wnt signaling pathways as important for contributing to the variation in these traits, particularly at older age. This study contributes to our understanding of senescence and nominates genes in these pathways as potential targets to treat age-related decline in physical performance.

Introduction

Most multicellular organisms exhibit senescence, a decline in physiological function with age (Finch, 1990; Rose, 1991). A number of studies have demonstrated that senescence has a genetic component, that the rate of age-specific decline varies dramatically among individuals and species, and this variation has a genetic basis (Finch, 1990; Rose, 1991). However, the genetic basis of natural variation in senescence remains poorly understood.

Physical performance traits such as speed, endurance, and strength, are important indicators of health that exhibit senescence in a wide variety of organisms, including humans (Fried et al. 2001; Boehm and Nabel 2002; Crackower et al. 2002; Oudit et al. 2003; Espinoza and Walston 2005; Der Sarkissian et al. 2006). In humans, decline in physical performance traits with age predicts hospitalization, morbidity, and mortality (Fried et al. 2001). Previous studies in both flies (Chapter 2) and humans (Wang et al. 2008; Buford et al. 2012, 2016) have demonstrated significant, genetically based, variation among individuals in the effect of age on the decline in physical ability.

Although studies of the actual genes that influence age-specific physical performance traits are limited, one gene, the angiotensin-converting enzyme (ACE), has been associated with physical performance (Mongomery et al. 1998; Wang et al. 2008) and longevity in humans (Petranovic et al. 2012). Human ACE is an essential enzyme that regulates the renin-

angiotensin system (Abadir et al. 2012) and ACE inhibitors (ACE*i*), such as Lisinopril, are commonly prescribed for hypertension in the elderly. While ACE*i* are prescribed for treating high blood pressure, ACE*i* have also been reported to have beneficial side effects, improving different measures of physical performance in the elderly (Buford et al. 2012, 2016). In these cases, the positive effect of ACE*i* may result from alterations in body composition and the metabolism of skeletal muscle, and not from the intended effect of ACE*i* to control blood pressure (Carter et al. 2005, Cabello-Verrugio 2015).

Despite the findings outlined above, beneficial effects of ACE*i* on physical performance traits are not always observed. Gray et al. (2009) did not find an association between ACE*i* use and the incidence of frailty in women ages 65 years and older. Shrikrishna et al. (2014), found no effect of ACE*i* on improving quadriceps strength in patients with COPD. Witham et al. (2014), found no detectable effect of ACE*i* usage on improving grip strength in the elderly. Use of ACE*i* has also been reported to have detrimental effects on certain physical performance traits (Tinetti et al. 2014a; George and Verghese 2017). For example, in the study by George and Vergese (2017), elderly individuals taking ACE*i* had a slower walking gait speed compared to patients that were taking other types of antihypertensive medicine.

The inconsistent findings of these studies on the effect of ACE*i* on physical performance measures is likely due to a number of factors, both experimental and biological. Undoubtedly, some inconsistency results from the different experimental designs used in each study. These differences

include variation in the samples sizes used, variation in the durations of the studies, differences in age distributions and sexes of individuals used, the types of ACE*i* treatments individuals received, and the types of traits examined. A biological source of inconsistency among studies might be from variation among individual differences in the response to ACE*i* treatment, which is likely to have a genetic component. For example, genetic variation in the *ACE* gene alone has been associated with a wide range of patient outcomes in cardiovascular disease and response to ACE*i* (Taylor et al. 1996; Chung et al. 2010). More recently, genomic studies suggest that variation in a number of additional genes as well as variable metabolomic responses contribute to variation in the response of blood pressure to ACE*i* treatment (Flaten and Monte 2017).

These studies suggest that genetic variation is an important component of the variable responses of individuals to ACE*i*. Unfortunately, incomplete understanding of the genes and genetic networks that regulate the responses of physiological traits to ACE*i* treatment limits our ability to design effective treatment. Such knowledge would allow the design of pharmacological and other interventions to account for genetic differences among individuals, and so enhance treatment efficacy while reducing risk (Giocomoni et al. 2007; Wang et al. 2008; Wang et al. 2011).

In this study, I use the fruit fly, *D. melanogaster,* to test the hypothesis that the inconsistent responses to ACE*i* treatment depend, in part, on age and genotype of the individual and to identify evolutionarily conserved loci that

modulate the response of physical performance traits to drug treatment. *D. melanogaster* is a well-established model for studying muscle physiology and function (Jones and Grotewiel 2011; Demontis et al. 2013), aging (Durham et al. 2014), and drug treatment (Akif et al. 2010; Kasai and Cagan 2010). In particular, flies exhibit age-related decline in physical performance (Grotewiel et al. 2005) which is one of the traits theorized to be enhanced by ACE*i* treatment. The closest *Drosophila* ortholog to mammalian *ACE*, angiotensin-converting enzyme (*Ance*) (Coates et al. 2000), has been identified as a candidate gene contributing to natural variation in lifespan (Durham et al. 2014). In addition, the mechanism by which Lisinopril binds to fly Ance is similar to that of human ACE (Akif et al. 2010).

To gain insight into the genetic basis of natural variation in physical performance and drug response at the molecular genetic level, I measured two aspects of physical performance of young and old flies together with drug response in non-mated males, using 126 lines from the *Drosophila* Genetic Reference Panel (DGRP). The DGRP is a set of 205 *Drosophila* lines derived from nature that have been completely sequenced (Mackay et al. 2012). We used the DGRP lines to carry out genome wide association (GWA) studies to identify polymorphisms, candidate genes and gene networks that contributed to the variation in age-specific walking speed and endurance, both of which are indicators of human and fly frailty (Chapter 2). I carried out the GWA using two treatments, one in which flies were fed a regular diet, and one in which the regular diet was supplemented with the ACE*i*, Lisinopril. This

allowed us to measure the effects of Lisinopril treatment on these traits as the flies aged, and identify candidate genes that influenced the response of these traits to Lisinopril treatment.

Materials and Methods

i. ***Drosophila* stocks and maintenance**

Virgin males of 126 distinct genotypes from the *Drosophila* Genetic Reference Panel (Mackay et al. 2012) were used for all physical performance assays. Control groups were fed standard food medium (solid ingredients: 82.9% cornmeal, 16.5% yeast, and 3.4% agar). Treated groups were fed 1mM Lisinopril according to drug dosage and administration protocols previously described (Chapter 2). Flies were maintained in vials at 25^{o}C and approximately 55% relative humidity under a 12-hour light and dark cycle. All physical performance assays were completed between 8a.m. and 2p.m.

ii. **Physical Performance Assays**

Climbing speed and endurance assays previously described (Chapter 2) were used to test age-related decline of physical performance. For each assay I used 30 flies for each of the 126 genotypes, per age, per treatment. An individual fly was only tested in one measure of performance at one age; independent flies were used in each line, age, and treatment combination. In brief, climbing speed was tested by aspirating an individual fly from a vial and placing it into the bottom of a Costar® 25-mL in 2/10, non-pyrogenic

serological pipet, marked at nine and 27 centimeters. The start time was measured once the body of fly passed the zero mark on the bottom of the pipet. Each trial was ended when the fly either reached a distance of nine centimeters on the pipet, paused for greater than five seconds, or dropped to the bottom. Endurance was measured using same technique as that for climbing speed but was calculated based on the distance traveled in 15 seconds.

iii. GWA Gene Ontology

To assess the functional relatedness of the candidate genes identified in each GWA, I performed Gene Ontology enrichment analysis of candidate genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (Dennis et al. 2003; Huang Da et al. 2009). I did four separate GO analyses, one for each trait (climbing speed and endurance) for each age separately (one and five weeks of age). For each GO analysis, I combined candidate genes identified in the control and Lisinopril treated conditions.

iv. Network analysis and Gene Ontology

To prioritize candidate genes for the follow up study, we performed network analyses using candidate genes implicated by the GWA using the *igraph* package in R (R Core Team). We first generated separate networks for climbing speed and endurance at each age. For network analysis within each

age, we combined the genes identified as candidates from GWA of flies in control and Lisinopril treatments. We identified computationally predicted networks of genetically interacting genes, allowing one missing gene (non-candidate) in between the candidate genes (i.e. a gene connecting two candidate genes, but not carrying a variant associated with the trait). We used candidate genes significant at $P < 10^{-6}$ and mapped them to the physical and genetic interaction databases downloaded from Flybase release r5.57 using the *igraph* package in R (Fochler et al. 2017; R Core Team). Genes in these networks are represented as nodes, whereas edges between nodes represent interactions. We extracted subnetworks from the global networks whose edges either directly connected candidate genes or were bridged by only one gene that was not in the list of candidate genes. We tested whether the maximum subnetwork was significantly greater than would be expected by chance using a permutation procedure (Antonov et al. 2008). Briefly, we randomly selected *n* genes that could be mapped to the global networks, where *n* is the number of significant genes mapped to the global network. The size of the largest subnetwork was then computed. This procedure was repeated 1,000 times, and the *P* value was calculated as (A + 1)/1,001, where A was the number of permutations in which the size of the largest subnetwork was equal or greater than the size of the largest subnetwork with the observed gene list (Carbone et al. 2017). Human orthologs were obtained using the DRSC Integrative Ortholog Prediction Tool with all available prediction tools, excluding low scores of less than 2 (DIOPT, version 5.4;

http://www.flyrnai.org/diopt; (Hu et al. 2011). A gene interaction network for human orthologs was constructed using R-Spider (http://www.bioprofiling.de) (Antonov 2011). We performed Gene Ontology enrichment analysis of connected candidate genes in each network DAVID as described in the GWA Gene Ontology section above.

v. Statistical Analyses

Climbing and endurance data were analyzed using ANCOVA (PROC GLM, SAS V9.3) using wet fly mass as a covariate. I used the following model to assess the influence of genotype, treatment, and age on climbing speed and endurance: $y = c + m + g + t + a + all\ interactions + E$, where c is a constant, g tested for differences among DGRP lines, t tested the effects of Lisinopril treatment, a is the effect of age, and E is error. None of the interactions between mass and the main effects in the model were significant so interaction terms involving mass were dropped from the model.

To identify candidate SNPs that contribute to variation in the phenotypes, I submitted the least squares line means of each trait (corrected for body mass) to the DGRP analysis pipeline (http://dgrp.gnets.ncsu.edu/). GWA was completed on 123 of the 126 lines assayed, based on the sequence data available within the pipeline at the time of analysis. The DGRP Freeze 2 Release GWA analysis uses simple linear model ANOVAs on approximately 4.8 million SNPs using the model y = u + M + E, where M is the effect of the SNP and E is the error variance (Mackay et al. 2012). The output

included all candidate SNPs associated with each respective phenotype at a nominal $P < 10^{-6}$ and provided information on site class for each SNP. SNPs located in coding regions were identified as missense, nonsense, or synonymous variants (Mackay et al. 2012).

Results

i. Genetic variation in age-specific climbing speed, endurance, and drug response

Climbing Speed

Climbing speed varied significantly among genotypes (Fig. 3.1) ($P < 0.0001$). There was also a significant effect of age on climbing speed as young flies climbed 50% faster than old flies ($P < 0.0001$; Week 1: 1.54 ± 0.01, Week 5: 0.77 ± 0.01). However, the effect of age on climbing speed depended on genotype ($P < 0.0001$, Fig. 3.2A, B).

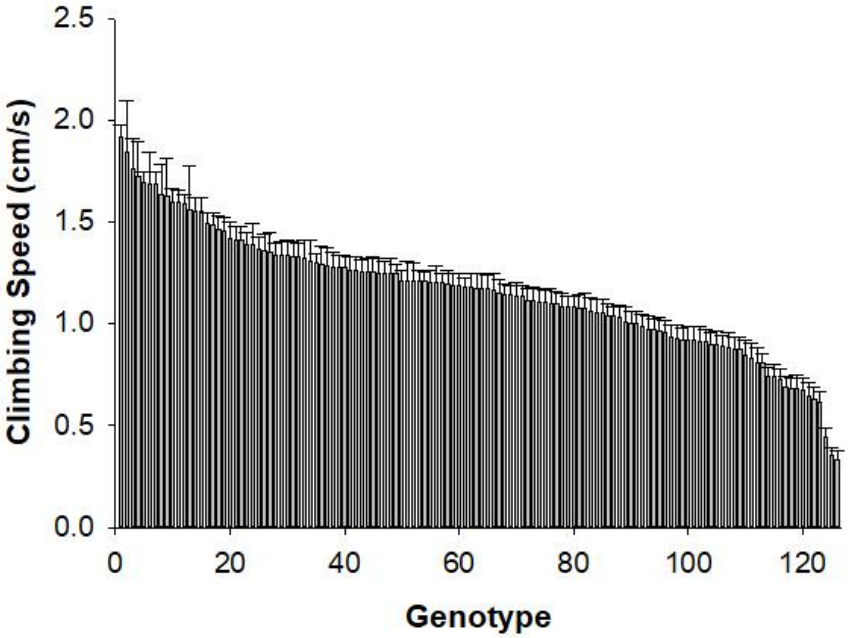
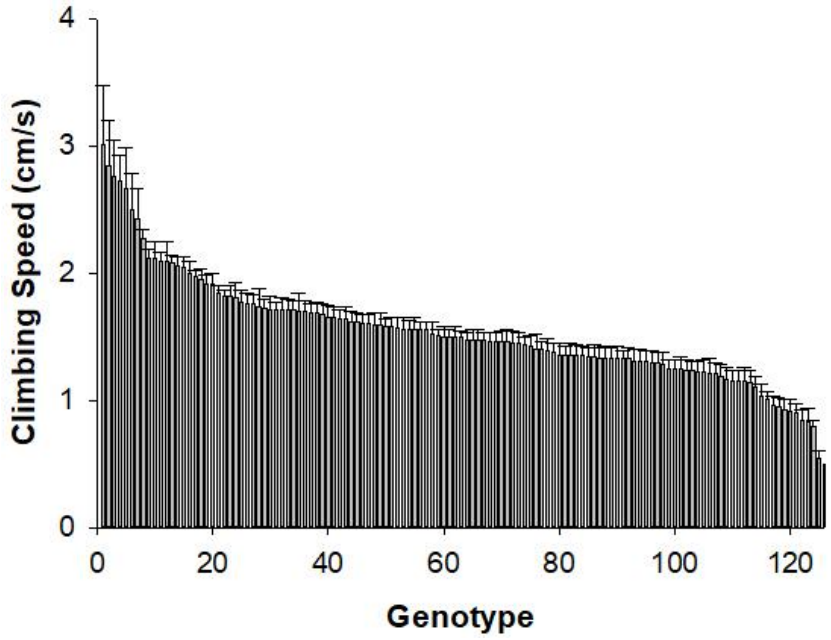


Figure 3.1. Climbing speed varies with genotype. Data are the mean climbing speed for each DGRP line tested (± one S.E.) independent of age and treatment. Genotypes are ranked from fastest to slowest.

A



B

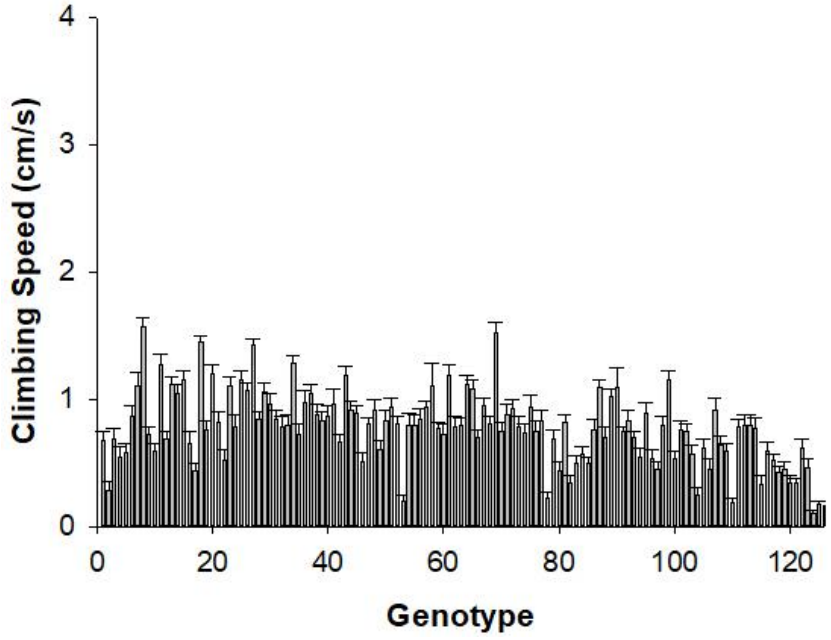


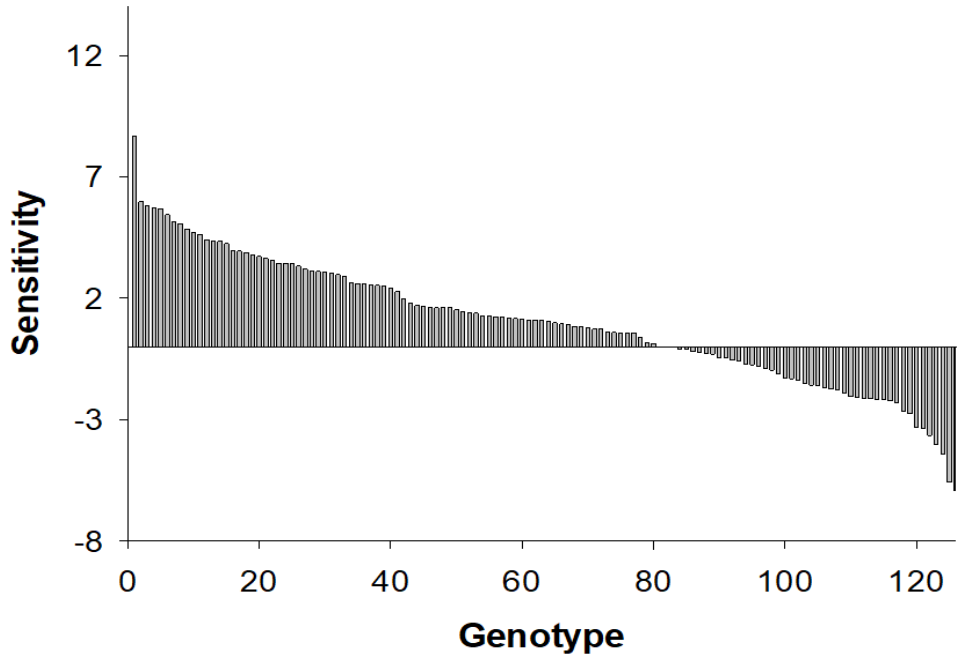
Figure 3.2. The effects of age on climbing speed depends on genotype. There is a significant genotype by age interaction. **(A)** Genetic variation in climbing speed at one week of age; genotypes are ranked from fastest to

slowest. **(B)** Variation among genotypes in climbing speed at five weeks of age; genotypes are ranked based on climbing speed at one week of age. Data are the mean climbing speeds for each DGRP line tested (± one S.E.) at each age; ANCOVA.

Flies treated with Lisinopril, independent of age and genotype, climbed 8% faster than untreated flies (P = 0.0141; Lisinopril treated: 1.20 ± 0.01 cm/s, Control mean 1.10 ± 0.01 cm/s,). The effect of Lisinopril treatment on climbing speed also varied significantly among genotypes (P = 0.0069). The three-way interaction between age, Lisinopril treatment and genotype approached significance (P = 0.0502).

We used a modified sensitivity index of Falconer (1990) to examine the age-specific responses of each genotype to the effects of Lisinopril treatment reflected in the above interactions. This method calculates a sensitivity value for each genotype by taking the difference in the mean climbing speed between Lisinopril treated and untreated flies, and dividing this value by the average difference in treated and untreated flies across all genotypes (Fig. 3.3A, B). Genotypes exhibited extensive variation in their sensitivity to Lisinopril treatment at each age, and most genotypes climb faster when treated with Lisinopril, as indicated by positive sensitivity values (P < 0.05). Genotypes also exhibited a greater range of sensitivity to Lisinopril treatment at older ages, compared to younger ages (P < 0.05) (Figs. 3.3A, B).

A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-8
Sensitivity
0
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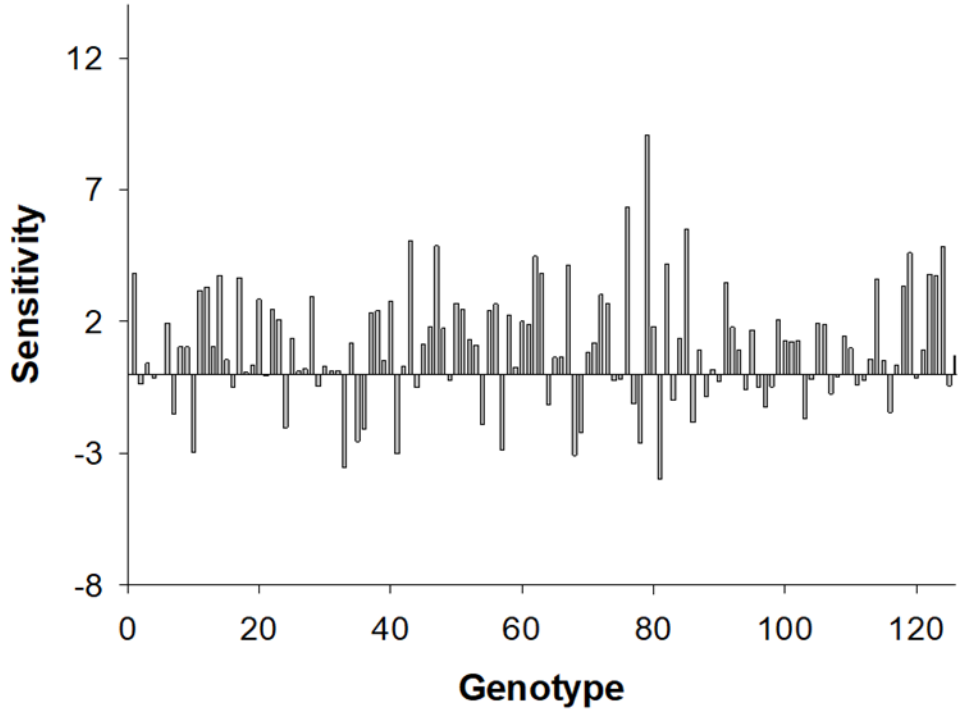
60
80
100
120
Genotype

B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Genotype

Figure 3.3. Sensitivity of each genotype to Lisinopril treatment depends on both age and treatment. (**A**) There is significant variation among genotypes in the sensitivity of climbing speed to Lisinopril treatment at one week of age. Genotypes are ranked by sensitivity at one week of age from most to least sensitive. (**B**) There is significant variation among genotypes in the sensitivity of climbing speed to Lisinopril treatment at five weeks of age. Genotypes ranked based on their sensitivity to Lisinopril treatment at one week of age. In both figures positive values indicate greater speed in Lisinopril treatment relative to no treatment controls, negative values indicate slower speeds relative to no treatment controls; ANCOVA.

Endurance

Endurance varied significantly among genotypes ($P < 0.0001$) (Fig. 3.4). As was the case for climbing speed, physical performance also declined with age ($P < 0.0001$). Younger flies were able to climb over twice the distance that older flies reached during the 15 second interval in the endurance test (distance reached by one week old flies: 16.57 $\pm$ 0.14 cm, distance reached by five week old flies: 7.34 $\pm$ 0.12 cm).

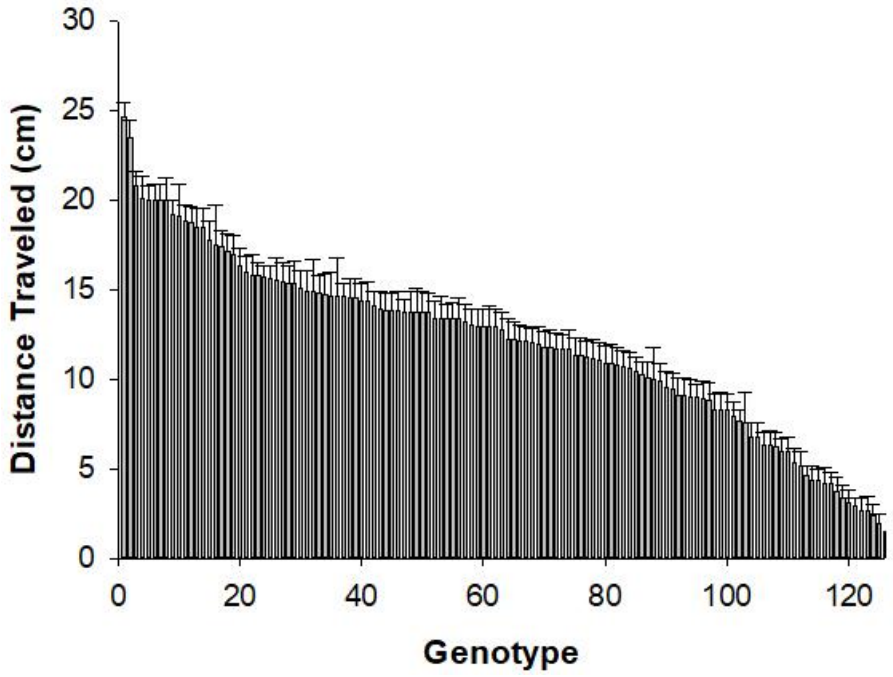
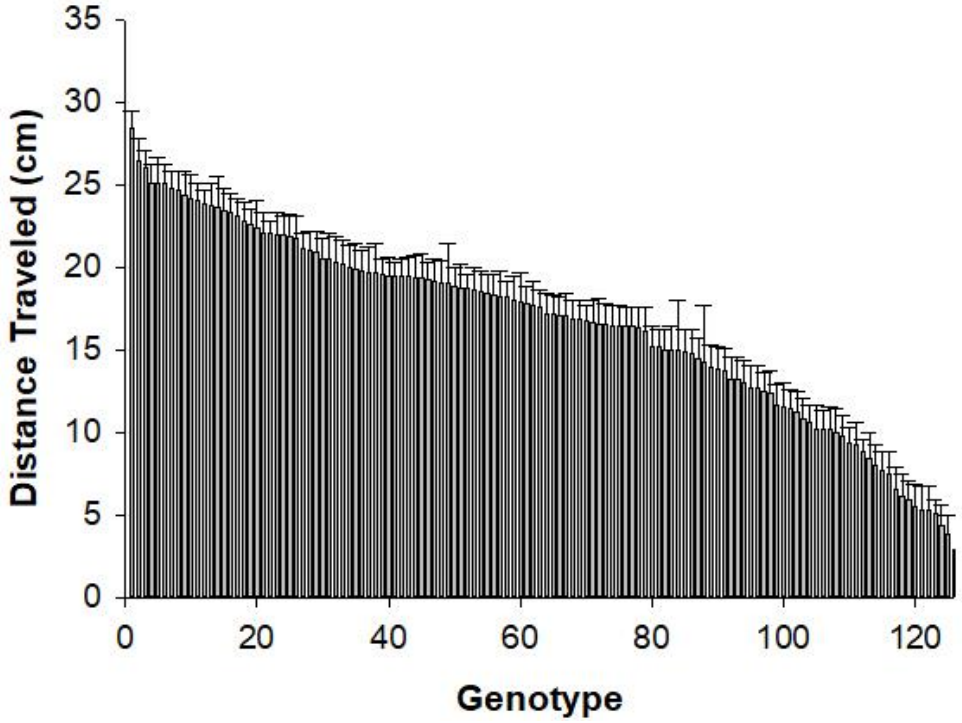


Figure 3.4. Endurance varies with genotype. Data are the mean distance reached in 15 seconds for each DGRP line tested (± one S.E.) independent of age and treatment. Genotypes are ranked from longest to shortest distance traveled.

The effect of age on endurance varied extensively among genotypes (Fig. 3.5A, B) Lisinopril treatment, independent of age and genotype, positively influenced endurance ($P < 0.0001$). Flies treated with Lisinopril climbed 10% farther than controls (Lisinopril treated: 12.53 ± 0.14 cm/s, Control mean 11.39 ± 0.14 cm/s). As in the results for climbing speed, the effect of Lisinopril treatment on endurance varied significantly among genotypes ($P < 0.0001$). There was also a significant three-way interaction ($P < 0.0001$), indicating that the effect of age on endurance depended both on genotype and Lisinopril treatment.

A


Distance Traveled (cm)
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0
20
40
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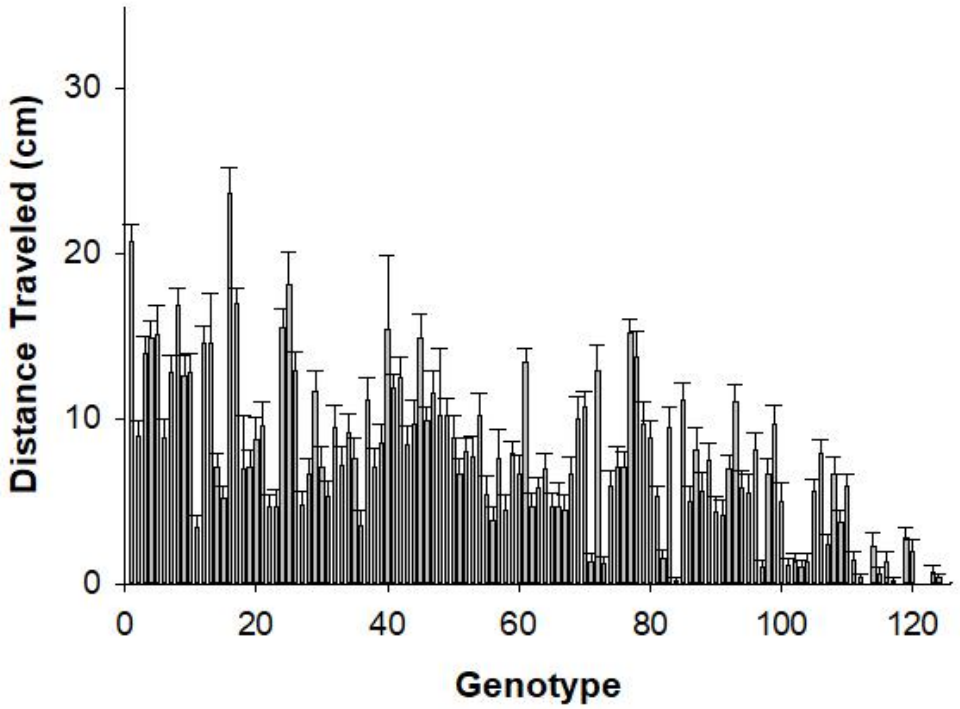
80
100
120
Genotype

B


Distance Traveled (cm)
30
20
10
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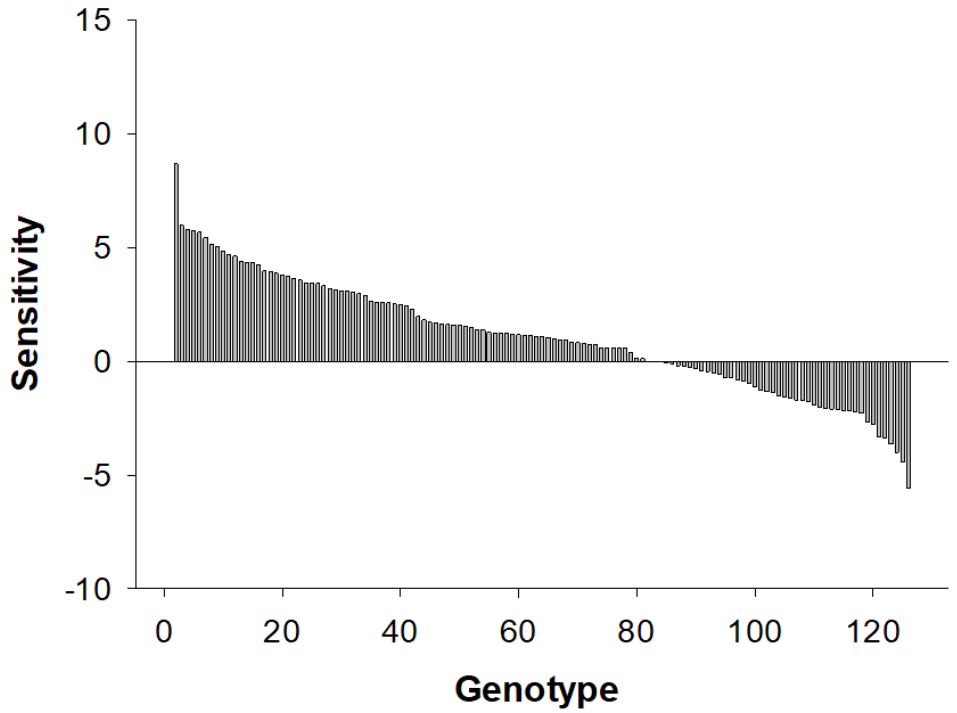
0
20
40
60
80
100
120
Genotype

Figure 3.5. The effects of age on endurance depends on genotype. There is a significant genotype by age interaction. **(A)** Variation among genotypes in endurance at one week of age; genotypes are ranked from longest to shortest distance reached. **(B)** Variation among genotypes in endurance at five weeks of age; genotypes are ranked based on endurance from longest to shortest distance reached at one week of age. Data are the mean distance reached for each DGRP line tested (± one S.E.) at each age; ANCOVA.

As was the case for climbing speed, effects of Lisinopril treatment on endurance varies with both age and treatment. There was significant variation among genotypes in the sensitivity of endurance to Lisinopril treatment at each age ($P < 0.0001$) (Fig. 3.6A, B). Comparing the sensitivities of each genotype across ages (Fig. 3.6A and B) also indicates that the effect of age on sensitivity to the drug varies dramatically among genotypes ($P < 0.0001$).

To investigate the genetic basis of these age- and treatment- specific differences, we carried out genome-wide association analyses using the DGRP.

A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Sensitivity
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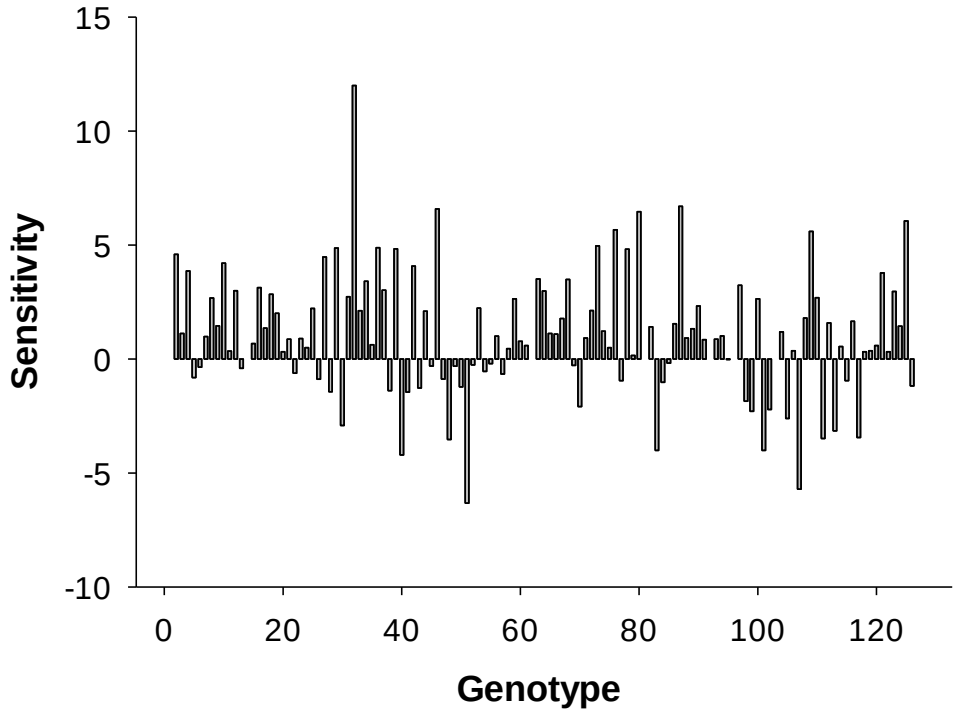
Genotype

B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Sensitivity
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40
60
80
100
120
Genotype

Figure 3.6. Sensitivity of each genotype to Lisinopril treatment depends on both age and treatment. (**A**) There is significant variation among genotypes in the sensitivity of endurance to Lisinopril treatment at one week of age. Genotypes are ranked by sensitivity at one week of age from most to least sensitive. (**B**) There is significant variation among genotypes in the sensitivity of endurance to Lisinopril treatment at five weeks of age. Genotypes ranked based on their sensitivity to Lisinopril treatment at one week of age. In both figures positive values indicate longer distance reached in Lisinopril treatment relative to no treatment controls, negative values indicate shorter distance reached relative to no treatment controls; ANCOVA.

ii. GWA results

We next utilized the DGRP analysis pipeline (http://dgrp.gnets.ncsu.edu) to associate variation in climbing speed, endurance, and drug response with allelic variation at just over 4.8 million SNPs (Huang et al. 2014; Mackay et al. 2012). We analyzed the following four conditions for climbing speed and for endurance: one-week old flies fed control food, one-week old flies fed Lisinopril-treated food, five-week old flies fed control food, and five-week old flies fed Lisinopril-treated food.

GWA Climbing speed

In one-week old, control flies, we identified eight indels and 66 candidate SNPs within or nearby (less than 5,000 bp away from) 45 genes affecting climbing speed ($P < 10^{-6}$) (Appendix A1). For climbing speed in one-week old, Lisinopril-treated flies, we identified eight indels and 126 candidate SNPs within or nearby (less than 5,000 bp away from) 67 genes, affecting climbing speed ($P < 10^{-6}$) (Appendix A1).

For climbing speed in five-week old, control flies, we identified 12 indels and 200 candidate SNPs within or nearby (less than 5,000 bp away from) 99 genes, affecting climbing speed ($P < 10^{-6}$) (Appendix A1). For climbing speed in five-week old, Lisinopril-treated flies, we identified six indels and 38 candidate SNPs within or nearby (less than 5,000 bp away from) 14 genes (Appendix A1), affecting climbing speed ($P < 10^{-6}$).

GWA Endurance

In one-week old, control flies, we identified three indels and 26 candidate SNPs within or nearby (less than 5,000 bp away from) nine genes (Appendix A2), affecting endurance ($P < 10^{-6}$). For endurance in one-week old, Lisinopril-treated flies, we identified three indels and 25 candidate SNPs within or nearby (less than 5,000 bp away from) nine genes (Appendix A2), affecting endurance ($P < 10^{-6}$).

For endurance in five-week old, control flies, we identified 12 indels and 150 candidate SNPs within or nearby (less than 5,000 bp away from) 49

genes (Appendix A2), affecting endurance ($P < 10^{-6}$). For endurance in five-week old, Lisinopril-treated flies, we identified six indels and 38 candidate SNPs within or nearby (less than 5,000 bp away from) 14 genes (Appendix A2), affecting endurance ($P < 10^{-6}$).

Candidate SNPs influencing climbing speed, endurance, and drug response are age specific

The GWA revealed little overlap in candidate polymorphisms influencing climbing speed between treatments within each age. Only 20 out of 75 total genes were identified as candidates affecting young control and treated flies (Appendix A1, A2, B) and zero of 100 genes were found in common comparing the old control and Lisinopril-treated flies. Comparing young and old flies within treatments, no genes were found in common between young and old control flies (Appendix A1, A2, B) and young and old flies that were treated with Lisinopril.

iii. GWA Gene Ontology analysis

GO analysis of candidate genes for climbing speed at one week of age found no functional categories overrepresented. GO analysis of climbing speed candidates at five weeks of age identified five functional categories that were overrepresented. The largest functional category was transmembrane proteins (33 genes) following by those with immunoglobulin domains, receptors, and zinc finger proteins.

Analysis of candidate genes for endurance at young ages found no functional categories overrepresented. Based on the candidates identified for endurance at five weeks of age, one functional category was overrepresented in the gene list. The major GO category was transmembrane helix represented by 13 genes, only two of which have been named, *Equilibrative nucleoside transporter 3* (*Ent3*), and *Tetraspanin 42Eg* (*Tsp42Eg*).

iv. Network analysis

Climbing speed

We first analyzed networks of genes associated with variation in climbing speed and endurance, combining genes identified by GWA when flies were maintained on control or Lisinopril containing food. Analysis of climbing speed candidates revealed a network comprised of 33 interacting genes with 17 candidate genes and 16 non-candidate genes for young flies (Fig. 3.7A). Seventy-six percent of these genes have a human orthologs. *Small nuclear ribonucleoprotein* (*Snr1*) is the most interconnected gene in the network. *Snr1* has been associated with dendrite morphogenesis (Parrish et al. 2006), dendrite guidance (Tea and Luo 2011) and regulation of transcription (Bonnay et al. 2014). Gene ontology analysis showed enrichment of organ morphogenesis, metamorphosis, RNA metabolic process, programmed cell death and oogenesis. In addition, six genes were involved in heart development (Appendix 3.1). We identified network of 28

candidate genes and 60 missing genes associated with variation in climbing speed in old flies (Fig. 3.7B). This network was enriched for genes associated with cell differentiation, epithelium development, organ and tissues morphogenesis as well as regulation of signal transduction (Appendix 3.2). Amazingly, 90% of these genes have known human orthologs. *Ras-homologous* (*Rho1*), *Smooth* (*S*), *Grunge* (*Gug*), and *Echinoid* (*ed*) were among the genes with the highest number of interactions. *Rho1* is a GTPase signaling protein, that plays a role in actin cytoskeleton organization, morphogenesis and wound repair (Hall 1998; Abreu-Blanco et al. 2014). *Gug* is involved in segmentation, embryonic pattern specification (Zhang et al. 2002) and is a negative regulator of EGFR signaling pathway (Charroux et al. 2006). *S* is a type II transmembrane protein that plays a role in growth regulation (Lee et al. 2001), cell survival (Montrasio et al. 2007) and behavioral response to ethanol (Corl et al. 2009). *ed* participates in cell-cell adhesion (Wei et al. 2005), as well as in multiple signaling pathways including EGFR, Notch, and Hippo during organogenesis (Bai et al. 2001; Yue et al. 2012). This network also has similar GO categories as those at young age including tissue and organ morphogenesis, appendage development, as well as regulation of neurogenesis, including nervous system development, signal transduction and chemotaxis. Four of the candidate genes, *Antennapedia* (*Antp*), *Axin* (*Axn*), *numb*, and *Tailup* (*tup*) and 13 non-candidate genes have been associated with heart development [*Armadillo* (*arm*), *Decapentaplegic* (*dpp*), *Epidermal growth factor receptor* (*Egfr*), *Frizzled* (*Fz*), *Hedgehog* (*hh*),

Matrix metalloproteinase-2 (*Mmp2*), *Myospheroid* (*mys*), *Pointed* (*pnt*), *Ras oncogene at 85D* (*Ras85D*), *Shotgun* (*shg*), *Slit* (*sli*), *Ultrabithorax* (*Ubx*), and *Wingless* (*Wg*)]. Three of these were also in the network for young flies (*Egfr*, *dpp*, and *Ras85D*). We also noted that several genes are in the Wnt signaling pathway.

We noticed that several genes in the networks were present in both ages (i.e. *mib1*, *klu*), missing in the network of young flies but present in the network for old flies, or missing in the network of old flies but present in the network for young flies (i.e. *Snr1, S)*. Thus, we created a genetically interacting network of candidate genes associated with variation in climbing speed for both ages combined. We found a significant network of 11 candidate genes (Fig. 3.7C, $P = 0.0030$), with *Snr1* and *S* as hub genes. Tube development, appendage morphogenesis and development, regulation of transcription, and RNA metabolic process were significantly enriched GO categories in this set of genes (Appendix 3.3).

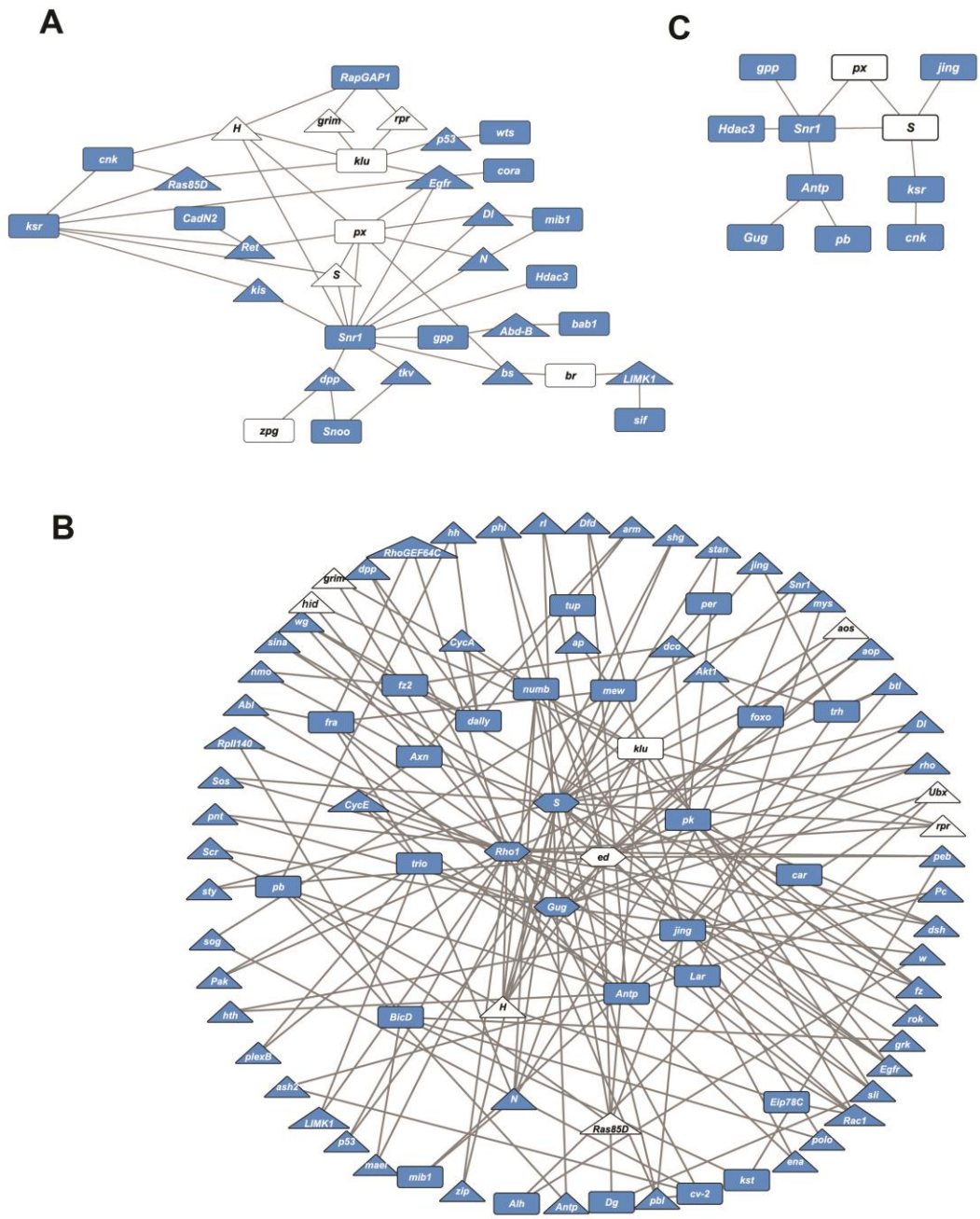

Figure 3.7. Genetic networks for climbing speed. (**A**) A genetic network for climbing speed at one week of age. (**B**) A genetic network for climbing speed at five weeks of age. (**C**) A genetic network for climbing speed combining both age groups. The networks were derived from candidate genes identified in GWA analyses for climbing speed, using data from flies on

control and Lisinopril treated food within an age group (**A**, **B**) or using data from both ages (**C**). Boxes indicate candidate genes identified in the GWA. Genes in triangles are non-candidate genes not identified in GWA but provide a connection between two genes that were in the GWA. Boxes and triangles with a blue background contain genes with human orthologs. Genes in boxes or triangles with a white background have no identified human orthologs.

Endurance

The analyses of candidate genes associated with variation in the endurance phenotype revealed a network comprised of 9 interacting genes with five candidate genes and four non-candidate genes for young flies (Fig. 3.8A). Gene ontology analysis showed enrichment of neuron development, including genes associated with development of the nervous system, cell morphogenesis, and oogenesis (Appendix 3.4). We identified a network of seven candidate genes and four non-candidate genes associated with variation in endurance phenotype in old flies (Fig. 3.8B). This network was enriched for genes associated with cell differentiation, epithelium development, organ and tissues morphogenesis as well as regulation of signal transduction (Appendix 3.5). Among the genes in these networks, 70% have known human orthologs. Next, we created genetically interacting network of candidate genes associated with variation in endurance phenotype in both young and old flies. We found a network of 50 interacting genes with 16 candidate genes (Fig. 3.8C).

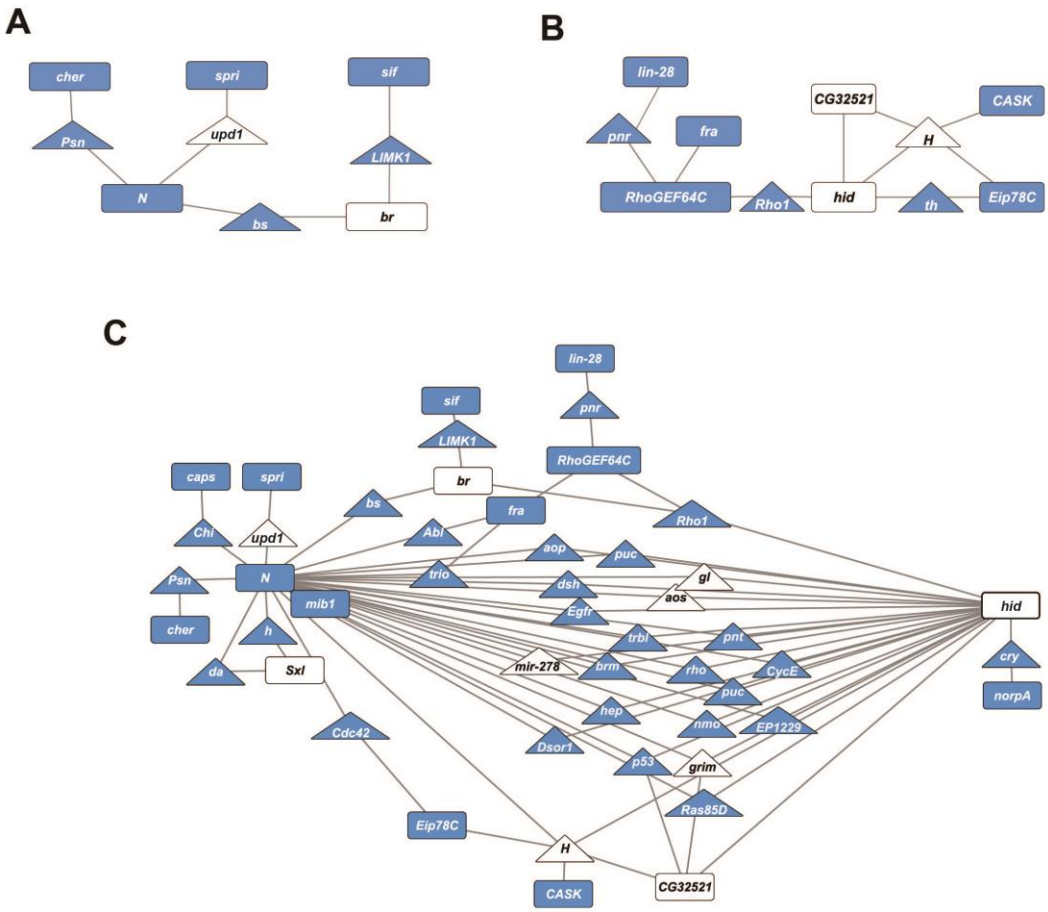

Figure 3.8. Genetic networks for endurance. (**A**) A genetic network for endurance at one week of age. (**B**) A genetic network for endurance at five weeks of age. (**C**) A genetic network for endurance combining both age groups. The networks were derived from candidate genes identified in GWA analyses for endurance, using data from flies on control and Lisinopril treated food within an age group (**A**, **B**) or using data from both ages (**C**). See legend Fig. 3.7 for description of symbols in figure.

Notch (*N*) and *head involution defective* (*hid*) genes were the two most connected genes in the network. The gene *hid* has been previously associated with apoptosis (Bilak and Su 2009), gravitaxis (Armstrong et al. 2006), transmembrane transport (Mackenzie et al. 1999), and cGMP transport (Evans et al. 2008). *Notch* regulates both neurogenesis and cell cycle activity. Notch signaling is a highly evolutionarily conserved pathway across species (Raphael Kopan and Ilagan 2009; Zacharioudaki and Bray 2014). This network was enriched for genes associated with oogenesis, sensory organ development, epithelium development, organ and tissues morphogenesis, and neuron and nervous system development (Appendix 3.6). These are the same categories that we observed previously when ages were analyzed separately. Despite the fact that we did not have any overlap nor at the SNP nor at the gene levels for endurance phenotype in young *versus* old flies, we were able to construct genetic network of interacting genes that was enriched for a wide range of biological processes.

Finally, we combined genes associated with both phenotypes for all ages and constructed a genetic interaction network of 31 genes (P =0.001, Fig. 3.9) with *N*, *S* and *Snr1* genes being the hub genes.

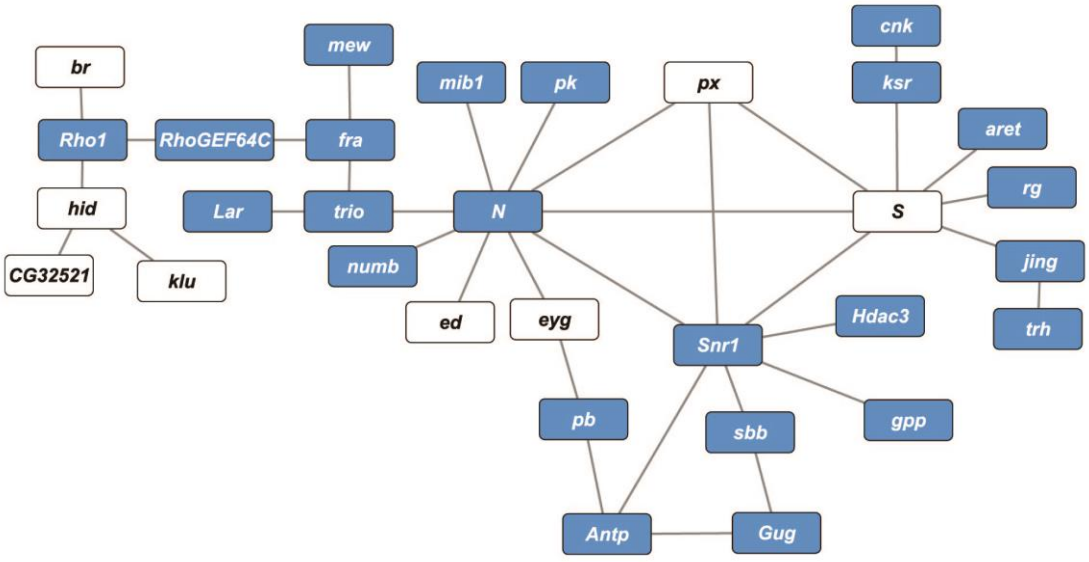

Figure 3.9. Genetic networks for climbing speed and endurance combined. The network was derived from candidate genes identified in GWA analyses for climbing speed and endurance at both ages, using data from flies on control and Lisinopril treated food. See legend Fig. 3.7 for description of symbols in figure.

N was the most interconnected gene associated with the endurance phenotype, while *S* and *Snr1* were the most highly connected in the network for the climbing speed phenotype. Thus, this network elucidates the architecture of the genetic network connecting both phenotypes. Not surprisingly, we found similar enriched GO categories such as organ morphogenesis, appendage development, regulation of signal transduction and nervous system development (Appendix 3.7).

Among the genes in these networks about 70-90% have human orthologs. This gives us the ability to construct a human genetic interaction network based on the *Drosophila* interaction networks associated with variation climbing speed and endurance (Fig. 3.10A, B) (Antonov 2011). Sixteen genes formed the network associated with climbing speed phenotype ($P < 0.005$; Fig. 3.10A). *P21 Activated Kinase-1* (*PAK1*), *P21 Activated Kinase-1* (*PAK2*), and *Plexin B1* (*PLXNB1*) were the most interconnected genes. The resulting network of orthologous genes associated with variation in endurance phenotype in flies consist of 18 orthologs for corresponding genes from the *Drosophila* network ($P < 0.005$; Fig. 3.10B). *Cell Division Cycle 42* (*CDC42*) and *Mitogen-Activated Protein Kinase Kinase 1* (*MAP2K1*) are the most interconnected genes in the network. Taking into account evolutionarily conserved pathways, we found similar enriched GO categories that we saw previously in flies, including cell development and morphogenesis, neuron and nervous system development (Appendix 3.8A, B).

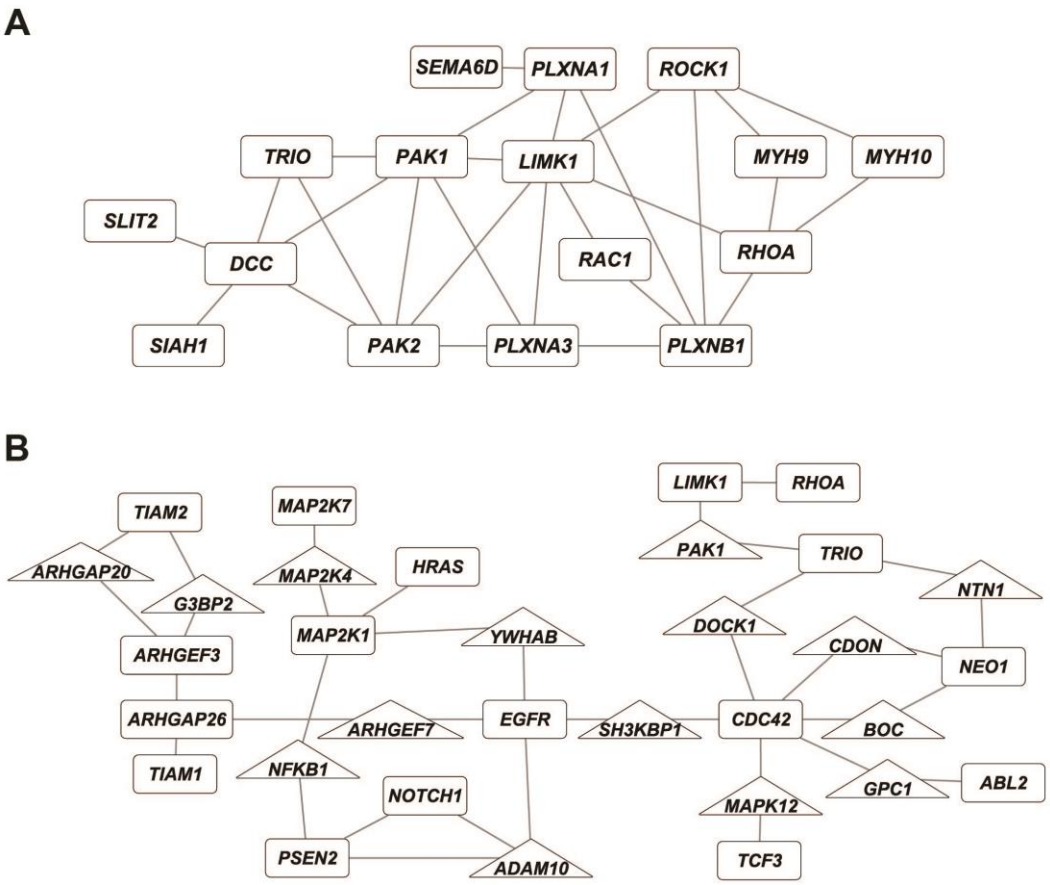

Figure 3.10. Genetic networks of human orthologs for climbing speed and endurance. (**A**) A genetic network of human genes for climbing speed (**B**) A genetic network of human genes for endurance. The networks were derived using interactions of human orthologs of candidate genes identified in GWA analyses for climbing speed and endurance. Genes in boxes are human orthologs of genes identified in the GWA of *Drosophila*. Genes in triangles are non-candidate genes not identified in GWA but provide a connection between human orthologs of two genes that were in the GWA.

Conclusions

Age-related decline in physical performance, including age-related locomotor impairment (ARLI) is a general characteristic of senescence (Grotewiel et al. 2005) and an important indicator of frailty in humans (Fried et al. 2001). The influence of age on ARLI has a genetic component but the genes involved are largely unknown. In this study, I used GWA to identify genes and genetic networks influencing age-specific climbing speed and endurance using *Drosophila* as a model. I also characterized genetic variation in the response of these traits to the ACE*i*, Lisinopril. While Lisinopril is commonly prescribed for hypertension, it has also been implicated to improve physical performance traits in the elderly. I used GWA to identify genes and genetic networks that contribute to the age-specific responses of climbing speed and endurance. I also used GWA to identify genes that contribute to variation of these traits when treated with Lisinopril. My results identified candidate genes and genetic pathways that may contribute to both the positive, and potentially negative, effects on physical performance that have been attributed to use of Lisinopril and other ACE*i*.

i. Most polymorphisms influencing physical performance traits are not shared across ages

I found that the genetic basis of climbing and endurance differ across ages as there was little overlap in the genes or polymorphisms that were significantly associated with either trait across ages. For climbing speed, only

two genes, *mib1* and *klu*, were identified as candidate genes at both ages. *mib1* is a regulator of the Notch signaling pathway which plays a role in stem cell muscle maintenance (Luo et al., 2005). The gene *klu* has been implicated in stem cell maintenance and cell division (Gabilondo et al., 2014). Age-specific effects of polymorphisms on complex phenotypes are commonly found in other mapping studies in both *Drosophila* (Leips et al. 2006; Felix et al. 2012; Durham et al. 2014; Carbone et al. 2016) and humans (Medina-Gomez et al. 2012; Dumitrescu et al. 2013; Simino et al. 2014; Winkler et al. 2015). This does not mean that the genes influencing phenotypes differs completely across ages. This is because the results of GWA are sensitive to the distribution of phenotypes in the mapped population, and this distribution changes with age. Rather my results, and those of the mapping studies noted above, imply that the relative influence of polymorphisms on phenotypes changes as organisms age. Age-specific effects of RNAi on phenotypes (Chapter 4) lends support for this idea. Studies aimed at understanding the mechanisms that give rise to these age-specific genetic effects are needed.

While there was little overlap in the candidate genes for climbing speed identified by GWA across ages, 14 genes were part of the climbing speed networks at each age. These included the two genes identified as candidates at both ages, *mib1* and *klu*, and an additional 12 that were recruited into the network. *Snr1* was identified by GWA as a climbing speed candidate in young flies but appeared as a recruited gene in the network of older flies (Fig. 3.7). The remaining 11, *rpr*, *grim*, *p53*, *LIMK1*, *DL*, *dpp*, *RAS85D*, *N*, *Ret*, *H*, and

Egfr, were recruited into each network during the network construction. Interestingly, nine of the 12 are involved in programmed cell death (*rpr*, *grim*, *p53*, *DL*, *dpp*, *RAS85D*, *N*, *klu,* and *Egfr*) and seven of these specifically involved in stem cell fate (*p53*, *DL*, *dpp*, *RAS85D*, *N*, *klu*, and *Egfr*). To our knowledge, only one other study (Jordan et al. 2012) has mapped genes influencing negative geotaxis behavior, a similar phenotype to climbing speed. Their study GWA with two week old flies from the DGRP lines to identify genes involved in the sensitivity of locomotor phenotypes to oxidative stress, a factor often proposed to contribute to senescence (Abadir et al. 2012). They identified a number of genes influencing negative geotaxis behavior, and GO analysis indicated that the influence of their candidates on locomotion were through their effects on neural connectivity and function. None of the candidate genes in the Jordan et al. (2012) study were identified as candidates in my study. This is likely due to the fact that the phenotypes, ages, and conditions in which the flies were reared were vastly different between studies.

ii. Treatment with Lisinopril alters the genetic basis of variation in climbing speed and endurance

Similar to my finding that genetic influences on climbing speed and endurance were age-specific, candidate genes contributing to variation in these traits differed between Lisinopril and control conditions in most cases. Of the 114 candidates influencing climbing speed at one week of age, only 28 genes contributed to the variation in control and Lisinopril treatments [*Ankrin-*

repeat SH3-domain Proline-rich-region containing Protein (*ASPP*), *CG12147*, *CG14669*, *CG14764*, *CG2258*, *CG42741*, *CG8312*, *CG9527*, CG9990, CR43864, *Caherin-N2* (*CadN2*), *Calcineurin-A1* (*CanA1*), *ER Degradation-enhancing Alpha-mannosidase-like 1* (*Edem1*), *Ecdysone-induced Protein 28/29kD* (*Eip71CD*), *Liprin-beta*, *Maltase A7* (*Mal-A7*), *Pancreatic eIF-2alpha Kinase* (*PEK*), *Polymerase DNA-directed Delta Interacting Protein 2* (*POLDIP2*), *Ribosomal protein L35A* (*RpL35A*), *Snr1*, *Bric-a-brac 1* (*bab1*), *Grappa* (*gpp*), *Hikaru genki* (*hig*), *Huntingtin* (*htt*), and *Long non-coding RNA: iab-8* (*iab-8*)]. At five weeks of age, of the 128 candidates identified, only 14 were identified in both conditions [*Antp*, *Bicaudal D* (*BicD*), *CG17716*, *CG42340*, *CG42458*, *CG5065*, *Ecdysone-induced Protein 78C* (*Eip78C*), *Ionotropic Receptor 67a* (*Ir67a*), *Seminal Fluid Protein 24Bc* (*Sfp24Bc*), *bves*, *numb*, *Shaking B* (*shakB*), slowdown (*slow*), and *Stargazin-like Protein* (*stg1*)]. For endurance, of the 79 genes identified as candidates at one week of age, none were identified as candidates in both control and Lisinopril treatments. At five weeks of age, of the 82 genes identified as candidates, only two genes were candidates in the control and Lisinopril treatment, *Eip78C* and *capricious* (*caps*). One interpretation of these results is that Lisinopril somehow ameliorates the influence of genes that only influenced the physical performance traits in the control condition. GO analysis of genes identified in the control but not Lisinopril treated groups did not identify any pathways or biological processes overrepresented these gene lists. However, experimental focus on the "silencing" effect of Lisinopril on those genes in

future studies may provide insight into the mechanism of drug action on physical performance traits.

While GO analyses on the list of genes that affected physical performance in both control and Lisinopril treatments above did not identify any overrepresented biological processes or GO terms, many of these genes have been implicated in locomotion and/or muscle development, maintenance and function. The *htt* gene, the *Drosophila* ortholog of the *huntingtin* gene (*HTT*) in humans, is important for maintaining mobility in adult flies and loss of gene leads to a neurodegenerative phenotype (Zhang et al. 2009). *Antp* is a member of the *Ant* Hox gene complex and is involved in a number of developmental processes including muscle cell fate specification (Enriquez et al. 2010). RNAi of the gene POLDIP2, influences sarcomere and myofibril morphology, and reduces flight capability (Schnorrer et al. 2010). Disruption of *BicD* produces defects in locomotion (Li et al. 2010). The *numb* is an inhibitor of Notch signaling, a signaling pathway also implicated as important for locomotion phenotypes in the network analyses. The *shakB* produces an innexin protein. Innexins are important in forming gap junctions which allow the passage of ions and small molecules between cells. In adult flies, it is expressed in tergotrochanteral muscle motor neurons as well. Mutations in this gene cause defects in jump response (Baird et al. 1990), light response (Krishnan et al. 1993) and flight capability (Trimarchi and Murphey 1997). Finally, *slow* is involved in muscle attachment (Gilsohn and Volk 2010). Each

of these candidate genes are implicated in age-related physical performance and should be the targets of future research.

iii. Genetic network analysis suggests signaling pathways and epigenetic regulation are important for maintaining physical performance

Genes in both the Notch and Wnt signaling pathways appeared in many of the networks affecting both traits, particularly old age climbing speed, and so these pathways should be the focus of future studies. Notch is involved in many developmental process and in adults is important for homeostasis and regulation of stem cell lineages (Liu et al. 2010). Genes in the Notch pathway were also a significant component of the human gene network identified in this study. Likewise, Wnt signaling has also been implicated in development and stem cell maintenance and in particular shown to influence age related deterioration of muscle function (Brack 2007). Many genes in the Wnt signaling pathway were also found in the networks including *Axn*, *Wg*, *Fz*, (*Rpl35A*, and *Nemo*. Validation of the effects of some of these candidate genes on physical performance is the subject of Chapter 4 of this dissertation.

The network analyses also pointed to genes involved in epigenetic regulation as candidates that may influence age-related physical performance. Muscle stem cells exhibit epigenetic changes with age (Liu et al., 2013), which may be an underlying cause of the loss of skeletal muscle

mass or function with age. Evidence from this comes from a paper by Schnorrer et al. (2010) which identified human gene Hoxa9 as contributing to the regenerative decline in muscle with age. Misexpression of Hoxa9 with age due to epigenetic changes in muscle stem cells was associated with age-related functional decline of muscle cells. The most similar gene in flies to *Hoxa9* is *Abdominal B* (*Abd-B*). *Abd-B* was part of the network of genes contributing to the variation in climbing speed at young age (Fig. 3.7A) and the network constructed of the genes when combined across ages (Fig. 3.7C). A further argument for epigenetic regulation comes from the fact that SNR-1 has a SET domain which is associated with histone lysine methylation. SNR-1 appears as a hub gene in many of the interaction networks. SNR-1 interacts with *gpp* which interacts with both Abd-B and the histone deacetylase gene HDAC. These results suggest that some of the genetically based differences in age-specific physical performance could be to differences in epigenetic regulation in aging organisms.

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Chapter 4

Functional Tests to Validate Candidate Genes Proposed to be Involved in Age- and Genotype-specific Physical Performace and Drug Response

Content of this chapter, in its entirety, is being submitted for publication.

Abstract

Understanding the genetic basis of age-related physical performance is an important goal of studies of aging. In this study, I used the GAL4-UAS system in *Drosophila* to reduce the expression of candidate genes identified in a genome wide association study to validate the influence of these genes on climb speed and endurance. I also compared the effects of the ACE-inhibitor, Lisinopril, on these traits when gene expression was reduced to test the hypothesis that the effects of Lisinopril on physical performance traits were mediated through genes in the Wnt signaling pathway. I found that each of the genes tested, *Axn*, *Nemo*, *Wg*, and *Fz* influenced climbing speed and endurance in an age specific manner. I also found that beneficial effects of Lisinopril on these performance traits were abolished when the expression of these genes was reduced. My results support the findings of the GWA reported in Chapter 3 and suggest an important role for the Wnt signaling pathway in maintaining age-specific physical performance traits. The results also suggest that the effects of Lisinopril on physical performance are dependent, at least in part, on Wnt signaling.

Introduction

The decline in physical performance ability may be partially explained by aging in skeletal muscle. In mammals, such senescence is characterized by an increase in fibrous connective tissue (Goldspink et al. 1994) and an impairment of muscle regenerative potential (Grounds 1998; Conboy and Rando 2005). Previous studies have examined the cellular and molecular mechanism of this age dependent increase in skeletal muscle fibrosis in rodents (Brack et al. 2007; Eliezer and Brack 2016; Rajasekaran et al. 2017). Muscle stem cells (satellite cells) from aged mice tend to convert from a myogenic to a fibrogenic lineage as they begin to proliferate and this conversion is mediated by factors in the systemic environment of old animals.

This lineage conversion has been shown to be associated with an activation of the canonical Wnt signaling pathway in aged myogenic progenitors and can be suppressed by Wnt inhibitors (Brack et al. 2007). In addition, components of serum from aged mice that bind to the Frizzled family of proteins, which are Wnt receptors, may account for the elevated Wnt signaling in aged cells (Brack et al. 2007). Wnt signaling has also been implicated in age-associated changes in many tissues (Fujimaki et al. 2015) and has been implicated to play a key role in homeostasis. These studies support further study of the role of the Wnt signaling pathway in aging phenotypes.

Here, I test the effects of altered expression in four genes in the Wnt signaling pathway on physical performance ability. In *Drosophila*, I tested the

climbing speed and endurance in one-week and five-week old genotypes using the skeletal adult muscle-specific driver, *dj667*-Gal4 and RNAi against *Axn*, *Frizzled*, *Nemo*, and *Wingless*. I hypothesize that, individually, knockdown of these four genes in skeletal muscle will reduce climbing speed and endurance across all ages and that Lisinopril treatment will have no effect on these traits.

Materials and Methods

i. Expression of *dj667*-Gal4 with age

Prior to evaluating the effect of candidate genes on age-specific climbing speed and endurance using the GAL4/UAS system to activate RNAi against each gene, I first tested the change in expression of *dj667*-Gal4 with age. This was to ensure that the effects of RNAi, if any, were not due changes in the expression of the Gal4 driver with age. Primers used for Gal4 were made using Primer3 and were as follows: Forward, TCACAGTGTGCAATCCCATT, Reverse, CGATAGTTGCAGAACCGACA. I used the expression of *rp49,* an endogenous housekeeping gene in *Drosophila* to normalize the expression of GAL4 at each age. Primers used for the control gene, *rp49*, were as follows: Forward, GTGAAGAAGCGCACCAAGCAC, Reverse, ACGCACTCTGTTGTCGATACCC (Saadin and Starz-Gaiano 2016). All other qPCR methods are as previously described (Chapter 2).

ii. Validation of candidate genes by assessing climbing speed and endurance of RNAi lines

To evaluate the effect of candidate genes on age-specific climbing speed and endurance, I used the GAL4/UAS system to activate RNAi against each gene (Table 4.1). These genes were chosen because at old age, they influenced both climbing speed and response to Lisinopril. Three of the genes, *frizzled*, *Nemo*, and *Wingless*, were identified as “non-hub" genes in the network affecting climbing speed of flies at five weeks of age. In this case, "non-hub" indicates that there were four or fewer connections to other genes in the network. One gene, *Axn*, was identified as a “hub” candidate gene and was connected to seven other genes in this network. In addition, all four genes are in the Wnt signaling pathway which is known to have roles in muscle stem cell development, maintenance (Brack et al. 2006), and aging (Eliezer and Brack 2016). Furthermore, each gene chosen has a human ortholog and has multiple RNAi stocks available (http://flystocks.bio.indiana.edu) to use for confirmation.

As a test of the age-specific effects identified in the GWA, I compare the effect of the knockdown of each gene on climbing speed and endurance at one and five weeks of age. To test the hypothesis that Lisinopril is affecting these traits through the action of these candidates, I also compared age-specific climbing and endurance of control and RNAi knockdown flies on control food versus Lisinopril-treated food.

We used eight RNAi lines, two stocks for each gene, generated by the Transgenic RNAi Project (TRiP) at Harvard Medical School (http://www.flyrnai.org) (Table 4.1). We used two stocks per each RNAi construct to control for potential off-target effects and for potential effects of the transgene insertion site on the phenotype. Because two stocks are less likely to have the same off targets, using multiple lines provides greater assurance that the intended target is likely causing the phenotype. Also, because the two stocks containing the same RNAi construct were inserted into two different locations in the genome, comparable phenotypic effects in both RNAi stocks provides assurance that it is the RNA knockdown that is influencing the trait, instead of the potential disruption of other genes near the site of the RNAi construct.

Table 4.1. List of RNAi TRiP lines used to validate candidate genes.

Gene Name and Stock	Stock Number	FlyBase Genotype	Human Ortholog
Axin stock 1	31705	y^1 v^1; P{TRiP.HM04012}attP2	*AXIN1*
Axin stock 2	62434	y^1 v^1; P{TRiP.HMJ23888}attP40/CyO	*AXIN1*
Frizzled stock 1	31036	y^1 v^1; P{TRiP.JF01481}attP2	*FZD1* and *FZD7*
Frizzled stock 2	34321	y^1 sc* v^1; P{TRiP.HMS01308}attP2	*FZD1* and *FZD7*
Nemo stock 1	41586	y^1 v^1; P{TRiP.GL00703}attP2	*NLK*
Nemo stock 2	25793	y^1 v^1; P{TRiP.JF01799}attP2	*NLK*
Wingless stock 1	31310	y^1 v^1; P{TRiP.JF01257}attP2	*WNT1*
Wingless stock 2	31249	y^1 v^1; P{TRiP.JF01480}attP2	*WNT1*

I used the standard genetic background control lines for these stocks that also contains the attP2 or attP40 landing site, as designated by the TRiP

project. The first control is *dj667*-Gal4 x y^1 v^1; P{CaryP}attP2 (stock #36303). The second control is y^1v^1; P{y[+t7.7]=CaryP}attP40 (stock #36304). I also used a third control, *dj667*-Gal4 x y^1 sc* v^1; P{VALIUM20-mCherry}attP2 (stock #35785), to control for activation of RNAi machinery.

We crossed males of these TRiP and control lines with virgin females harboring the *dj667*-Gal4 driver to knockdown expression of individual candidate genes in adult skeletal muscle in the offspring. We measured climbing speed and endurance at one week and five weeks of age using the same procedures used to assay the DGRP lines (Chapter 3).

iii. Statistical analysis

To determine which candidate genes had an effect on each trait, we used ANOVA to test for differences among crosses in the focal trait using the model $y = g + \varepsilon$, where g is the genotype of the cross and ε is the error. Each analysis was followed by a post-hoc Dunnett's test (Dunnet 1955) which allowed us to compare all the RNAi lines against the single control line (the attP2 control stock) while correcting the results for multiple testing.

Results

i. Expression of *dj667*-Gal4 does not change with age

To ensure that the effects of RNAi, if any, were not due changes in the expression of the Gal4 driver with age, I used qPCR to analyze the expression of GAL4 in one- and five-week old *dj667*-Gal4 males (Fig. 4.1)

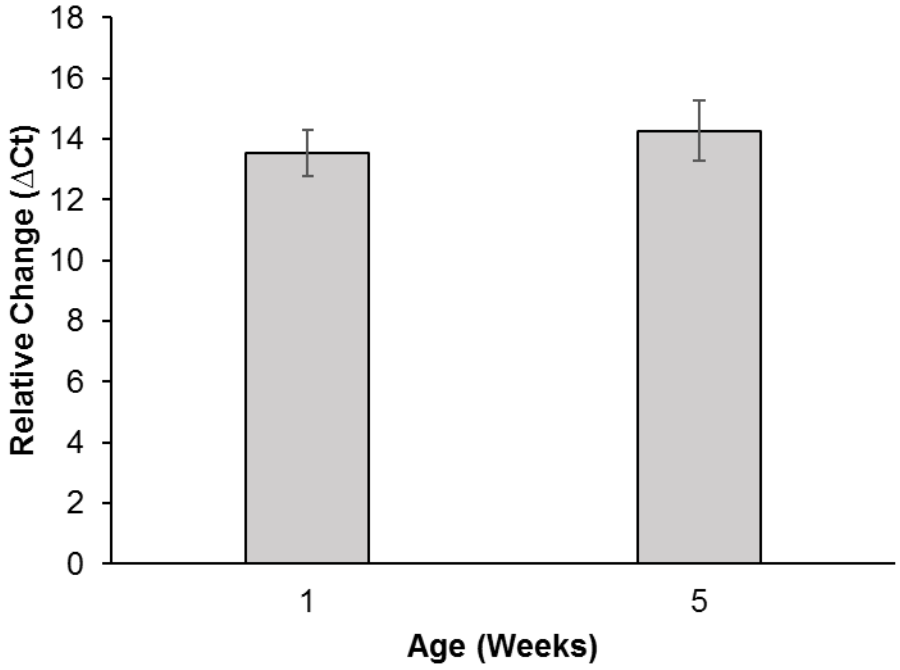


Figure 4.1. Expression of *dj667*-Gal4 does not significantly change with age. Age has no significant effect on expression of *dj667*-Gal4 relative to housekeeping gene *rp49*.

ii. RNAi validates the contribution of candidate genes to physical performance

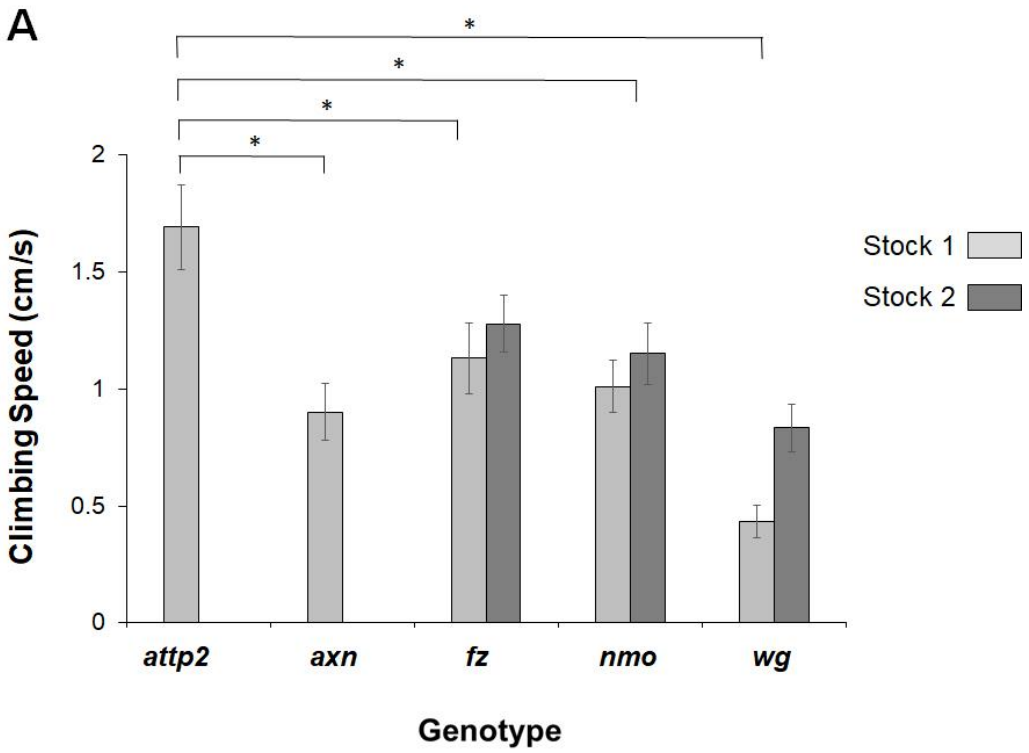
To confirm the influence of four candidate genes on climbing speed, endurance, and/or drug response, I repeated the age-specific physical performance assays using offspring from crosses of four candidate UAS–RNAi lines and three control lines with virgin females of *dj667*-Gal4. Genes were chosen based on GWA candidate polymorphisms which had varying age-specific effects on climbing speed or endurance and drug response, network analysis, shared Wnt pathway, identification of human orthologs, and availability of TRiP RNA*i* stocks (http://www.flyrnai.org).

Validation of candidate genes affecting climbing speed

When compared with the control lines, all of the candidate genes influenced climbing at young age using ANOVA with a post hoc Dunnett's test ($n = 240$, $P < 0.05$) (Fig. 4.2A). Stock two of the *frizzled* RNAi genotypes was not significantly different from the attP2 control stock, but the climbing speed was reduced in this genotype relative to the control, as were all other RNAi genotypes at week one of age.

The results for five week old flies were markedly different from the results at one week of age. At five weeks of age, flies with reduced expression of *Axn*, *Fz* and stock one of the *Nemo* were significantly faster with age compared with the control ($n = 240$, $P < 0.05$) (Fig. 4.2B). This result

is largely due to the fact that the climbing speed of the *attP2* control stock was dramatically reduced at five weeks of age.



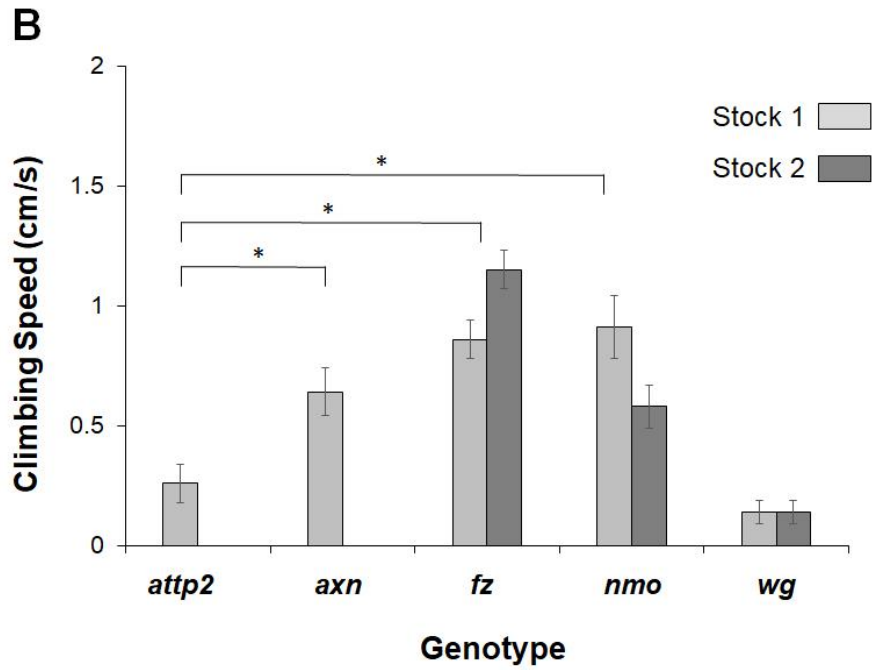
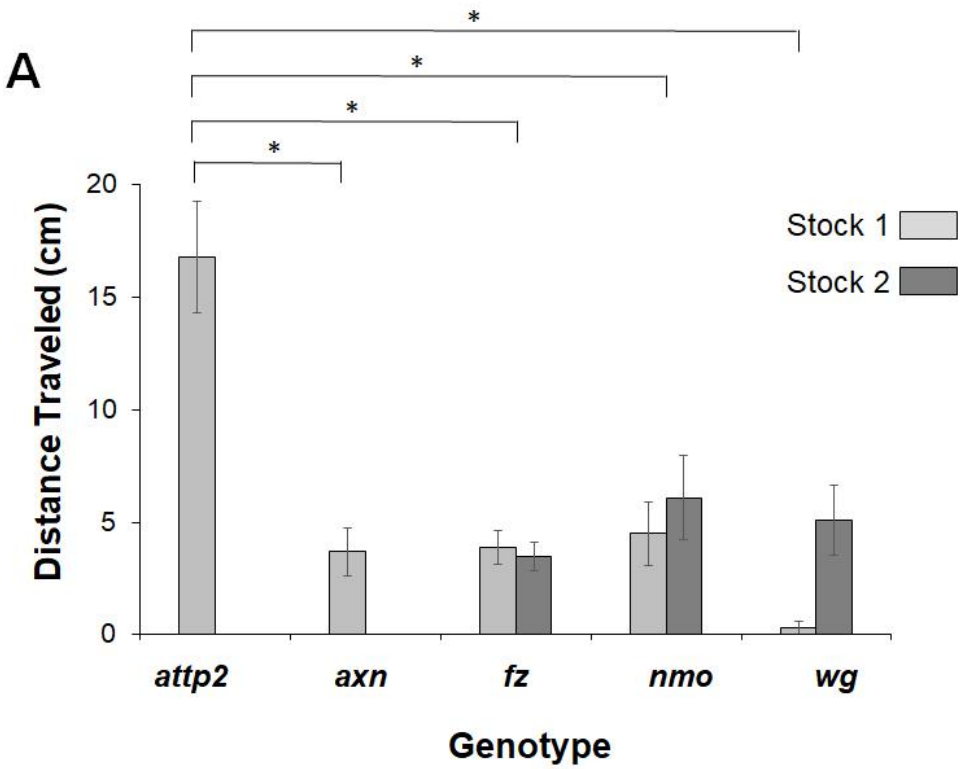


Figure 4.2. Climbing speed of *dj667*-Gal4 x UAS-RNAi F_1 offspring. (**A**) At one week of age, climbing speed of *Axn*, *Fz*, *Nemo, and Wingless* RNA*i* flies is reduced relative to the *attP2* control line. (**B**) At five weeks of age, climbing speed of *Axn*, *Fz*, and *Nemo* is higher than that of the *attP2* control. ANOVA, post hoc Dunnett's test; * $P < 0.05$.

While all of the RNAi flies also had reduced speed at five weeks of age, the climbing speed of flies with reduced expression of *Axn*, *Fz*, and *Nemo* was less affected by age than flies of the control genotype. In sum, these results both validated the influence of these genes on climbing speed and confirmed that they contribute to variation in climbing speed in an age-specific manner.

Validation of candidate genes affecting endurance

When compared with the control lines, all of the RNAi genotypes had reduced endurance at one week of age (Fig. 4.3A). At five weeks of age, only the RNAi *Fz* genotypes differed from the control (Fig. 4.3B). In this case, the RNAi *Fz* flies had increased endurance relative to the controls, which was similar to the results of climbing speed discussed above. A caveat with these results is the very low endurance of most of these genotypes at five weeks of age. Given the low endurance among all the lines, there was very little scope to detect differences in endurance among genotypes.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Distance Traveled (cm)
Stock 1
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fz
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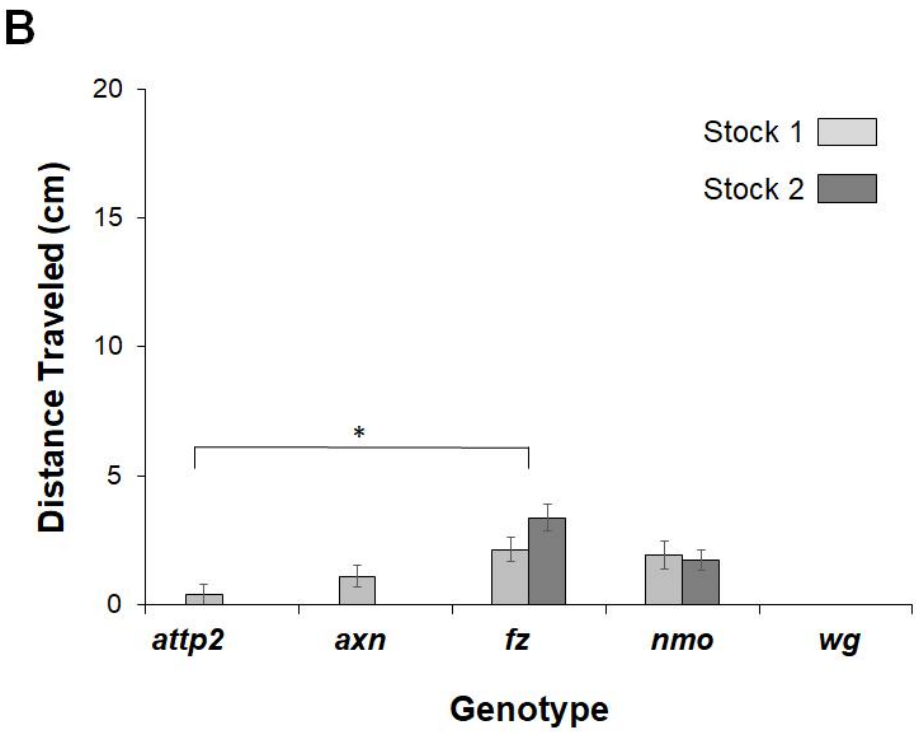
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Genotype

Figure 4.3. Endurance of F1 offspring from the dj667-Gal4 x UAS-RNAi crosses. (**A**) At one week of age, the endurance of *Axn*, *Fz*, *Nemo, and Wingless* RNA*i* flies is reduced relative to the *attP2* control line. (**B**) At five weeks of age, only the *Fz* RNA*i* genotype differed significantly from the *attP2* control. *Fz* RNAi flies had significantly greater endurance than the *attP2* control at five weeks of age. ANOVA, post hoc Dunnett's test; * $P < 0.05$.

iii. RNAi implicates genes in the Wnt signaling pathway as mediating the effects of Lisinopril on climbing speed

In the *attP2* control line, flies on Lisinopril are faster at weeks one and week five of age, but only significantly faster at five weeks of age ($P = 0.0132$). In the *mCherry* control line, flies on Lisinopril are faster at week one of age ($P = 0.0010$) and at week five of age ($P = 0.0013$). Relative to the *attP2 control*, which controls for genetic background, climbing speed of untreated RNAi flies closely resemble that of Lisinopril-treated flies for all eight RNAi stocks at five weeks of age (Table 4.2).

Table 4.2. Effect of Lisinopril treatment on climbing speed of *dj667*-Gal4 x UAS-RNAi F_1 offspring at five weeks of age. C is control food, L is Lisinopril-treated food.

Gene Name and Stock	Treatment (C or L)	Mean Climbing Speed (cm/sec)	S.E.	*P*-value
attP2 control	C	0.26	0.08	0.0132
	L	0.58	0.09	
mCherry control	C	0.03	0.03	0.0013
	L	0.36	0.09	
Axn stock 1	C	0.64	0.10	ns
	L	0.67	0.08	
Axn stock 2	C	0.67	0.08	ns
	L	0.77	0.08	
frizzled stock 1	C	0.86	0.08	ns
	L	0.85	0.09	
frizzled stock 2	C	1.10	0.08	ns
	L	1.02	0.10	
Nemo stock 1	C	0.91	0.13	ns
	L	0.81	0.11	
Nemo stock 2	C	0.58	0.09	ns
	L	0.82	0.07	
Wingless stock 1	C	0.14	0.05	ns
	L	0.27	0.07	
Wingless stock 2	C	0.14	0.05	ns
	L	0.27	0.08	

When the expression of *Axn*, *Frizzled*, *Nemo*, or *Wingless* is reduced in skeletal muscle, I found no effect of Lisinopril on climbing speed. This could mean that Lisinopril provided no additional benefit beyond the improvements due to the RNAi; the effect of Lisinopril may be maxed out if flies are already faster due to the RNAi. Alternatively, it is possible that the beneficial effect of Lisinopril on climbing speed requires the expression of these genes, all of which are in the Wnt signaling pathway. I found that endurance is not significantly affected by Lisinopril treatment in any of the controls nor the RNAi lines.

Conclusions

The results of this study confirm the influence of *Axn*, *Fz*, *Nemo* and *Wg* genes on both climbing speed and endurance, and support the findings of the GWA, that polymorphisms in these loci contribute to the variation in these traits in natural populations. More generally, they confirm the influence of the Wnt signaling pathway on physical performance traits and support the hypothesis that expression of each of these genes in fly skeletal muscle influences both traits. The influence of these genes on the phenotype were age-dependent, also supported the findings of the GWA in this and other studies of age-dependent genetic effects in our laboratory (Felix et al. 2012; Durham et al. 2014).

One of the more interesting findings was that reducing the expression of most of the genes had different effects on the traits relative to the controls. At the younger age, reductions in gene expression led to reductions in speed and endurance relative to controls. However, at older ages, flies with reduced expression of most of these genes were faster and tended to have higher endurance than controls. These age-specific effects may in fact result from two different mechanisms. First, it is clear that the Wnt pathway regulates many aspects of the phenotype that are likely to influence climbing speed and endurance, such as the development of the nervous system, neuromuscular junctions and skeletal muscle development (Packard et al. 2002; von Maltzahn et al. 2012, Rosso and Inestrosa 2013; Rudolf et al. 2014). The GAL4-UAS method of RNA interference in this experiment does not allow for

age-specific control of gene knockdown, and so flies should experience the reduction of these genes in muscle tissue throughout development. Therefore, for the younger flies we would expect that genotypes with reduced expression of genes in the Wnt signaling pathway could have reduced muscle mass, and potentially reduced innervation of the musculature which should reduce the speed and endurance of young flies relative to the control flies with normal Wnt signaling.

The results for the older flies are more difficult to explain based, in part, on what is known about the influence of Wnt on aging. Several studies have suggested that changes in Wnt signaling influence senescence in various tissues, including the nervous system and skeletal muscle (Fujimaki et al. 2015). Wnt signaling is critical for the formation of neuromuscular junctions during development (Packard et al. 2002) and down-regulation of Wnt signaling in older individuals has been reported to decrease neurogenesis in the mammalian brain (Okamoto et al. 2011; Seib et al. 2013). At first glance then, this might suggest that one possible mechanistic link between Wnt signaling and locomotion is that reduced neurogenesis in the brain causes behaviorally associated changes in physical performance traits (Apple et al. 2017). However, reduction in Wnt genes in older flies, when there was a difference, produced flies that tended to be faster and have greater endurance than control flies. In addition, our test of the *dj667* driver indicated that reduced expression of the candidate genes may be confined to the muscle; however, qPCR using other tissue is needed to confirm this. One

possible explanation for our results then could be that higher Wnt signaling in older flies is detrimental to physical performance. Indeed, one study lends support to this conclusion. Brack et al. (2007) reported that increased Wnt signaling in advanced age reduced the regenerative capacity of muscle by altering muscle stem cell fate and increasing fibrosis (Brack et al. 2007). This suggests that an immediate follow up study to look at levels of fibrosis in the skeletal muscle of flies with reduced expression in these genes. An initial test of this hypothesis would be to look for increased level of protein aggregation in RNAi genotypes as was done in the experiment described in Chapter 2.

My results support the hypothesis that, individually, knockdown of these four genes in skeletal muscle will reduce climbing speed and endurance across all ages and that Lisinopril treatment will have no effect on these traits. It is likely that that Lisinopril provided no additional benefit beyond the improvements due to the RNAi; the effect of Lisinopril may be maxed out if flies are already faster due to the RNAi. Alternatively, the effects on climbing speed and endurance may be due in part through Lisinopril treatment. This would be supported by the observation that the beneficial effects of Lisinopril on physical performance traits in the control lines were not observable when expression of these genes was reduced in the RNAi genotypes. Support for this interpretation comes from other studies suggesting that the Wnt signaling pathway plays a critical role in fibrosis, and that ACE-inhibitors such as Lisinopril inhibit the formation of fibronectin (Cisternas et al. 2014). More work on this system is needed to confirm the

potential role of Wnt signaling on traits influencing physical frailty and to elucidate the mechanisms that explain how ACE-inhibitors act to ameliorate the deleterious effects of age on physical performance.

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Chapter 5

Conclusions

Introduction

Age-specific physical performance, resiliency, and lifespan are complex quantitative traits that are of primary importance in the context of human health span. These traits are sensitive to medication, a clinically relevant variable, and are likely influenced by hundreds of genes. Although physical performance and lifespan have been investigated for over a century, we still know little about the genes influencing natural variation in these traits. Additionally, how age and drug treatment affect the genetic architecture underlying physical performance and lifespan, subsequently influencing variation in these traits, is poorly understood. Further, the genetic mechanisms which maintain variation in resilience and in the plastic response of physical performance ability and lifespan to medications remain unclear.

Summary of dissertation

My doctoral research elucidated the genetic basis of natural variation in physical performance traits and lifespan and the responses of these traits to variation in drug treatment using *Drosophila melanogaster*. My research has contributed to studies in the biology of aging by providing novel methods to measure climbing, endurance, and strength in fruit flies. To my knowledge, this is also the first GWAS testing the effects of Lisinopril on age-related decline in the aforementioned traits in fruit flies. I have also provided a strong foundation for several future research avenues.

There were four main goals of my dissertation project: 1) to evaluate the age- and genotype-specific effects of Lisinopril treatment on physical

performance in *Drosophila*, 2) to identify candidate polymorphisms and their associated genes that influence age specific physical performance and to assess the extent to which this genetic variation is treatment-specific, 3) to identify candidate polymorphisms and their associated genes that influence the sensitivity of age specific climbing ability to drug treatment, and 4) to apply genetic information gained from goals 2 and 3 to identify gene networks and validate a subset of identified candidate genes, in muscle tissue, on climbing ability and response to Lisinopril. To accomplish these goals, I used the *Drosophila melanogaster* Genetic Reference Panel (DGRP) (Mackay et al. 2012) to complete a genome-wide association (GWA) study on physical performance traits in non-mated *Drosophila* males maintained on either standard food or Lisinopril-containing food.

Chapter 2 of this dissertation contains the findings of the physical performance (climbing speed, endurance, and strength), lifespan, and the effects of Lisinopril on these traits. In summary, I found significant variation in age-specific climbing speed and endurance and in lifespan among the three DGRP lines 229, 73, and 304 tested. I then compared changes in physical performance, Fly Physical Performance Index (FPP), and life span in my three fly lines to test the impact of genetic background on the effects of ACE inhibition. Lisinopril treatment influenced age-related decline of climbing speed, endurance, and strength that was dependent on genotype. Treatment of DGRP_229 flies significantly attenuated the decline of all three measures of physical performance: climbing speed, endurance, and strength. In

contrast, treated flies of DGRP_73 and DGRP_304 showed no effect on climbing speed nor endurance, but rather only on strength.

I then tested the effects of Lisinopril on the composite measure, FPP. I noted a decline in prevalence of LC performance in DGRP_229 and an increase in percentage of HC flies with treatment. While treatment with Lisinopril significantly extended the average life span of all lines, this reduction in mortality was associated with improvement of all 3 physical function measures only in DGRP_229. To investigate the apparent dissociation between individual measures of physical performance and rate of decline in my DGRP lines, I constructed a composite index (FPP) analogous to criteria used in humans (Fried et al. 2001) to identify worst performers (lowest quartile) of all three physical measures with age. My results indicate a strong relationship between genotype and performance capacity. Specifically, I observed the highest prevalence of low capacity performers in DGRP_304, which was associated with medium rate of decline in physical function but long life span. My results differ from some previous studies which show that high physical performance ability is directly and positively related to long life span (Roshanravan et al. 2017), while low physical capacity is directly and negatively related to short lifespan (Fried et al. 2001). However, my results are consistent with studies which demonstrate that physical performance can be inversely related to life span (van de Vijver et al. 2016) or not necessarily associated with life span at all.

I tested whether survivorship is affected by the expression of Ance in muscles. My results show that knockdown of skeletal muscle-specific Ance was associated with a significant increase in survivorship compared to untreated control males. Treatment of the RNAi knock down flies with Lisinopril had no added effects on survivorship.

At a molecular level, aging is associated with changes in muscle fiber type and accumulation of protein aggregates (Stefani and Dobson 2003), potentially leading to defects in physical performance. My data suggest that the differential effect of Lisinopril on climbing speed, endurance, and strength in the three lines is driven by differences in the accumulation of protein aggregates in muscles.

Results from the RNA-Seq experiment identified several genes that responded to Lisinopril treatment. Many of these have been implicated in some aspect of stress and immune responses. These include genes in the Turandot family, CHK kinases and genes involved in the humoral response to infection. This experiment also identified genes whose expression in response to Lisinopril depended on genotype in an age-specific manner. Many of these genes are also involved in stress responses, suggesting that genetically based variation in the phenotypic response to drug treatment may depend on the extent to which stress response pathways are activated in different genotypes.

Chapter 3 of this dissertation contains the characterization of natural variation and identification of candidate polymorphisms and genes involved in

age specific physical performance of flies as well the assessment of the extent to which this genetic variation is treatment-specific. I performed the climbing speed and endurance assays on 126 DGRP lines maintained on either control or Lisinopril-treated food.

I found that the genetic basis of climbing and endurance differ across ages as there was little overlap in the genes or polymorphisms that were significantly associated with either trait across ages. For climbing speed, only two genes, *mib1* and *klu*, were identified as candidate genes at both ages. *Mib1* is a regulator of the Notch signaling pathway (Lai et al., 2005) which plays a role in the maintenance of stem cell muscle (Liu et al., 2013). The gene *klu* has been implicated in stem cell maintenance and cell division (Gabilondo et al., 2014). Age-specific effects of polymorphisms on complex phenotypes are commonly found in other mapping studies in both *Drosophila* (Leips et al. 2006, Felix et al. 2012, Durham et al. 2014, Carbone et al. 2016) and humans (Medina-Gomez et al. 2012, Dumitrescu et al. 2013, Simino et al. 2014, Winkler et al. 2015). A general conclusion that can be drawn from these studies is that the genetic basis of naturally occurring variation in physiological traits changes as the organism ages. Further work is necessary to determine if this is true of other polygenic traits that change with age (e.g., behavior). From a practical perspective this suggests that pharmacological treatment of patients for particular disorders may need to be designed to target different genes or pathways for different aged individuals. Of course, my study and those listed above typically find that genotypes senescence at

different rates and so chronological age may be a poor predictor of physiological age. For example, in my study, genotypes differed a great deal in their FPP index even though they were the same age. As such, it will be important to develop reliable biomarkers of aging in the future so that treatment can be properly tailored to the physiological age and genotype of individuals.

While there was little overlap in the candidate genes for climbing speed identified by GWA across ages, 14 genes were part of the climbing speed networks at each age. These included the two genes identified as candidates at both ages, mib1 and klu, and an additional 12 that were non-candidates. Snr1 was identified by GWA as a climbing speed candidate in young flies but appeared as a non-candidate gene in the network of older flies. The remaining non-candidates are involved in programmed cell death, stem cell fate, regulation of cell growth, and developmental patterning. As far as we are aware, only one other study (Jordan et al. 2012) has mapped genes influencing negative geotaxis behavior, a similar phenotype to climbing speed. Their study GWA with two week old flies from the DGRP lines to identify genes involved in the sensitivity of locomotor phenotypes to oxidative stress, a factor often proposed to contribute to senescence (Pole et al., 2016).

Similar to my finding that genetic influences on climbing speed and endurance were age-specific, candidate genes contributing to variation in these traits differed between Lisinopril and control conditions in most cases. Of the 114 candidates influencing climbing speed at one week of age, only 28

genes contributed to the variation in control and Lisinopril treatments. At five weeks of age, of the 128 candidates identified, only 14 were identified in both conditions. For endurance, of the 79 genes identified as candidates at one week of age, none were identified as candidates in both control and Lisinopril treatments. At five weeks of age, of the 82 genes identified as candidates, only two genes were candidates in the control and Lisinopril treatment, *Eip78C* and *caps*. One interpretation of these results is that Lisinopril somehow ameliorates the influence of genes that only affected the physical performance traits in the control condition.

Genes in both the Notch and Wnt signaling pathways appeared in many of the networks affecting both traits, particularly old age climbing speed, and so these pathways should be the focus of future studies. Notch is involved in many developmental process and in adults is important for homeostasis and regulation of stem cell lineages (Liu et al, 2010). Genes in the Notch pathway were also a significant component of the human gene network identified in this study. Likewise, Wnt signaling has also been implicated in development and stem cell maintenance and in particular shown to influence age related deterioration of muscle function (Brack 2007). Many genes in the Wnt signaling pathway were also found in the *Drosophila* networks in this study including *Axn*, *Wg*, *Fz*, and *Nemo*. The fact that reduced expression of each of these genes resulted in similar phenotypes, whether they act to upregulate or downregulate Wnt signaling, suggests that other genes are contributing to the phenotypes.

The network analyses also pointed to genes involved in epigenetic regulation as candidates that may influence age-related physical performance. Muscle stem cells exhibit epigenetic changes with age, (Liu et al., 2013) which may be an underlying cause of the loss of skeletal muscle mass or function with age. Evidence from this comes from a paper by Schnorrer et al. (2010), which identified the gene *Hoxa9* as contributing to the regenerative decline in muscle with age. The most similar gene in flies to *Hoxa9* is *Abd-B*. *Abd-B* was part of the network of genes contributing to the variation in climbing speed at young age and the network constructed of the genes when combined across ages. A further argument for epigenetic regulation comes from the fact that *SNR-1* has a SET domain which is associated with histone lysine methylation. *SNR-1* appears as a hub gene in many of the interaction networks. *SNR-1* interacts with *gpp* which interacts with both *Abd-B* and the histone deacetylase gene *HDAC*. These results suggest that some of the genetically based differences in age-specific physical performance could be to differences in epigenetic regulation in aging organisms. Age-related epigenetic changes, broadly defined as changes in gene regulation without changes in DNA sequence, are recognized as important contributors to age-related physiological decline (senescence). Epigenetic changes that have been associated with senescence include alterations in DNA methylation, histone modifications and chromatin stability (reviewed in Lidzbarsky et al. 2018). Results from my network analyses suggest that some of the genetic differences in physical performance may be

attributable to differences in epigenetic characteristics among genotypes. A promising line of future research could be to characterize age-specific epigenetic changes among genotypes in the DGRP and look for an association with age-specific physical performance. This would allow us to use GWA to identify polymorphisms that contribute to age-related variation in the epigenome, potentially elucidating an important mechanism contributing to senescence.

Chapter 4 of this dissertation contains the validation of a subset of candidate genes, identified in Chapter 3. In brief, I used the GAL4-UAS system in *Drosophila* and eight RNAi lines to reduce the expression of candidate genes to validate the influence of these genes on climbing speed and endurance. I also compared the effects of the ACE-inhibitor, Lisinopril, on these traits when gene expression was reduced to test the hypothesis that the effects of Lisinopril on physical performance traits were mediated through genes in the Wnt signaling pathway. I found that each of the genes tested, *Axn*, *Nemo*, *Wg*, and *Fz* influenced climbing speed and endurance in an age specific manner. I also found that Lisinopril had no beneficial effects on these performance traits; it could be that the effects were potentially masked when the expression of these genes was reduced. My results support the findings of the GWA reported in Chapter 3, and suggest an important role for the Wnt signaling pathway in maintaining age-specific physical performance traits. The results also suggest that the effects of Lisinopril on physical performance are dependent, at least in part, on Wnt signaling. Overall, my dissertation

results contribute to identification of genetic bases of variation in physical performance, provide a foundation for predictions about treatment response of a patient, and provide novel genetic targets to extend health span in elderly humans.

Critical evaluation of the study

While this study successfully satisfied all the specific aims outlined in the introduction section, it was not without limitations. As a starting point, examined only flight muscles. Morphologically, there are two major muscle types in adult *Drosophila*: fibrillar muscles and tubular muscles. Fibrillar muscles are exclusively present as flight muscles and provide power for oscillatory flight. Tubular muscles, such as the jump muscles and leg muscles, are neurogenic and used for activities including climbing and the initiation of flight (Grotewiel et al. 2005). Although I specifically concentrated on the flight muscles, protein aggregation appears to be a general contributor to the decline of adult muscle function.

Future studies should assess the effects of Lisinopril treatment on protein aggregation in other muscle types. I also suggest assessment of protein aggregation in other locations, such as nervous or cardiac tissue as this might provide additional insight into the variable effects of Lisinopril on traits such as life span.

I note that we used RNAi to test the effects of candidate genes identified by the GWA. Polymorphisms associated with the traits may or may

not actually reduce gene expression. Alternatively, they may alter other functional aspects such as protein stability, or influence patterns of alternative splicing. However, we are less concerned with the functional effects of the polymorphisms in flies as such polymorphisms are unlikely to be the same as those contributing to natural variation in these traits in humans. As such our method is useful for nominating important genes and pathways for further study in vertebrates.

Future directions

This work has laid the foundation for an array of future investigations regarding the genetic architecture underlying natural variation in age-related decline of physical performance and the response of such traits to Lisinopril treatment. The most straightforward of these future endeavors is to functionally validate the candidate genes associated with each trait, age, and treatment in a tissue- and age-specific manner. This is currently in progress and I am using RNAi lines crossed with muscle-specific driver, *dj667*. Future experiments may use other drivers, such as cardiac-specific or brain-specific, which are also readily available from the Bloomington *Drosophila* Stock Center (http://flystocks.bio.indiana.edu).

Another logical next step in uncovering the path from genotype to phenotype is to investigate the transcriptional genetic networks associated with age-specific physical performance in the DGRP and to compare this data

with the GWA results from nucleotide sequence variation obtained in my dissertation work. The candidate SNPs could modulate transcription rates of target genes directly (i.e. transcription factors, transcription factor binding sites) or they could function as regulatory SNPs that indirectly influence transcription rates in a manner that leads to phenotypic variation. We expect the transcriptome to be highly variable (Ayroles et al. 2009).

GO analysis of genes identified in the control but not Lisinopril-treated groups did not identify any pathways or biological processes overrepresented these gene lists. This was also true for the GO analyses on the list of genes that affected physical performance in both control and Lisinopril treatments. A limitation of GO analyses is that they are based only on current knowledge of gene functions, so the GO results may be incomplete. As our knowledge of gene functions increases, the results of GO analyses change. As such, additional pathways and biological processes directly influenced by Lisinopril treatment may be identified in the future. A more direct approach would be to study the "silencing" effect of Lisinopril on candidate genes. This approach would elucidate genetic mechanisms by which drugs act on physical performance traits.

Many of these genes have been implicated in locomotion and/or muscle development, maintenance and function. For example, the gene *Htt* (*Huntingtin*), the *Drosophila* ortholog of the *huntingtin* gene (HTT) in humans, is important for maintaining mobility in adult flies and loss of gene leads to a neurodegenerative phenotype (Zhang et al. 2009). The gene *Antp*

(*Antennapedia*) is a member of the Antennapedia Hox gene complex and involved in a number of developmental processes including muscle cell fate specification (Enriquez et al. 2010). RNAi of the gene *POLDIP2*, influences sarcomere and myofibril morphology, and reduces flight capability (Schnorrer et al. 2010). Disruption of *BicD* produce defects in locomotion (Li et al. 2010). The gene *Numb* encodes an inhibitor of Notch signaling, a signaling pathway also implicated as important for locomotion phenotypes in the network analyses. The gene *ShakB* produces an innexin protein. In adult flies, it is expressed in muscle motor neurons and mutations in this gene cause defects in jump response (Baird et al. 1990), light response (Krishnan et al. 1993) and flight capability (Trimarchi and Murphey 1997). Finally, the gene *Slow* is involved in muscle attachment (Gilsohn and Volk 2010). As such, each of these genes are prime candidates for influencing age-related physical performance and should be the targets of future research.

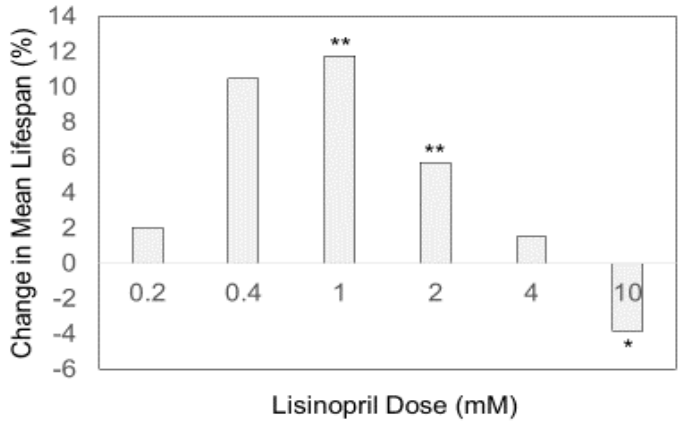
Since the DGRP sequence information is readily available and GWA can be accomplished, the DGRP serves as an excellent resource for identifying candidate SNPs associated with a host of phenotypes in many different environments. It is also useful for making comparisons between GWA investigations to uncover pleiotropic and epistatic relationships between genes. This raises an abundance of questions regarding the specificity of genes contributing to variation in a range of phenotypes and conditions: Are the patterns of increased genetic variation with age that I observed specific to physical performance measures, or does this increase also occur in other

traits that exhibit senescence like age-specific immunity or age-specific stress responses? Are the same genes and patterns of genetic variation observed in age-specific phenotypes in mated males? To what extent do the candidate genes or patterns of genetic variation overlap with variation in female age-specific physical performance? The DGRP is an excellent tool to address these questions from a systems genetics approach.

Finally, another avenue of future work is to dissect the specifics of how ACE inhibition increases lifespan and mediates age-related decline of physical performance. I used only one of many ACE*i*, Lisinopril. It is well known that other ACE inhibitors such as Enalapril (Inserra et al. 2009) and Angiotensin II receptor blockers such as Losartan (Kosmadakis et al. 2010) and Valsartan also contribute to extension of lifespan or reduce effects of senescence in mammals (Liern et al. 2004; Benigni et al. 2009, 2010; Dal-Ros et al. 2010). Future studies could include these drugs, or a combination of them, to test how individual genetic backgrounds will respond to a given treatment. Understanding the mechanisms of response to medications has been a long standing goal in clinical research. Identifying genes and mechanisms that influence this response have far reaching impacts on our understanding of how to increase human health span.

Appendices

Appendix 2.1. Determination of Lisinopril dose response curve. Drug dose is optimal, minimal, and least toxic when DGRP_229 flies are treated with 1mM Lisinopril. Drug dose of 0.2 and 0.4 mM have no effect on lifespan. Drug dose of 10mM is toxic and significantly reduced lifespan. Data are deviation from control (0 mM). *$P < 0.05$, **$P < 0.001$.



Appendix 2.2. Genes differentially expressed between untreated and Lisinopril-treated flies. (A) one week and (B) five weeks of age. FBGN: FlyBase Gene Number, Symbol: Gene name, Location: Location in genome, 1wk.DrugEffect.logFC = Fold change in expression. Positive values indicate increased gene expression in response to Lisinopril treatment.

A. One week of age

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0053665	*CG33665*	X:6372171-6372882	-5.45	0.0304
FBgn0266405	*CR45045*	3R:13493299-13493547	-3.19	0.0177
FBgn0039685	*Obp99b*	3R:25541976-25542618	-1.06	0.0219
FBgn0015035	*Cyp4e3*	2L:9747839-9750071	-1.04	< 0.0001
FBgn0050121	*CR30121*	2R:14626636-14629163	-0.98	0.0219
FBgn0085256	*CG34227*	2R:7135631-7136115	-0.91	0.0182
FBgn0039312	*CG10514*	3R:21104149-21105568	-0.73	0.0049
FBgn0260429	*CG42524*	2R:11501359-11544719	-0.62	0.0177
FBgn0039313	*CG11892*	3R:21107270-21108959	-0.55	0.0300
FBgn0031276	*CG12506*	2L:773547-774017	-0.54	0.0177
FBgn0033027	*TpnC4*	2R:1390942-1395371	-0.51	0.0114
FBgn0086359	*Invadolysin*	3R:5970390-5984856	-0.51	0.0007
FBgn0032727	*CG10623*	2L:18955481-18957311	-0.51	0.0177
FBgn0036935	*CG14186*	3L:20091184-20096927	-0.48	0.0219
FBgn0038717	*CG17751*	3R:15432976-15435044	-0.48	0.0186
FBgn0033188	*Drat*	2R:3413623-3420486	-0.46	0.0122
FBgn0032167	*CG5853*	2L:9940773-9947648	-0.41	0.0122
FBgn0036833	*CG3819*	3L:18913530-18915208	-0.39	0.0344
FBgn0032864	*CG2493*	2L:20461585-20463444	0.51	0.0084
FBgn0032615	*CG6012*	2L:16860029-16861489	0.68	0.0017
FBgn0259748	*CG42397*	3L:11966185-11966890	0.78	0.0007

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0022700	*Cht4*	2R:16952884-16954592	0.95	0.0133
FBgn0034187	*CG6967*	2R:12950280-12954051	1.47	0.0306
FBgn0028396	*TotA*	3R:16696758-16697422	1.49	0.0134
FBgn0044812	*TotC*	3R:16698710-16699302	1.97	0.0026

B. Five weeks of age

FBGN	SYMBOL	LOCATION	5wk.DrugEffect.logFC	5wk.DrugEffect.FDR
FBgn0264987	*CR44138*	3R:9202625-9203117	-2.01	0.0044
FBgn0013772	*Cyp6a8*	2R:10774676-10776515	-1.86	< 0.0001
FBgn0033204	*CG2065*	2R:3551291-3552774	-1.68	0.0000
FBgn0038082	*CG5724*	3R:8565364-8567139	-1.65	0.0002
FBgn0037936	*CG6908*	3R:7514103-7515773	-1.56	0.0006
FBgn0039091	*CG10182*	3R:19412842-19415541	-1.53	< 0.0001
FBgn0010038	*GstD2*	3R:8197693-8198426	-1.48	< 0.0001
FBgn0028940	*Cyp28a5*	2L:13977302-13979569	-1.40	0.0183
FBgn0023495	*Lip3*	3R:9195962-9197626	-1.21	0.0142
FBgn0052079	*CG32079*	3L:11018661-11020432	-1.20	0.0541
FBgn0039319	*CG13659*	3R:21126613-21128039	-1.19	0.0048
FBgn0035176	*CG13905*	3L:902898-903810	-1.17	< 0.0001
FBgn0028396	*TotA*	3R:16696758-16697422	-1.14	0.0081
FBgn0015039	*Cyp9b2*	2R:3017782-3019644	-1.13	0.0061
FBgn0033207	*CG12826*	2R:3557832-3558608	-1.11	0.0165
FBgn0005660	*Ets21C*	2L:544925-552923	-1.09	< 0.0001
FBgn0035343	*CG16762*	3L:2474667-2475614	-1.07	0.0061
FBgn0037724	*Fst*	3R:5470700-5471876	-1.07	0.0000
FBgn0010041	*GstD5*	3R:8201455-8202294	-1.06	< 0.0001
FBgn0001258	*ImpL3*	3L:6252205-6255794	-1.02	0.0447
FBgn0033302	*Cyp6a14*	2R:4451692-4454707	-1.02	0.0001
FBgn0051272	*CG31272*	3R:6582018-6585120	-1.02	< 0.0001

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0266431	*CG45061*	X:9765131-9765735	-0.99	0.0520
FBgn0039006	*Cyp6d4*	3R:18524041-18526029	-0.98	0.0032
FBgn0051809	*CG31809*	2L:16853620-16858714	-0.97	0.0251
FBgn0015035	*Cyp4e3*	2L:9747839-9750071	-0.96	< 0.0001
FBgn0035791	*CG8539*	3L:7490311-7491931	-0.96	0.0015
FBgn0259164	*CG42269*	3L:6059396-6064645	-0.94	0.0003
FBgn0033978	*Cyp6a23*	2R:10763334-10765159	-0.86	0.0095
FBgn0010241	*Mdr50*	2R:10134471-10140102	-0.85	0.0234
FBgn0039326	*CG10562*	3R:21146775-21148479	-0.85	0.0061
FBgn0053301	*CG33301*	2L:10049580-10051201	-0.85	0.0005
FBgn0052523	*CG32523*	X:21088905-21089862	-0.82	0.0259
FBgn0038455	*CG14907*	3R:12476939-12478041	-0.81	0.0001
FBgn0035445	*CG12014*	3L:3378268-3380083	-0.81	0.0229
FBgn0022700	*Cht4*	2R:16952884-16954592	-0.81	0.0046
FBgn0085227	*CG34198*	2R:15569951-15570482	-0.79	0.0004
FBgn0030347	*CG15739*	X:11767352-11769379	-0.78	0.0001
FBgn0037934	*CG6830*	3R:7506694-7510064	-0.76	0.0009
FBgn0039316	*CG11893*	3R:21115611-21117088	-0.76	0.0267
FBgn0015037	*Cyp4p1*	2R:5127546-5129644	-0.76	< 0.0001
FBgn0029831	*CG5966*	X:5882824-5886673	-0.75	0.0044
FBgn0036362	*CG10725*	3L:13431054-13432109	-0.75	0.0011
FBgn0005664	*Cry*	2L:11944129-11947131	-0.74	0.0002
FBgn0038475	*Keap1*	3R:12899664-12905667	-0.73	0.0243
FBgn0033981	*Cyp6a21*	2R:10772769-10774452	-0.73	0.0223
FBgn0013984	*InR*	3R:17395970-17445043	-0.71	0.0299
FBgn0042101	*CG18744*	3R:3622800-3624493	-0.71	0.0002
FBgn0015351	*CG14906*	3R:12475703-12476879	-0.71	0.0016
FBgn0267339	*p38c*	3R:19974742-19975925	-0.70	0.0062
FBgn0034279	*CG18635*	2R:13655313-13657494	-0.70	0.0032

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0038353	*CG5399*	3R:11520963-11522864	-0.68	< 0.0001
FBgn0038299	*Spn88Eb*	3R:11032425-11034484	-0.65	0.0138
FBgn0032805	*CG10337*	2L:19545376-19546959	-0.65	0.0255
FBgn0037391	*CG2017*	3R:1683805-1688127	-0.64	0.0481
FBgn0039209	*CG13624*	3R:20383090-20408586	-0.63	0.0325
FBgn0037563	*CG11672*	3R:4115117-4116300	-0.63	0.0005
FBgn0040350	*CG3690*	X:842618-845933	-0.63	0.0044
FBgn0033659	*Damm*	2R:7752681-7753888	-0.61	0.0004
FBgn0041184	*Socs36E*	2L:18138675-18152417	-0.60	0.0165
FBgn0040256	*Ugt86Dd*	3R:6952319-6954050	-0.60	0.0514
FBgn0032451	*spict*	2L:12704725-12706683	-0.59	0.0002
FBgn0041337	*Cyp309a2*	2L:2564886-2573037	-0.59	0.0225
FBgn0032900	*CG14401*	2L:20869854-20871565	-0.59	0.0088
FBgn0010786	*l(3)02640*	3L:1334889-1339415	-0.58	0.0015
FBgn0003499	*sr*	3R:13916525-13961305	-0.58	0.0410
FBgn0082995	*snoRNA:Psi28S-1837b*	3L:5757213-5757349	-0.58	0.0179
FBgn0005612	*Sox14*	2R:19866324-19872700	-0.58	0.0020
FBgn0002069	*Aats-asp*	2R:8764509-8770673	-0.57	0.0041
FBgn0033787	*CG13321*	2R:8840617-8843217	-0.57	0.0001
FBgn0037960	*mthl5*	3R:7709660-7712890	-0.56	0.0507
FBgn0003328	*scb*	2R:11136289-11146003	-0.56	0.0008
FBgn0034709	*Swim*	2R:18077322-18090176	-0.55	0.0104
FBgn0051216	*Naam*	3R:15497172-15524464	-0.55	0.0232
FBgn0050489	*Cyp12d1-p*	2R:7004730-7009389	-0.55	0.0180
FBgn0263316	*Mrp4*	3R:7394972-7401659	-0.54	0.0008
FBgn0040299	*Myo28B1*	2L:7666047-7689459	-0.54	0.0038
FBgn0020416	*Idgf1*	2L:16445278-16446793	-0.54	0.0041
FBgn0265186	*CG44251*	2R:8835853-8839798	-0.54	0.0137
FBgn0033051	*dream*	2R:1907816-1910649	-0.53	0.0189

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0030893	*RhoGAP16F*	X:17987178-17991446	-0.53	0.0385
FBgn0036196	*CG11658*	3L:11692596-11701653	-0.53	0.0210
FBgn0038638	*CG7702*	3R:14495112-14500401	-0.52	0.0279
FBgn0063493	*GstE7*	2R:14294429-14295193	-0.52	0.0032
FBgn0033205	*CG2064*	2R:3553333-3554848	-0.52	0.0165
FBgn0038842	*hdly*	3R:16740018-16754935	-0.51	0.0033
FBgn0030332	*CG9360*	X:11657507-11658623	-0.51	0.0221
FBgn0051683	*CG31683*	2L:20447734-20449964	-0.51	0.0398
FBgn0044047	*Ilp6*	X:2225526-2228691	-0.51	0.0447
FBgn0038730	*CG6300*	3R:15597104-15598947	-0.51	0.0398
FBgn0001987	*Gli*	2L:15756002-15762758	-0.51	0.0018
FBgn0033397	*Cyp4p3*	2R:5129825-5131915	-0.50	0.0138
FBgn0040208	*Kat60*	3R:1053011-1057931	-0.50	0.0055
FBgn0000053	*ade3*	2L:7014623-7023898	-0.50	0.0187
FBgn0033226	*CG1882*	2R:3700009-3702991	-0.50	0.0038
FBgn0030808	*RhoGAP15B*	X:16837323-16850267	-0.50	0.0055
FBgn0261984	*Ire1*	3R:15679630-15687013	-0.49	0.0265
FBgn0028983	*Spn55B*	2R:14013852-14015715	-0.49	0.0288
FBgn0035715	*CG10103*	3L:6747117-6749597	-0.49	0.0062
FBgn0039321	*CG10550*	3R:21132129-21134113	-0.48	0.0164
FBgn0265185	*CG44250*	2R:8837909-8839798	-0.48	0.0038
FBgn0033127	*Tsp42Ef*	2R:2912515-2915587	-0.47	0.0126
FBgn0038325	*Atg4b*	3R:11179442-11182646	-0.47	0.0454
FBgn0036493	*CG7255*	3L:15297355-15304295	-0.47	0.0099
FBgn0024947	*NTPase*	2L:2880704-2885850	-0.46	0.0374
FBgn0026577	*CG8677*	2L:21221312-21232059	-0.46	0.0098
FBgn0025628	*CG4199*	X:1960468-1967516	-0.46	0.0274
FBgn0000473	*Cyp6a2*	2R:2667254-2668996	-0.45	0.0267
FBgn0053126	*NLaz*	2L:1359837-1361821	-0.45	0.0210

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0025692	*CG3814*	2R:8762669-8764471	-0.45	0.0374
FBgn0004228	*mex1*	3L:15510524-15512692	-0.45	0.0447
FBgn0266410	*CG45050*	3R:5165938-5188716	-0.44	0.0116
FBgn0030894	*CG7192*	X:17991591-17995347	-0.44	0.0454
FBgn0032694	*MESR3*	2L:18617256-18661776	-0.44	0.0345
FBgn0051004	*mesh*	3R:27004111-27021484	-0.43	0.0396
FBgn0035049	*Mmp1*	2R:20558817-20575707	-0.43	0.0248
FBgn0266376	*CR45018*	X:13198038-13202037	-0.43	0.0410
FBgn0030929	*CG15043*	X:18387226-18388201	-0.43	0.0267
FBgn0000636	*Fas3*	2L:18320102-18393441	-0.41	0.0249
FBgn0015589	*Apc*	3R:24658023-24670470	-0.41	0.0460
FBgn0038803	*CG5191*	3R:16386587-16398722	-0.40	0.0432
FBgn0086346	*ALiX*	3R:23522188-23525832	-0.40	0.0447
FBgn0004657	*mys*	X:7955678-7964270	-0.40	0.0299
FBgn0052369	*CG32369*	3L:7770205-7802925	-0.40	0.0429
FBgn0029507	*Tsp42Ed*	2R:2897003-2899244	-0.40	0.0541
FBgn0031450	*Hrs*	2L:2739986-2743377	-0.39	0.0520
FBgn0035670	*CG10472*	3L:6054472-6056010	0.44	0.0396
FBgn0027538	*beta4GalNAcTA*	2R:10146535-10148655	0.47	0.0541
FBgn0016675	*Lectin-galC1*	2L:19417447-19418274	0.48	0.0229
FBgn0003930	*snRNA:U4:39B*	2L:21215036-21215178	0.48	0.0177
FBgn0036110	*Cpr67Fb*	3L:10887273-10887793	0.49	0.0085
FBgn0038398	*sxe2*	3R:11909600-11912944	0.50	0.0061
FBgn0039755	*CG15531*	3R:26020512-26021950	0.53	0.0068
FBgn0029084	*gom*	2R:18027440-18028869	0.53	0.0454
FBgn0083014	*snoRNA:Psi18S-996*	3L:261803-261953	0.55	0.0447
FBgn0032144	*CG17633*	2L:9769060-9770409	0.55	0.0274
FBgn0082957	*snoRNA:Psi28S-3405d*	3R:24428278-24428417	0.56	0.0321
FBgn0031141	*CG1304*	X:20835554-20836446	0.57	0.0429

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0032615	*CG6012*	2L:16860029-16861489	0.58	0.0237
FBgn0004191	*snRNA:U2:34ABa*	2L:13211925-13212116	0.60	0.0396
FBgn0034647	*pirk*	2R:17548472-17550500	0.61	0.0258
FBgn0036321	*CG14120*	3L:12878139-12882344	0.61	0.0143
FBgn0035619	*CG10592*	3L:5585841-5588372	0.62	0.0140
FBgn0028920	*CG8997*	2L:13843915-13845325	0.63	0.0442
FBgn0033593	*Listericin*	2R:7129250-7129699	0.63	0.0127
FBgn0004187	*snRNA:U1:95Cc*	3R:19652056-19652219	0.65	0.0301
FBgn0035666	*Jon65Aii*	3L:6045421-6046315	0.68	0.0184
FBgn0032913	*CG9259*	2L:21089375-21090999	0.68	0.0005
FBgn0031249	*CG11911*	2L:320279-321248	0.68	0.0418
FBgn0026169	*snoRNA:Psi18S-1820*	2R:20063751-20063890	0.68	0.0002
FBgn0259998	*CG17571*	2L:20255988-20257192	0.69	0.0274
FBgn0264552	*CG43931*	3L:20890657-20891601	0.70	0.0165
FBgn0004556	*Dbp73D*	3L:16721596-16723918	0.71	0.0398
FBgn0039030	*CG6660*	3R:18749074-18750247	0.71	0.0098
FBgn0086670	*snoRNA:Psi28S-2622*	3L:1488906-1489045	0.71	0.0005
FBgn0039769	*CG15534*	3R:26257786-26260861	0.74	0.0036
FBgn0039184	*CG6432*	3R:20162644-20166367	0.77	< 0.0001
FBgn0259952	*Sfp24Bb*	2L:3669261-3669775	0.77	< 0.0001
FBgn0039313	*CG11892*	3R:21107270-21108959	0.82	0.0459
FBgn0085320	*CG34291*	3R:22251012-22251503	0.82	0.0418
FBgn0038700	*CG3734*	3R:15213583-15215588	0.83	0.0065
FBgn0038986	*CG5278*	3R:18384064-18388294	0.84	0.0252
FBgn0265922	*CR44711*	X:21454829-21455602	0.86	0.0274
FBgn0038130	*CG8630*	3R:9105443-9110249	0.89	0.0008
FBgn0263763	*CG43680*	3L:15046098-15046451	0.90	0.0126
FBgn0050049	*CG30049*	2R:8243176-8246592	0.94	0.0546
FBgn0050043	*CG30043*	2R:8246941-8250670	0.98	0.0432

FBGN	SYMBOL	LOCATION	1wk.DrugEffect.logFC	1wk.DrugEffect.FDR
FBgn0032105	*borr*	2L:9251472-9253118	0.99	0.0232
FBgn0043578	*PGRP-SB1*	3L:16720399-16721089	1.01	0.0002
FBgn0032266	*CG18302*	2L:10534322-10536587	1.03	0.0061
FBgn0027584	*CG4757*	3R:6986929-6989220	1.05	0.0010
FBgn0035620	*CG5150*	3L:5588642-5590522	1.06	0.0001
FBgn0035779	*CG8562*	3L:7391385-7393012	1.06	< 0.0001
FBgn0039312	*CG10514*	3R:21104149-21105568	1.11	0.0221
FBgn0083936	*Acp54A1*	2R:13020950-13021255	1.15	< 0.0001
FBgn0051832	*CG31832*	2L:15030928-15031750	1.19	0.0165
FBgn0034317	*CG14499*	2R:14075227-14075794	1.25	0.0267
FBgn0051089	*CG31089*	3R:21728365-21729900	1.28	0.0037
FBgn0032839	*CG10659*	2L:20024975-20025811	1.38	0.0022
FBgn0028853	*CG15263*	2L:15285000-15286047	1.51	< 0.0001
FBgn0039685	*Obp99b*	3R:25541976-25542618	1.73	0.0001
FBgn0028855	*CG15282*	2L:14712471-14713298	1.86	0.0180
FBgn0043576	*PGRP-SC1a*	2R:4597238-4597825	2.12	0.0007
FBgn0041579	*AttC*	2R:9281210-9282172	2.14	< 0.0001
FBgn0014865	*Mtk*	2R:11296351-11296618	2.15	0.0001
FBgn0052185	*edin*	3L:17487980-17488400	2.20	0.0002
FBgn0034407	*DptB*	2R:14754896-14755400	2.62	0.0002
FBgn0264541	*CG43920*	2R:13486350-13486758	2.83	0.0183
FBgn0265577	*CR44404*	2R:13558573-13558800	3.04	0.0001
FBgn0010388	*Dro*	2R:10633466-10634219	3.38	0.0001
FBgn0266405	*CR45045*	3R:13493299-13493547	3.45	< 0.0001
FBgn0004240	*Dpt*	2R:14753270-14753765	4.60	< 0.0001

Appendix 2.3. Results from the Gene Ontology Analysis of genes significantly responding to Lisinopril treatment in five-week old flies.

Gene Cluster 1 Enrichment Score: 6.43

Category	Term	Count	*P*-Value	Genes	Fold Enrichment
INTERPRO	IPR015897:CHK kinase-like	10	4.76E-22	FBGN0039312 FBGN0039316 FBGN0039313 FBGN0053301 FBGN0037934 FBGN0039319 FBGN0039326 FBGN0039321 FBGN0032913 FBGN0037936	193.83

Gene Cluster 2 Enrichment Score: 6.41

Category	Term	Count	*P*-Value	Genes	Fold Enrichment
GOTERM_MF_DIRECT	GO:0016705~oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	13	3.71E-25	FBGN0015039 FBGN0015037 FBGN0015035 FBGN0050489 FBGN0033397 FBGN0041337 FBGN0000473 FBGN0033302 FBGN0033978 FBGN0028940 FBGN0039006 FBGN0033981 FBGN0013772	94.17
GOTERM_CC_DIRECT	GO:0005789~endoplasmic reticulum membrane	12	5.48E-18	FBGN0015039 FBGN0028940 FBGN0015037 FBGN0015035 FBGN0033397 FBGN0039006 FBGN0041337 FBGN0000473 FBGN0033981 FBGN0013772 FBGN0033302 FBGN0033978	38.38
COG_ONTOLOGY	Secondary metabolites biosynthesis, transport, and catabolism	13	1.22E-14	FBGN0015039 FBGN0015037 FBGN0015035 FBGN0050489 FBGN0033397 FBGN0041337 FBGN0000473 FBGN0033302 FBGN0033978 FBGN0028940 FBGN0039006	12.56

				FBGN0033981 FBGN0013772	
GOTERM_BP_DIRECT	GO:0046680~response to DDT	7	1.41E-13	FBGN0028940 FBGN0050489 FBGN0039006 FBGN0000473 FBGN0033981 FBGN0013772 FBGN0033302	181.25
GOTERM_BP_DIRECT	GO:0046701~insecticide catabolic process	6	1.97E-11	FBGN0028940 FBGN0039006 FBGN0000473 FBGN0033981 FBGN0013772 FBGN0033302	183.87
GOTERM_BP_DIRECT	GO:0031000~response to caffeine	2	0.013024	FBGN0000473 FBGN0013772	70.48

Gene Cluster 3 Enrichment Score: 3.38

Category	Term	Count	*P*-Value	Genes	Fold Enrichment
INTERPRO	IPR002347:Glucose/ribitol dehydrogenase	5	6.52E-10	FBGN0033204 FBGN0033205 FBGN0030332 FBGN0051809 FBGN0032615	154.16
INTERPRO	IPR016040:NAD(P)-binding domain	5	4.56E-08	FBGN0033204 FBGN0033205 FBGN0030332 FBGN0051809 FBGN0032615	54.24
GOTERM_MF_DIRECT	GO:0016491~oxidoreductase activity	5	1.81E-07	FBGN0033204 FBGN0033205 FBGN0030332 FBGN0051809 FBGN0032615	38.48

Gene Cluster 4 Enrichment Score: 3.21

Category	Term	Count	PValue	Genes	Fold Enrichment
GOTERM_BP_DIRECT	GO:0009617~response to bacterium	5	2.68E-10	FBGN0010388 FBGN0014865 FBGN0041579 FBGN0028396 FBGN0004240	191.23

Appendix 2.3 (continued)

Gene Cluster 5 Enrichment Score: 2.05

Category	Term	Count	PValue	Genes	Fold Enrichment
GOTERM_BP_DIRECT	GO:0006508~proteolysis	8	2.69E-10	FBGN0035779 FBGN0035670 FBGN0052523 FBGN0031141 FBGN0035666 FBGN0035791 FBGN0259998 FBGN0031249	20.25
INTERPRO	IPR009003:Trypsin-like cysteine/serine peptidase domain	6	1.53E-07	FBGN0035670 FBGN0052523 FBGN0031141 FBGN0035666 FBGN0259998 FBGN0031249	26.10
GOTERM_MF_DIRECT	GO:0004252~serine-type endopeptidase activity	6	4.81E-07	FBGN0035670 FBGN0052523 FBGN0031141 FBGN0035666 FBGN0259998 FBGN0031249	20.72
UP_KEYWORDS	Serine protease	4	3.91E-05	FBGN0035670 FBGN0035666 FBGN0259998 FBGN0031249	35.51
GOTERM_MF_DIRECT	GO:0004181~metallocarboxypeptidase activity	2	0.0172	FBGN0035779 FBGN0035791	50.45

Gene Cluster 6 Enrichment Score: 0.33

Category	Term	Count	PValue	Genes	Fold Enrichment
GOTERM_CC_DIRECT	GO:0016021~integral component of membrane	26	1.48E-14	FBGN0040350 FBGN0032900 FBGN0032805 FBGN0052079 FBGN0024947 FBGN0038130 FBGN0040256 FBGN0034279 FBGN0029084 FBGN0039755 FBGN0033127 FBGN0050049 FBGN0038082 FBGN0050043 FBGN0039091 FBGN0039321 FBGN0029507 FBGN0036493	3.42

				FBGN0025692 FBGN0038638 FBGN0004228 FBGN0038986 FBGN0039030 FBGN0051272 FBGN0032451 FBGN0259164	
GOTERM_BP_DIRECT	GO:0006633~fatty acid biosynthetic process	3	3.00E-04	FBGN0038986 FBGN0039030 FBGN0039755	71.86
GOTERM_CC_DIRECT	GO:0005887~integral component of plasma membrane	6	0.006438	FBGN0033127 FBGN0040350 FBGN0051272 FBGN0259164 FBGN0029507 FBGN0036493	3.93
GOTERM_MF_DIRECT	GO:0016717~oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water	2	0.009764	FBGN0038130 FBGN0039755	94.73
GOTERM_MF_DIRECT	GO:0004768~stearoyl-CoA 9-desaturase activity	2	0.011152	FBGN0038130 FBGN0039755	82.89
GOTERM_MF_DIRECT	GO:0008514~organic anion transmembrane transporter activity	2	0.016684	FBGN0040350 FBGN0051272	55.26
KEGG_PATHWAY	dme01040:Biosynthesis of unsaturated fatty acids	2	0.019501	FBGN0038130 FBGN0039755	38.22
GOTERM_MF_DIRECT	GO:0102338~3-oxo-lignoceronyl-CoA synthase activity	2	0.027664	FBGN0038986 FBGN0039030	33.15
GOTERM_MF_DIRECT	GO:0102337~3-oxo-cerotoyl-CoA synthase activity	2	0.027664	FBGN0038986 FBGN0039030	33.15
GOTERM_MF_DIRECT	GO:0102336~3-oxo-arachidoyl-CoA synthase activity	2	0.027664	FBGN0038986 FBGN0039030	33.15
PIR_SUPERFAMILY	PIRSF002419:tetraspanin	2	0.029777	FBGN0033127 FBGN0029507	16.79
INTERPRO	IPR007484:Peptidase M28	2	0.030973	FBGN0050049 FBGN0050043	30.51
KEGG_PATHWAY	dme00053:Ascorbate and aldarate metabolism	2	0.03236	FBGN0038082 FBGN0040256	22.93
KEGG_PATHWAY	dme00830:Retinol metabolism	2	0.033426	FBGN0038082 FBGN0040256	22.19
GOTERM_MF_DIRECT	GO:0015020~glucuronosyltransferase activity	2	0.03988	FBGN0038082 FBGN0040256	22.86

Appendix 2.4. Genes that exhibited significant genotype by Lisinopril interactions. (A) one week and (B) five weeks of age

A. One week of age

FBGN	SYMBOL	FDR corrected p-value
FBgn0035620	*CG5150*	< 0.0001
FBgn0029172	*Fad2*	< 0.0001
FBgn0039685	*Obp99b*	< 0.0001
FBgn0065100	*snmRNA:254*	< 0.0001
FBgn0086672	*snoRNA:Or-aca5*	< 0.0001
FBgn0083014	*snoRNA:Psi18S-996*	< 0.0001
FBgn0086659	*snoRNA:Psi18S-176*	< 0.0001
FBgn0086603	*snoRNA:Or-CD2*	< 0.0001
FBgn0086601	*snoRNA:Psi28S-3327c*	< 0.0001
FBgn0035619	*CG10592*	< 0.0001
FBgn0083015	*snoRNA:Psi18S-920*	< 0.0001
FBgn0086658	*snoRNA:Psi28S-1180*	< 0.0001
FBgn0263461	*snoRNA:CG32479-a*	< 0.0001
FBgn0083007	*snoRNA:Psi28S-1135f*	< 0.0001
FBgn0082986	*snoRNA:Psi28S-2562*	< 0.0001
FBgn0263474	*scaRNA:PsiU2-38.40.42*	< 0.0001
FBgn0263462	*snoRNA:Dek-a*	< 0.0001
FBgn0083013	*snoRNA:Psi28S-1060*	< 0.0001
FBgn0083005	*snoRNA:Psi28S-1175a*	< 0.0001
FBgn0263477	*scaRNA:PsiU1-6*	< 0.0001
FBgn0086671	*snoRNA:Psi28S-2876*	< 0.0001
FBgn0085256	*CG34227*	< 0.0001
FBgn0263476	*snoRNA:CG32479-b*	< 0.0001
FBgn0083027	*snoRNA:Psi18S-531*	< 0.0001
FBgn0065055	*snoRNA:Psi28S-2648*	< 0.0001
FBgn0083057	*snoRNA:Psi18S-110*	< 0.0001
FBgn0065073	*snoRNA:229*	< 0.0001
FBgn0044812	*TotC*	< 0.0001
FBgn0082988	*snoRNA:Psi28S-2442b*	< 0.0001
FBgn0065058	*snoRNA:684*	< 0.0001
FBgn0003930	*snRNA:U4:39B*	< 0.0001
FBgn0065046	*snoRNA:U3:9B*	< 0.0001
FBgn0039312	*CG10514*	< 0.0001

FBGN	SYMBOL	FDR corrected p-value
FBgn0028396	*TotA*	< 0.0001
FBgn0086669	*snoRNA:Psi18S-841a*	< 0.0001
FBgn0082974	*snoRNA:Psi28S-3305c*	< 0.0001
FBgn0004183	*snRNA:U1:82Eb*	< 0.0001
FBgn0082963	*snoRNA:Psi28S-3378*	< 0.0001
FBgn0263487	*scaRNA:PsiU5-44*	0.0001
FBgn0026169	*snoRNA:Psi18S-1820*	0.0001
FBgn0086670	*snoRNA:Psi28S-2622*	0.0001
FBgn0039313	*CG11892*	0.0001
FBgn0083058	*snoRNA:Psi18S-1086*	0.0002
FBgn0083039	*snoRNA:Psi18S-301*	0.0003
FBgn0082957	*snoRNA:Psi28S-3405d*	0.0003
FBgn0266455	*CG45080*	0.0003
FBgn0086662	*snoRNA:Psi28S-3186*	0.0003
FBgn0032638	*CG6639*	0.0003
FBgn0086667	*snoRNA:Psi28S-3342*	0.0004
FBgn0263489	*unsRNA:d-a*	0.0005
FBgn0086359	*Invadolysin*	0.0005
FBgn0010241	*Mdr50*	0.0005
FBgn0263488	*unsRNA:CG10576-a*	0.0005
FBgn0083046	*snoRNA:Psi18S-1389a*	0.0006
FBgn0082922	*snoRNA:Or-aca4*	0.0006
FBgn0065064	*snoRNA:Psi28S-291*	0.0007
FBgn0082983	*snoRNA:Psi28S-2626*	0.0008
FBgn0082923	*snoRNA:Or-aca3*	0.0008
FBgn0032913	*CG9259*	0.0008
FBgn0015035	*Cyp4e3*	0.0010
FBgn0039769	*CG15534*	0.0011
FBgn0086661	*snoRNA:Psi28S-2566*	0.0013
FBgn0082959	*snoRNA:Psi28S-3405b*	0.0013
FBgn0003923	*snRNA:U2:38ABb*	0.0015
FBgn0003931	*snRNA:U4:38AB*	0.0018
FBgn0259971	*CG42481*	0.0028
FBgn0083048	*snoRNA:Psi18S-1377d*	0.0029
FBgn0004187	*snRNA:U1:95Cc*	0.0029
FBgn0082973	*snoRNA:Psi28S-3308*	0.0032
FBgn0082954	*snoRNA:Psi28S-3571*	0.0043
FBgn0086666	*snoRNA:Psi28S-2179*	0.0043
FBgn0263485	*scaRNA:PsiU2-55*	0.0045
FBgn0053665	*CG33665*	0.0058

FBGN	SYMBOL	FDR corrected p-value
FBgn0083987	*snRNA:U11*	0.0058
FBgn0083040	*snoRNA:Psi18S-1854c*	0.0059
FBgn0038135	*CG8773*	0.0064
FBgn0033027	*TpnC4*	0.0067
FBgn0083047	*snoRNA:Psi18S-1377e*	0.0073
FBgn0003938	*snRNA:U5:63BC*	0.0080
FBgn0001168	*h*	0.0084
FBgn0082994	*snoRNA:Psi28S-1837c*	0.0088
FBgn0086602	*snoRNA:Psi28S-3436b*	0.0104
FBgn0082960	*snoRNA:Psi28S-3405a*	0.0114
FBgn0034331	*CG15067*	0.0124
FBgn0082999	*snoRNA:Psi28S-1192c*	0.0137
FBgn0034229	*CG4847*	0.0141
FBgn0266405	*CR45045*	0.0141
FBgn0086600	*snoRNA:Psi18S-1347c*	0.0141
FBgn0004431	*LysX*	0.0141
FBgn0263018	*snRNA:U4atac:82E*	0.0176
FBgn0033782	*sug*	0.0223
FBgn0083041	*snoRNA:Psi18S-1854b*	0.0267
FBgn0038700	*CG3734*	0.0334
FBgn0039311	*CG10513*	0.0339
FBgn0261454	*scaRNA:MeU4-A65*	0.0345
FBgn0003916	*snRNA:U1:21D*	0.0345
FBgn0040759	*CG13177*	0.0346
FBgn0038930	*CG5778*	0.0346
FBgn0263484	*snoRNA:Pi4KIIalpha-a*	0.0350
FBgn0083049	*snoRNA:Psi18S-1377c*	0.0360
FBgn0082980	*snoRNA:Psi28S-2996*	0.0360
FBgn0082961	*snoRNA:Psi28S-3385b*	0.0364
FBgn0264347	*CR43803*	0.0367
FBgn0036321	*CG14120*	0.0390
FBgn0040609	*CG3348*	0.0392
FBgn0264330	*CG43789*	0.0392
FBgn0035779	*CG8562*	0.0392
FBgn0004191	*snRNA:U2:34ABa*	0.0392
FBgn0043791	*CG8147*	0.0411
FBgn0028948	*CG15253*	0.0416
FBgn0263472	*snoRNA:2R:9445205*	0.0424
FBgn0039114	*Lsd-1*	0.0448
FBgn0065076	*snoRNA:185*	0.0454

FBGN	SYMBOL	FDR corrected p-value
FBgn0010651	*l(2)08717*	0.0509
FBgn0044810	*TotX*	0.0513
FBgn0262565	*CR43105*	0.0530
FBgn0002869	*MtnB*	0.0530

B. Five weeks of age

FBGN	SYMBOL	FDR corrected p-value
FBgn0039091	*CG10182*	0.0001
FBgn0036831	*CG6839*	0.0001
FBgn0037724	*Fst*	0.0001
FBgn0010038	*GstD2*	0.0001
FBgn0023495	*Lip3*	0.0001
FBgn0028987	*Spn28F*	0.0001
FBgn0036833	*CG3819*	0.0002
FBgn0031910	*CG15818*	0.0002
FBgn0053530	*Acp53C14c*	0.0008
FBgn0036024	*CG18180*	0.001
FBgn0015035	*Cyp4e3*	0.0018
FBgn0033302	*Cyp6a14*	0.0018
FBgn0031654	*Jon25Bii*	0.0018
FBgn0086670	*snoRNA:Psi28S-2622*	0.0036
FBgn0262150	*CG42876*	0.0058
FBgn0026169	*snoRNA:Psi18S-1820*	0.0074
FBgn0020506	*Amyrel*	0.0079
FBgn0010041	*GstD5*	0.0086
FBgn0029831	*CG5966*	0.0133
FBgn0003358	*Jon99Ci*	0.0133
FBgn0036023	*CG18179*	0.0145
FBgn0036362	*CG10725*	0.0262
FBgn0035791	*CG8539*	0.0332
FBgn0263830	*CG40486*	0.0376
FBgn0031653	*Jon25Biii*	0.0398
FBgn0010381	*Drs*	0.0408
FBgn0015584	*Acp53Ea*	0.0443
FBgn0015351	*CG14906*	0.0443
FBgn0000565	*Eip71CD*	0.0443

Appendix 2.5. Gene ontology analysis of genes whose expression exhibited significant genotype by drug treatment interaction. (A) one week and (B) five weeks of age

A. One week of age

Gene Cluster 1 Enrichment Score: 2.85

Category	Term	Count	*P*-Value	Genes	Fold Enrichment
INTERPRO	IPR015897:CHK kinase-like	4	9.43E-08	FBGN0039311 FBGN0039312 FBGN0039313 FBGN0032913	161.52
INTERPRO	IPR004119: Protein of unknown function DUF227	4	9.43E-08	FBGN0039311 FBGN0039312 FBGN0039313 FBGN0032913	161.52

Gene Cluster 2 Enrichment Score: 2.53

Category	Term	Count	*P*-Value	Genes	Fold Enrichment
GOTERM_MF_DIRECT	GO:0004035~alkaline phosphatase activity	3	3.55E-06	FBGN0043791 FBGN0035619 FBGN0035620	343.85
GOTERM_BP_DIRECT	GO:0016311~dephosphorylation	3	3.03E-05	FBGN0043791 FBGN0035619 FBGN0035620	120.17
GOTERM_CC_DIRECT	GO:0009986~cell surface	3	6.13E-05	FBGN0043791 FBGN0035619 FBGN0035620	84.60
KEGG_PATHWAY	dme00790:Folate biosynthesis	3	7.93E-05	FBGN0043791 FBGN0035619 FBGN0035620	73.38
UP_KEYWORDS	Hydrolase	3	0.012891	FBGN0043791 FBGN0035619 FBGN0035620	5.87

Appendix 2.5. (continued)

Gene Cluster 3 Enrichment Score: 1.94

Category	Term	Count	*P*-Value	Genes	Fold Enrichment
INTERPRO	IPR010825:Stress-inducible humoral factor Turandot	3	4.64E-07	FBGN0028396 FBGN0044810 FBGN0044812	915.33
GOTERM_BP_DIRECT	GO:0034605~cellular response to heat	3	1.74E-06	FBGN0028396 FBGN0044810 FBGN0044812	488.7111
GOTERM_BP_DIRECT	GO:0009617~response to bacterium	3	1.71E-05	FBGN0028396 FBGN0044810 FBGN0044812	159.3623
UP_KEYWORDS	Innate immunity	3	2.15E-05	FBGN0028396 FBGN0044810 FBGN0044812	142.7795
GOTERM_BP_DIRECT	GO:0006979~response to oxidative stress	3	6.92E-05	FBGN0028396 FBGN0044810 FBGN0044812	79.68116
GOTERM_BP_DIRECT	GO:0034644~cellular response to UV	2	0.001273	FBGN0028396 FBGN0044812	523.619

B. Five weeks of age

Cluster 1 Enrichment Score: 3.03

Category	Term	Count	*P*-Value	Genes	Fold Enrichment
GOTERM_BP_DIRECT	GO:0006508~proteolysis	6	1.47E-07	FBGN0031653 FBGN0036024 FBGN0035791 FBGN0003358 FBGN0031654 FBGN0036023	19.29123
GOTERM_MF_DIRECT	GO:0004252~serine-type endopeptidase activity	5	3.95E-06	FBGN0031653 FBGN0036024 FBGN0003358 FBGN0031654 FBGN0036023	22.10476

Appendix 3.1. Enriched gene ontology categories for candidate climbing speed genes at one week of age. Overrepresented gene ontology categories among candidate genes identified in the week one climbing speed GWA analysis. Each annotation cluster contains genes with similar biological processes. Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8.

Annotation Cluster 1 Enrichment Score: 11.00

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009887~organ morphogenesis	24	7.73E-16	6.95	5.39E-13	5.39E-13
GO:0009886~post-embryonic morphogenesis	20	5.62E-15	9.41	3.93E-12	1.96E-12
GO:0007552~metamorphosis	20	8.36E-15	9.22	5.78E-12	1.93E-12
GO:0048563~post-embryonic organ morphogenesis	18	6.65E-14	10.34	4.62E-11	1.15E-11
GO:0007560~imaginal disc morphogenesis	18	6.65E-14	10.34	4.62E-11	1.15E-11
GO:0007444~imaginal disc development	20	6.73E-14	8.22	4.67E-11	9.34E-12
GO:0048707~instar larval or pupal morphogenesis	19	7.44E-14	9.12	5.16E-11	8.60E-12
GO:0002165~instar larval or pupal development	20	8.47E-14	8.12	5.88E-11	8.40E12
GO:0035120~post-embryonic appendage morphogenesis	17	1.29E-13	11.30	8.93E-11	1.12E-11
GO:0035114~imaginal disc-derived appendage morphogenesis	17	1.83E-13	11.05	1.27E-10	1.41E-11
GO:0035107~appendage morphogenesis	17	2.03E-13	10.97	1.41E-10	1.41E-11
GO:0048737~imaginal disc-derived appendage development	17	2.18E-13	10.92	1.51E-10	1.37E-11
GO:0048736~appendage development	17	2.59E-13	10.80	1.79E-10	1.50E-11
GO:0060429~epithelium development	23	4.04E-13	5.67	2.81E-10	2.16E-11
GO:0009791~post-embryonic development	20	7.29E-13	7.20	5.06E-10	3.37E-11
GO:0060562~epithelial tube morphogenesis	18	8.55E-13	8.85	5.93E-10	3.71E-11
GO:0048569~post-embryonic organ development	18	9.03E-13	8.82	6.27E-10	3.69E-11
GO:0048731~system development	30	1.26E-12	3.30	8.77E-10	4.87E-11
GO:0002009~morphogenesis of an epithelium	19	1.69E-12	7.60	1.17E-09	6.17E-11
GO:0035239~tube morphogenesis	18	2.07E-12	8.38	1.44E-09	7.19E-11
GO:0035295~tube development	20	2.39E-12	6.74	1.66E-09	7.91E-11
GO:0048729~tissue morphogenesis	19	2.51E-12	7.43	1.74E-09	7.91E-11
GO:0008586~imaginal disc-derived wing vein morphogenesis	9	5.61E-12	48.99	3.89E-09	1.69E-10
GO:0048513~animal organ development	24	1.14E-11	4.47	7.89E-09	3.29E-10
GO:0060541~respiratory system development	13	2.05E-11	14.59	1.43E-08	5.70E-10
GO:0035220~wing disc development	16	2.56E-11	9.03	1.77E-08	6.82E-10
GO:0007389~pattern specification process	16	1.17E-10	8.11	8.14E-08	2.71E-09
GO:0045165~cell fate commitment	15	2.53E-10	8.71	1.76E-07	5.49E-09
GO:0001708~cell fate specification	9	8.97E-10	26.49	6.23E-07	1.89E-08
GO:0003002~regionalization	14	7.05E-09	7.64	4.89E-06	1.19E-07
GO:0007447~imaginal disc pattern formation	7	9.97E-07	19.80	6.92E-04	1.03E-05
GO:0009790~embryo development	12	5.71E-06	5.31	0.003954	4.77E-05
GO:0016477~cell migration	9	6.87E-06	8.35	0.004754	5.54E-05
GO:0048870~cell motility	9	1.29E-05	7.66	0.008918	9.84E-05
GO:0048646~anatomical structure formation involved in morphogenesis	10	2.59E-04	4.36	0.164366	0.001319

Annotation Cluster 2 Enrichment Score: 7.66

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0090596~sensory organ morphogenesis	14	7.15E-11	11.10	4.96E-08	1.77E-09
GO:0045165~cell fate commitment	15	2.53E-10	8.71	1.76E-07	5.49E-09
GO:0001654~eye development	14	9.85E-10	8.98	6.84E-07	2.01E-08
GO:0007423~sensory organ development	15	1.43E-09	7.63	9.90E-07	2.75E-08

Annotation Cluster 3 Enrichment Score: 6.04

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051252~regulation of RNA metabolic process	20	1.40E-09	4.68	9.73E-07	2.78E-08
GO:0019219~regulation of nucleobase-containing compound metabolic process	20	3.12E-09	4.47	2.16E-06	5.69E-08
GO:0010556~regulation of macromolecule biosynthetic process	20	4.60E-09	4.37	3.19E-06	8.19E-08
GO:0031326~regulation of cellular biosynthetic process	20	9.16E-09	4.19	6.36E-06	1.51E-07
GO:0034654~nucleobase-containing compound biosynthetic process	20	2.01E-08	4.00	1.40E-05	3.10E-07
GO:0010468~regulation of gene expression	20	2.44E-08	3.95	1.70E-05	3.61E-07
GO:0018130~heterocycle biosynthetic process	20	4.10E-08	3.83	2.84E-05	5.80E-07
GO:0019438~aromatic compound biosynthetic process	20	4.52E-08	3.81	3.14E-05	6.28E-07
GO:1901362~organic cyclic compound biosynthetic process	20	7.10E-08	3.71	4.93E-05	9.67E-07
GO:0016070~RNA metabolic process	20	1.97E-06	3.02	0.001364	1.92E-05
GO:0090304~nucleic acid metabolic process	20	1.02E-05	2.71	0.007048	7.95E-05
GO:0010629~negative regulation of gene expression	11	1.49E-05	5.43	0.010309	1.11E-04
GO:0044271~cellular nitrogen compound biosynthetic process	20	4.39E-05	2.46	0.030004	2.87E-04
GO:0034645~cellular macromolecule biosynthetic process	20	5.33E-05	2.43	0.036298	3.36E-04
GO:0009059~macromolecule biosynthetic process	20	5.67E-05	2.42	0.038565	3.54E-04
GO:0010467~gene expression	21	8.00E-05	2.26	0.05398	4.74E-04
GO:0010605~negative regulation of macromolecule metabolic process	11	8.59E-05	4.43	0.057877	5.01E-04
GO:0006139~nucleobase-containing compound metabolic process	20	1.01E-04	2.33	0.067885	5.71E-04
GO:0044260~cellular macromolecule metabolic process	25	4.46E-04	1.76	0.266176	0.002193

Annotation Cluster 4 Enrichment Score: 5.63

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010623~programmed cell death involved in cell development	11	6.61E-11	20.09	4.58E-08	1.70E-09
GO:0012501~programmed cell death	14	1.00E-10	10.80	6.95E-08	2.40E-09
GO:0022412~cellular process involved in reproduction in multicellular organism	16	2.44E-07	4.64	1.69E-04	3.20E-06
GO:0007281~germ cell development	15	7.09E-07	4.67	4.92E-04	7.81E-06
GO:0048477~oogenesis	14	8.88E-07	5.06	6.16E-04	9.63E-06
GO:0007276~gamete generation	16	9.45E-07	4.18	6.56E-04	1.01E-05
GO:0007292~female gamete generation	14	9.95E-07	5.01	6.90E-04	1.05E-05
GO:0032989~cellular component morphogenesis	15	1.28E-06	4.45	8.86E-04	1.27E-05
GO:0030707~ovarian follicle cell development	10	2.03E-06	8.04	0.001407	1.93E-05
GO:0002064~epithelial cell development	10	4.55E-06	7.29	0.00315	3.90E-05
GO:0016477~cell migration	9	6.87E-06	8.35	0.004754	5.54E-05
GO:0019953~sexual reproduction	16	9.17E-06	3.49	0.006343	7.23E-05
GO:0000902~cell morphogenesis	13	1.13E-05	4.43	0.007841	8.75E-05
GO:0048870~cell motility	9	1.29E-05	7.66	0.008918	9.84E-05
GO:0090132~epithelium migration	7	2.66E-05	11.22	0.018325	1.91E-04
GO:0001667~ameboidal-type cell migration	7	4.43E-05	10.25	0.030265	2.87E-04
GO:0035265~organ growth	5	8.00E-05	20.91	0.053994	4.70E-04
GO:0009798~axis specification	7	1.18E-04	8.59	0.078558	6.39E-04
GO:0006796~phosphate-containing compound metabolic process	10	0.006967	2.73	0.992189	0.02548

Annotation Cluster 5 Enrichment Score: 5.60

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009966~regulation of signal transduction	15	3.49E-08	5.95	2.42E-05	5.04E-07
GO:0007165~signal transduction	20	1.06E-07	3.62	7.33E-05	1.41E-06
GO:0007166~cell surface receptor signaling pathway	14	2.67E-07	5.62	1.85E-04	3.37E-06
GO:0019538~protein metabolic process	16	0.041256	1.59	1	0.119865

Annotation Cluster 6 Enrichment Score: 4.22

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0035215~genital disc development	7	1.03E-08	42.07	7.18E-06	1.67E-07
GO:0007548~sex differentiation	7	1.06E-06	19.61	7.33E-04	1.08E-05
GO:0046661~male sex differentiation	5	2.78E-06	48.08	0.001929	2.58E-05
GO:0061458~reproductive system development	6	7.76E-06	20.86	0.005372	6.19E-05
GO:0048608~reproductive structure development	6	7.76E-06	20.86	0.005372	6.19E-05

GO:0048732~gland development	8	1.39E-05	9.46	0.009596	1.05E-04
GO:0035112~genitalia morphogenesis	4	3.27E-05	60.74	0.022467	2.25E-04
GO:0030539~male genitalia development	4	4.47E-05	54.95	0.030568	2.87E-04
GO:0048806~genitalia development	4	1.63E-04	36.06	0.106739	8.48E-04
GO:0090598~male anatomical structure morphogenesis	3	0.001454	50.91	0.635601	0.006409
GO:0048808~male genitalia morphogenesis	3	0.001454	50.91	0.635601	0.006409
GO:0007484~imaginal disc-derived genitalia development	3	0.003673	32.06	0.922213	0.014487
GO:0008406~gonad development	3	0.014147	16.03	0.999949	0.047544
GO:0045137~development of primary sexual characteristics	3	0.014147	16.03	0.999949	0.047544

Annotation Cluster 7 Enrichment Score: 4.11

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0012501~programmed cell death	14	1.00E-10	10.80	6.95E-08	2.40E-09
GO:0043067~regulation of programmed cell death	11	1.64E-09	14.49	1.14E-06	3.07E-08
GO:0043068~positive regulation of programmed cell death	7	2.53E-07	24.93	1.75E-04	3.25E-06
GO:0097190~apoptotic signaling pathway	6	2.83E-07	40.26	1.97E-04	3.45E-06
GO:0010942~positive regulation of cell death	7	4.44E-07	22.69	3.08E-04	5.22E-06
GO:0032270~positive regulation of cellular protein metabolic process	7	1.15E-04	8.63	0.076809	6.29E-04
GO:0051247~positive regulation of protein metabolic process	7	1.48E-04	8.24	0.097714	7.79E-04
GO:0043069~negative regulation of programmed cell death	5	3.50E-04	14.28	0.215652	0.001734
GO:0060548~negative regulation of cell death	5	6.52E-04	12.12	0.364044	0.003074
GO:0070997~neuron death	3	0.007188	22.78	0.993305	0.026005
GO:1901214~regulation of neuron death	3	0.007942	21.64	0.996048	0.028556
GO:0010212~response to ionizing radiation	3	0.008331	21.11	0.996991	0.029788

Annotation Cluster 8 Enrichment Score: 3.64

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045165~cell fate commitment	15	2.53E-10	8.71	1.76E-07	5.49E-09
GO:0001708~cell fate specification	9	8.97E-10	26.49	6.23E-07	1.89E-08
GO:0045596~negative regulation of cell differentiation	9	4.86E-09	21.46	3.38E-06	8.44E-08
GO:0035215~genital disc development	7	1.03E-08	42.07	7.18E-06	1.67E-07
GO:2000027~regulation of organ morphogenesis	8	2.17E-08	24.55	1.51E-05	3.27E-07
GO:0010721~negative regulation of cell development	7	2.72E-07	24.63	1.89E-04	3.37E-06
GO:0001709~cell fate deGO Category #	8	4.34E-07	15.92	3.01E-04	5.19E-06

and Termination					
GO:0051960~regulation of nervous system development	10	6.47E-07	9.22	4.49E-04	7.24E-06
GO:0007447~imaginal disc pattern formation	7	9.97E-07	19.80	6.92E-04	1.03E-05
GO:0007548~sex differentiation	7	1.06E-06	19.61	7.33E-04	1.08E-05
GO:0048859~formation of anatomical boundary	6	1.09E-06	30.91	7.56E-04	1.10E-05
GO:0009880~embryonic pattern specification	9	1.98E-06	9.87	0.001371	1.91E-05
GO:0030707~ovarian follicle cell development	10	2.03E-06	8.04	0.001407	1.93E-05
GO:0060284~regulation of cell development	9	2.34E-06	9.65	0.001623	2.20E-05
GO:0035214~eye-antennal disc development	6	3.11E-06	25.09	0.002154	2.84E-05
GO:0061326~renal tubule development	6	3.34E-06	24.73	0.002314	3.01E-05
GO:0072002~Malpighian tubule development	6	3.34E-06	24.73	0.002314	3.01E-05
GO:0030718~germ-line stem cell population maintenance	6	3.84E-06	24.04	0.002662	3.42E-05
GO:0002064~epithelial cell development	10	4.55E-06	7.29	0.00315	3.90E-05
GO:0010648~negative regulation of cell communication	9	5.26E-06	8.66	0.003647	4.46E-05
GO:0050767~regulation of neurogenesis	8	5.89E-06	10.79	0.004076	4.86E-05
GO:0001655~urogenital system development	6	6.08E-06	21.91	0.004213	4.97E-05
GO:0072001~renal system development	6	6.08E-06	21.91	0.004213	4.97E-05
GO:0016477~cell migration	9	6.87E-06	8.35	0.004754	5.54E-05
GO:0048870~cell motility	9	1.29E-05	7.66	0.008918	9.84E-05
GO:0048732~gland development	8	1.39E-05	9.46	0.009596	1.05E-04
GO:0010160~formation of organ boundary	5	1.59E-05	31.36	0.011008	1.18E-04
GO:0035218~leg disc development	6	2.45E-05	16.49	0.016844	1.79E-04
GO:0090132~epithelium migration	7	2.66E-05	11.22	0.018325	1.91E-04
GO:0007507~heart development	6	2.93E-05	15.88	0.02015	2.08E-04
GO:0072358~cardiovascular system development	6	3.07E-05	15.74	0.021049	2.15E-04
GO:0072359~circulatory system development	6	3.07E-05	15.74	0.021049	2.15E-04
GO:0048645~organ formation	5	3.26E-05	26.23	0.022388	2.26E-04
GO:0008284~positive regulation of cell proliferation	5	3.51E-05	25.76	0.024032	2.36E-04
GO:0007431~salivary gland development	7	3.51E-05	10.69	0.024057	2.34E-04
GO:0035272~exocrine system development	7	3.51E-05	10.69	0.024057	2.34E-04
GO:0001667~ameboidal-type cell migration	7	4.43E-05	10.25	0.030265	2.87E-04
GO:0042067~establishment of ommatidial planar polarity	5	5.25E-05	23.27	0.035765	3.34E-04
GO:0048872~homeostasis of number of cells	4	5.93E-05	50.17	0.040313	3.67E-04
GO:0016331~morphogenesis of embryonic epithelium	6	6.58E-05	13.42	0.044669	4.01E-04
GO:0035265~organ growth	5	8.00E-05	20.91	0.053994	4.70E-04

Annotation Cluster 9 Enrichment Score: 3.32

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010604~positive regulation of macromolecule metabolic process	13	4.79E-07	5.99	3.32E-04	5.54E-06
GO:0031325~positive regulation of cellular metabolic process	13	6.28E-07	5.84	4.36E-04	7.14E-06
GO:0051254~positive regulation of RNA metabolic process	7	0.001308	5.47	0.59686	0.005844
GO:0010557~positive regulation of macromolecule biosynthetic process	7	0.001926	5.07	0.737562	0.008326
GO:0045935~positive regulation of nucleobase-containing compound metabolic process	7	0.001926	5.07	0.737562	0.008326
GO:0010628~positive regulation of gene expression	7	0.002322	4.89	0.800738	0.009848
GO:0009891~positive regulation of biosynthetic process	7	0.003333	4.55	0.901446	0.013459
GO:0031328~positive regulation of cellular biosynthetic process	7	0.003333	4.55	0.901446	0.013459
GO:0051173~positive regulation of nitrogen compound metabolic process	7	0.003523	4.50	0.913662	0.014059
GO:0001067~regulatory region nucleic acid binding	5	0.004069	7.28	0.171001	0.089506

Annotation Cluster 10 Enrichment Score: 2.94

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0032989~cellular component morphogenesis	15	1.28E-06	4.45	8.86E-04	1.27E-05
GO:0048666~neuron development	13	4.29E-06	4.87	0.002975	3.72E-05
GO:0000902~cell morphogenesis	13	1.13E-05	4.43	0.007841	8.75E-05
GO:0031175~neuron projection development	11	2.66E-05	5.09	0.018267	1.92E-04
GO:0000904~cell morphogenesis involved in differentiation	10	7.12E-05	5.16	0.048184	4.29E-04

Annotation Cluster 11 Enrichment Score: 2.94

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010629~negative regulation of gene expression	11	1.49E-05	5.43	0.010309	1.11E-04
GO:0046620~regulation of organ growth	5	4.31E-05	24.45	0.029484	2.85E-04

Annotation Cluster 12 Enrichment Score: 2.82

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0035556~intracellular signal transduction	12	4.29E-06	5.47	0.002973	3.77E-05
GO:0009967~positive regulation of signal transduction	8	6.20E-05	7.49	0.042113	3.81E-04

Annotation Cluster 13 Enrichment Score: 2.65

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0061458~reproductive system development	6	7.76E-06	20.86	0.005372	6.19E-05
GO:0048608~reproductive structure development	6	7.76E-06	20.86	0.005372	6.19E-05

Appendix 3.2. Enriched gene ontology categories for candidate climbing speed genes at five weeks of age. Overrepresented gene ontology categories among candidate genes identified in the week five climbing speed GWA analysis. Each annotation cluster contains genes with similar biological processes. Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8.

Annotation Cluster 1 Enrichment Score: 37.09

GO Category # and GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048729~tissue morphogenesis	60	5.33E-45	8.98	5.93E-42	5.93E-42
GO:0048513~animal organ development	75	2.26E-44	5.35	2.51E-41	1.25E-41
GO:0002009~morphogenesis of an epithelium	59	3.31E-44	9.04	3.68E-41	1.23E-41
GO:0009887~organ morphogenesis	65	6.95E-44	7.21	7.72E-41	1.93E-41
GO:0035295~tube development	61	1.48E-42	7.87	1.64E-39	2.73E-40
GO:0060429~epithelium development	66	9.35E-41	6.23	1.04E-37	1.48E-38
GO:0048736~appendage development	48	1.54E-39	11.68	1.71E-36	1.90E-37
GO:0060562~epithelial tube morphogenesis	52	1.57E-39	9.79	1.74E-36	1.74E-37
GO:0048737~imaginal disc-derived appendage development	47	2.24E-38	11.57	2.49E-35	2.26E-36
GO:0035239~tube morphogenesis	52	2.50E-38	9.27	2.78E-35	2.31E-36
GO:0007560~imaginal disc morphogenesis	48	1.77E-37	10.56	1.97E-34	1.41E-35
GO:0048563~post-embryonic organ morphogenesis	48	1.77E-37	10.56	1.97E-34	1.41E-35
GO:0035114~imaginal disc-derived appendage morphogenesis	46	3.17E-37	11.45	3.52E-34	2.20E-35
GO:0035107~appendage morphogenesis	46	4.29E-37	11.37	4.77E-34	2.81E-35
GO:0048569~post-embryonic organ development	50	8.20E-37	9.38	9.12E-34	5.06E-35
GO:0035120~post-embryonic appendage morphogenesis	45	2.63E-36	11.46	2.92E-33	1.54E-34
GO:0007444~imaginal disc development	52	1.27E-35	8.18	1.42E-32	7.08E-34
GO:0048731~system development	81	3.91E-35	3.41	4.35E-32	2.07E-33
GO:0009886~post-embryonic morphogenesis	49	1.06E-34	8.83	1.18E-31	5.13E-33
GO:0002165~instar larval or pupal development	51	4.15E-34	7.93	4.61E-31	1.92E-32
GO:0048707~instar larval or pupal morphogenesis	48	7.66E-34	8.82	8.52E-31	3.41E-32
GO:0007552~metamorphosis	48	4.99E-33	8.47	5.54E-30	2.05E-31
GO:0009791~post-embryonic development	52	8.74E-33	7.17	9.71E-30	3.47E-31
GO:0035220~wing disc development	42	1.86E-29	9.08	2.06E-26	6.44E-28

Annotation Cluster 2 Enrichment Score: 35.63

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007389~pattern specification process	54	5.63E-43	10.49	6.25E-40	1.25E-40
GO:0003002~regionalization	51	1.88E-40	10.65	2.09E-37	2.61E-38
GO:0009880~embryonic pattern specification	31	1.24E-25	13.02	1.38E-22	3.07E-24

Annotation Cluster 3 Enrichment Score: 32.86

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048468~cell development	73	9.59E-38	4.57	1.07E-34	8.20E-36
GO:0030154~cell differentiation	78	3.01E-32	3.36	3.34E-29	1.15E-30
GO:0007399~nervous system development	67	9.19E-31	4.19	1.02E-27	3.30E-29

Annotation Cluster 4 Enrichment Score: 26.84

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060284~regulation of cell development	37	1.45E-33	15.20	1.61E-30	6.18E-32
GO:0050767~regulation of neurogenesis	33	2.98E-31	17.04	3.31E-28	1.10E-29
GO:0051960~regulation of nervous system development	35	1.79E-28	12.36	1.99E-25	5.70E-27
GO:0031344~regulation of cell projection organization	19	5.41E-17	16.15	6.01E-14	8.01E-16

Annotation Cluster 5 Enrichment Score: 26.06

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007166~cell surface receptor signaling pathway	52	4.19E-35	7.99	4.66E-32	2.12E-33
GO:0007165~signal transduction	63	3.77E-29	4.37	4.19E-26	1.27E-27
GO:0010648~negative regulation of cell communication	31	6.37E-24	11.42	7.08E-21	1.39E-22
GO:0009966~regulation of signal transduction	42	1.83E-23	6.38	2.03E-20	3.91E-22
GO:0009968~negative regulation of signal transduction	29	2.72E-22	11.44	3.02E-19	5.30E-21

Annotation Cluster 6 Enrichment Score: 24.71

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0032989~cellular component morphogenesis	52	1.06E-28	5.91	1.18E-25	3.46E-27
GO:0000904~cell morphogenesis involved in differentiation	42	6.61E-28	8.30	7.34E-25	2.04E-26
GO:0006935~chemotaxis	33	3.50E-27	12.79	3.89E-24	9.98E-26
GO:0000902~cell morphogenesis	48	3.92E-27	6.27	4.35E-24	1.09E-25
GO:0048666~neuron development	46	9.92E-27	6.60	1.10E-23	2.62E-25
GO:0097485~neuron projection guidance	31	3.82E-25	12.55	4.24E-22	9.22E-24
GO:0031175~neuron projection development	41	6.42E-25	7.26	7.13E-22	1.52E-23
GO:0048858~cell projection morphogenesis	38	9.75E-21	6.30	1.08E-17	1.69E-19
GO:0032990~cell part morphogenesis	38	1.79E-20	6.19	1.99E-17	3.02E-19

Annotation Cluster 7 Enrichment Score: 21.50

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007292~female gamete generation	47	5.72E-27	6.44	6.36E-24	1.55E-25
GO:0048477~oogenesis	46	4.51E-26	6.37	5.01E-23	1.14E-24
GO:0007281~germ cell development	46	2.28E-23	5.49	2.53E-20	4.69E-22
GO:0022412~cellular process involved in reproduction in multicellular organism	46	4.39E-22	5.11	4.88E-19	8.41E-21
GO:0007276~gamete generation	47	3.56E-21	4.70	3.96E-18	6.60E-20
GO:0019953~sexual reproduction	50	1.31E-20	4.18	1.45E-17	2.23E-19
GO:0002064~epithelial cell development	30	2.66E-19	8.37	2.95E-16	4.22E-18
GO:0030707~ovarian follicle cell development	28	2.99E-18	8.62	3.33E-15	4.56E-17

Annotation Cluster 8 Enrichment Score: 19.928

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045165~cell fate commitment	39	2.45E-26	8.67	2.73E-23	6.34E-25
GO:0001708~cell fate specification	20	8.50E-21	22.55	9.44E-18	1.50E-19
GO:0001709~cell fate	18	7.87E-15	13.72	8.76E-12	1.03E-13

Annotation Cluster 9 Enrichment Score: 19.60

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060541~respiratory system development	32	2.84E-27	13.76	3.15E-24	8.30E-26
GO:0048870~cell motility	33	8.76E-25	10.76	9.73E-22	2.03E-23
GO:0016477~cell migration	31	1.85E-23	11.01	2.06E-20	3.88E-22
GO:0001667~ameboidal-type cell migration	25	3.81E-21	14.02	4.23E-18	6.83E-20
GO:0090132~epithelium migration	23	1.95E-19	14.12	2.17E-16	3.14E-18
GO:0002064~epithelial cell development	30	2.66E-19	8.37	2.95E-16	4.22E-18
GO:0030707~ovarian follicle cell development	28	2.99E-18	8.62	3.33E-15	4.56E-17
GO:0016331~morphogenesis of embryonic epithelium	18	1.01E-15	15.42	1.11E-12	1.37E-14
GO:0007297~ovarian follicle cell migration	17	1.53E-13	12.87	1.70E-10	1.82E-12

Annotation Cluster 10 Enrichment Score: 17.512

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007423~sensory organ development	42	1.16E-27	8.18	1.29E-24	3.48E-26
GO:0001654~eye development	36	2.32E-24	8.84	2.58E-21	5.27E-23
GO:0090596~sensory organ morphogenesis	32	1.26E-22	9.71	1.40E-19	2.50E-21

GO:0042067~establishment of ommatidial planar polarity	18	1.73E-21	32.08	1.93E-18	3.26E-20
GO:0001736~establishment of planar polarity	20	3.64E-21	23.51	4.04E-18	6.63E-20
GO:0007164~establishment of tissue polarity	20	3.64E-21	23.51	4.04E-18	6.63E-20
GO:0016318~ommatidial rotation	12	4.84E-16	45.72	4.93E-13	6.22E-15
GO:0008544~epidermis development	11	3.84E-10	17.87	4.26E-07	3.14E-09
GO:0001737~establishment of imaginal disc-derived wing hair orientation	8	3.73E-09	31.57	4.14E-06	2.71E-08
GO:0035316~non-sensory hair organization	9	1.41E-08	19.50	1.57E-05	9.95E-08

Annotation Cluster 11 Enrichment Score: 16.22

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007507~heart development	18	5.40E-17	18.25	6.00E-14	8.11E-16
GO:0072358~cardiovascular system development	18	6.34E-17	18.08	1.23E-13	1.67E-15
GO:0072359~circulatory system development	18	6.34E-17	18.08	1.23E-13	1.67E-15

Annotation Cluster 12 Enrichment Score: 15.31

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0048645~organ formation	16	5.14E-19	32.14	5.71E-16	7.93E-18
GO:0048859~formation of anatomical boundary	14	1.35E-15	27.62	1.48E-12	1.81E-14
GO:0010160~formation of organ boundary	12	1.67E-13	28.82	1.85E-10	1.95E-12

Annotation Cluster 13 Enrichment Score: 15.30

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048732~gland development	26	3.79E-20	11.77	4.21E-17	6.19E-19
GO:0048565~digestive tract development	18	1.02E-16	17.60	1.23E-13	1.55E-15
GO:0055123~digestive system development	18	1.02E-16	17.60	1.23E-13	1.55E-15
GO:0035272~exocrine system development	21	1.82E-16	12.28	2.47E-13	3.11E-15
GO:0007431~salivary gland development	21	1.82E-16	12.28	2.47E-13	3.11E-15
GO:0022612~gland morphogenesis	14	1.29E-09	9.79	1.44E-06	1.00E-08

Annotation Cluster 14 Enrichment Score: 10.49

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0055123~digestive system development	18	1.02E-16	17.60	1.23E-13	1.55E-15
GO:0048565~digestive tract development	18	1.02E-16	17.60	1.23E-13	1.55E-15
GO:0072002~Malpighian tubule development	14	3.13E-14	22.10	3.48E-11	3.95E-13
GO:0061326~renal tubule development	14	3.13E-14	22.10	3.48E-11	3.95E-13
GO:0072001~renal system development	14	1.63E-13	19.58	1.81E-10	1.93E-12
GO:0001655~urogenital system development	14	1.63E-13	19.58	1.81E-10	1.93E-12
GO:0007422~peripheral nervous system development	14	1.67E-12	16.46	1.86E-09	1.82E-11
GO:0048546~digestive tract morphogenesis	12	3.13E-11	18.41	3.47E-08	2.82E-10
GO:0061525~hindgut development	10	2.95E-09	18.11	3.28E-06	2.17E-08
GO:0007442~hindgut morphogenesis	10	2.95E-09	18.11	3.28E-06	2.17E-08
GO:0035215~genital disc development	9	8.57E-09	20.72	9.52E-06	6.18E-08
GO:0007443~Malpighian tubule morphogenesis	9	1.02E-08	20.29	1.13E-05	7.29E-08
GO:0048619~embryonic hindgut morphogenesis	9	2.26E-08	18.41	2.51E-05	1.56E-07
GO:0035277~spiracle morphogenesis, open tracheal system	6	1.53E-06	28.82	0.001699	8.02E-06
GO:2001013~epithelial cell proliferation involved in renal tubule morphogenesis	4	1.09E-04	40.18	0.113941	4.32E-04

Annotation Cluster 15 Enrichment Score: 9.12

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007267~cell-cell signaling	31	6.47E-14	5.05	7.19E-11	7.99E-13
GO:0198738~cell-cell signaling by wnt	15	1.36E-11	12.19	1.51E-08	1.35E-10
GO:1905114~cell surface receptor signaling pathway involved in cell-cell signaling	15	1.66E-11	12.01	1.85E-08	1.62E-10
GO:0030178~negative regulation of Wnt signaling pathway	7	9.60E-07	20.35	0.001066	5.15E-06
GO:0030111~regulation of Wnt signaling pathway	8	1.86E-05	9.50	0.020468	8.37E-05

Annotation Cluster 16 Enrichment Score: 9.06

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0030036~actin cytoskeleton organization	19	1.46E-11	7.95	1.62E-08	1.43E-10
GO:0007010~cytoskeleton organization	25	1.49E-09	4.26	1.66E-06	1.15E-08
GO:1902589~single-organism organelle organization	27	2.97E-08	3.39	3.30E-05	2.03E-07

Annotation Cluster 17 Enrichment Score: 8.73

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0043067~regulation of programmed cell death	18	7.24E-12	9.08	8.05E-09	7.38E-11
GO:0012501~programmed cell death	22	9.53E-12	6.50	1.06E-08	9.54E-11
GO:0043068~positive regulation of programmed cell death	10	3.84E-08	13.64	4.26E-05	2.57E-07
GO:0010942~positive regulation of cell death	10	8.82E-08	12.41	9.80E-05	5.54E-07
GO:0097190~apoptotic signaling pathway	8	9.10E-08	20.56	1.01E-04	5.68E-07

Annotation Cluster 18 Enrichment Score: 8.57

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0022604~regulation of cell morphogenesis	24	3.29E-19	12.63	3.66E-16	5.15E-18
GO:0031344~regulation of cell projection organization	19	5.41E-17	16.15	6.01E-14	8.01E-16
GO:0060560~developmental growth involved in morphogenesis	15	4.73E-14	18.21	5.25E-11	5.90E-13
GO:0051962~positive regulation of nervous system development	15	1.83E-13	16.57	2.04E-10	2.12E-12
GO:0010769~regulation of cell morphogenesis involved in differentiation	14	8.10E-13	17.38	8.99E-10	9.09E-12
GO:0045597~positive regulation of cell differentiation	14	1.92E-12	16.28	2.14E-09	2.08E-11
GO:0001558~regulation of cell growth	13	2.11E-12	19.15	2.34E-09	2.25E-11
GO:0010720~positive regulation of cell development	13	1.73E-11	16.14	1.92E-08	1.67E-10
GO:0050769~positive regulation of neurogenesis	12	1.93E-11	19.22	2.14E-08	1.85E-10
GO:1990138~neuron projection extension	11	3.50E-11	22.51	3.89E-08	3.14E-10
GO:0050920~regulation of chemotaxis	9	5.88E-11	36.83	6.53E-08	5.14E-10
GO:0032535~regulation of cellular component size	15	9.62E-11	10.56	1.07E-07	8.29E-10
GO:0051272~positive regulation of cellular component movement	8	5.61E-10	40.18	6.24E-07	4.52E-09
GO:0048588~developmental cell growth	11	9.08E-10	16.42	1.01E-06	7.20E-09
GO:0008361~regulation of cell size	12	1.07E-09	13.39	1.19E-06	8.44E-09
GO:1902667~regulation of axon guidance	8	1.12E-09	36.83	1.25E-06	8.78E-09
GO:0030516~regulation of axon extension	8	1.12E-09	36.83	1.25E-06	8.78E-09
GO:0061387~regulation of extent of cell growth	8	1.12E-09	36.83	1.25E-06	8.78E-09
GO:0001737~establishment of imaginal disc-derived wing hair orientation	8	3.73E-09	31.57	4.14E-06	2.71E-08
GO:0050921~positive regulation of chemotaxis	6	1.26E-08	66.29	1.40E-05	8.89E-08
GO:0035316~non-sensory hair organization	9	1.41E-08	19.50	1.57E-05	9.95E-08
GO:0030307~positive regulation of cell growth	8	5.38E-08	22.10	5.98E-05	3.48E-07
GO:0031346~positive regulation of cell projection organization	8	2.68E-07	17.68	2.98E-04	1.58E-06

GO:0048639~positive regulation of developmental growth	10	5.11E-07	10.14	5.68E-04	2.87E-06
GO:1902669~positive regulation of axon guidance	5	7.34E-07	61.38	8.15E-04	4.00E-06
GO:0045773~positive regulation of axon extension	5	1.21E-06	55.24	0.001348	6.39E-06
GO:0032103~positive regulation of response to external stimulus	7	3.08E-06	16.81	0.003411	1.53E-05
GO:0045746~negative regulation of Notch signaling pathway	7	3.97E-06	16.11	0.004402	1.93E-05
GO:0008593~regulation of Notch signaling pathway	9	9.96E-06	8.43	0.011008	4.65E-05
GO:0035151~regulation of tube size, open tracheal system	6	8.77E-05	13.00	0.0928	3.55E-04
GO:0035150~regulation of tube size	6	1.26E-04	12.05	0.130837	4.99E-04
GO:0010770~positive regulation of cell morphogenesis involved in differentiation	5	1.38E-04	18.41	0.142293	5.44E-04
GO:0030010~establishment of cell polarity	5	0.001882	9.36	0.876736	0.006305
GO:0061339~establishment or maintenance of monopolar cell polarity	3	0.008773	20.72	0.999944	0.026111

Annotation Cluster 19 Enrichment Score: 8.37

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045596~negative regulation of cell differentiation	15	2.70E-12	13.70	3.00E-09	2.86E-11
GO:0010454~negative regulation of cell fate commitment	9	1.99E-11	41.43	2.21E-08	1.87E-10
GO:0010721~negative regulation of cell development	11	2.55E-09	14.82	2.83E-06	1.90E-08
GO:0051961~negative regulation of nervous system development	11	6.98E-08	10.57	7.75E-05	4.46E-07
GO:0050768~negative regulation of neurogenesis	9	2.28E-07	13.81	2.53E-04	1.35E-06
GO:0035157~negative regulation of fusion cell fate specification	4	2.76E-06	110.49	0.003065	1.40E-05

Annotation Cluster 20 Enrichment Score: 7.92

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0019219~regulation of nucleobase-containing compound metabolic process	40	8.13E-13	3.42	9.04E-10	9.04E-12
GO:0051252~regulation of RNA metabolic process	39	9.76E-13	3.50	1.08E-09	1.07E-11
GO:0010556~regulation of macromolecule biosynthetic process	39	8.55E-12	3.26	9.50E-09	8.63E-11
GO:0018130~heterocycle biosynthetic process	41	2.23E-11	3.01	2.48E-08	2.09E-10
GO:0019438~aromatic compound biosynthetic process	41	2.70E-11	2.99	3.00E-08	2.50E-10
GO:0031326~regulation of cellular biosynthetic process	39	2.98E-11	3.13	3.31E-08	2.71E-10
GO:0010468~regulation of gene expression	40	3.88E-11	3.03	4.31E-08	3.44E-10

GO:1901362~organic cyclic compound biosynthetic process	41	6.42E-11	2.91	7.13E-08	5.57E-10
GO:0034654~nucleobase-containing compound biosynthetic process	39	1.22E-10	2.99	1.36E-07	1.04E-09
GO:0016070~RNA metabolic process	40	1.11E-07	2.31	1.23E-04	6.87E-07
GO:0090304~nucleic acid metabolic process	41	6.44E-07	2.13	7.15E-04	3.52E-06
GO:0044260~cellular macromolecule metabolic process	60	2.18E-06	1.62	0.002419	1.12E-05
GO:0044271~cellular nitrogen compound biosynthetic process	41	8.41E-06	1.93	0.009304	3.96E-05
GO:0034645~cellular macromolecule biosynthetic process	40	3.05E-05	1.86	0.033269	1.33E-04
GO:0009059~macromolecule biosynthetic process	40	3.37E-05	1.85	0.036737	1.45E-04
GO:0006139~nucleobase-containing compound metabolic process	41	3.52E-05	1.83	0.038379	1.51E-04
GO:0010467~gene expression	42	9.27E-05	1.73	0.09785	3.73E-04
GO:0003677~DNA binding	24	1.39E-04	2.31	0.011996	0.006016

Annotation Cluster 21 Enrichment Score: 7.64

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0010647~positive regulation of cell communication	20	1.06E-10	6.54	1.18E-07	9.05E-10
GO:0009967~positive regulation of signal transduction	19	1.87E-10	6.82	2.08E-07	1.56E-09
GO:1902533~positive regulation of intracellular signal transduction	8	6.07E-04	5.46	0.490873	0.002196

Annotation Cluster 22 Enrichment Score: 7.27

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009798~axis specification	23	6.47E-17	10.81	1.23E-13	1.55E-15
GO:0007309~oocyte axis specification	12	6.60E-08	9.08	7.33E-05	4.24E-07
GO:0007308~oocyte construction	12	8.72E-08	8.84	9.68E-05	5.50E-07
GO:0009994~oocyte differentiation	13	1.51E-07	7.37	1.68E-04	9.15E-07
GO:0030718~germ-line stem cell population maintenance	9	2.28E-07	13.81	2.53E-04	1.35E-06
GO:0048469~cell maturation	13	3.55E-07	6.81	3.94E-04	2.04E-06
GO:0048599~oocyte development	12	3.72E-07	7.66	4.14E-04	2.13E-06
GO:0007314~oocyte anterior/posterior axis specification	8	2.99E-05	8.84	0.032669	1.31E-04
GO:0007310~oocyte dorsal/ventral axis specification	6	7.22E-05	13.53	0.077051	2.95E-04

Annotation Cluster 23 Enrichment Score: 7.12

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010604~positive regulation of macromolecule metabolic process	29	4.32E-13	5.12	4.80E-10	4.95E-12
GO:0031325~positive regulation of cellular metabolic process	28	5.41E-12	4.82	6.01E-09	5.62E-11
GO:0010628~positive regulation of gene expression	18	1.20E-07	4.82	1.33E-04	7.39E-07
GO:0051254~positive regulation of RNA metabolic process	17	1.46E-07	5.09	1.62E-04	8.96E-07
GO:0045935~positive regulation of nucleobase-containing compound metabolic process	17	4.08E-07	4.72	4.53E-04	2.31E-06
GO:0010557~positive regulation of macromolecule biosynthetic process	17	4.08E-07	4.72	4.53E-04	2.31E-06
GO:0031328~positive regulation of cellular biosynthetic process	17	1.74E-06	4.23	0.001931	9.03E-06
GO:0009891~positive regulation of biosynthetic process	17	1.74E-06	4.23	0.001931	9.03E-06
GO:0051173~positive regulation of nitrogen compound metabolic process	17	2.01E-06	4.18	0.002234	1.04E-05
GO:0003677~DNA binding	24	1.39E-04	2.31	0.011996	0.006016

Annotation Cluster 24 Enrichment Score: 6.92

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007548~sex differentiation	14	5.57E-12	15.02	6.19E-09	5.73E-11
GO:0048608~reproductive structure development	11	2.88E-09	14.64	3.20E-06	2.13E-08
GO:0061458~reproductive system development	11	2.88E-09	14.64	3.20E-06	2.13E-08
GO:0008406~gonad development	8	4.62E-07	16.37	5.13E-04	2.60E-06
GO:0045137~development of primary sexual characteristics	8	4.62E-07	16.37	5.13E-04	2.60E-06
GO:0030031~cell projection assembly	10	3.94E-06	7.95	0.004371	1.92E-05
GO:0050919~negative chemotaxis	3	0.009885	19.50	0.999984	0.02931

Annotation Cluster 25 Enrichment Score: 6.72

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051174~regulation of phosphorus metabolic process	16	4.30E-08	6.03	4.78E-05	2.83E-07
GO:0000165~MAPK cascade	11	3.91E-07	8.81	4.34E-04	2.23E-06
GO:0023014~signal transduction by protein phosphorylation	11	3.91E-07	8.81	4.34E-04	2.23E-06

Annotation Cluster 26 Enrichment Score: 6.48

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010453~regulation of cell fate commitment	11	5.77E-13	32.85	6.41E-10	6.54E-12
GO:0010454~negative regulation of cell fate commitment	9	1.99E-11	41.43	2.21E-08	1.87E-10
GO:0035157~negative regulation of fusion cell fate specification	4	2.76E-06	110.49	0.003065	1.40E-05
GO:0022407~regulation of cell-cell adhesion	6	3.57E-06	24.55	0.003957	1.76E-05
GO:0007162~negative regulation of cell adhesion	5	5.63E-06	39.46	0.006237	2.71E-05
GO:0035155~negative regulation of GO Category # and Term inal cell fate specification, open tracheal system	4	1.36E-05	73.66	0.015033	6.23E-05
GO:0022408~negative regulation of cell-cell adhesion	4	1.09E-04	40.18	0.113941	4.32E-04
GO:2000736~regulation of stem cell differentiation	5	1.38E-04	18.41	0.142293	5.44E-04

Annotation Cluster 27 Enrichment Score: 6.27

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0035160~maintenance of epithelial integrity, open tracheal system	6	3.89E-08	55.24	4.33E-05	2.59E-07
GO:0048871~multicellular organismal homeostasis	9	2.04E-07	14.01	2.27E-04	1.23E-06
GO:0001894~tissue homeostasis	8	1.08E-06	14.49	0.001201	5.75E-06
GO:0060249~anatomical structure homeostasis	10	9.61E-06	7.13	0.010624	4.51E-05

Annotation Cluster 28 Enrichment Score: 5.95

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0001763~morphogenesis of a branching structure	10	3.07E-08	13.99	3.41E-05	2.08E-07
GO:0061138~morphogenesis of a branching epithelium	9	3.49E-07	13.08	3.88E-04	2.02E-06
GO:0048754~branching morphogenesis of an epithelial tube	9	3.49E-07	13.08	3.88E-04	2.02E-06
GO:0030097~hemopoiesis	8	1.11E-05	10.28	0.012264	5.14E-05
GO:0035146~tube fusion	5	3.90E-05	25.11	0.042353	1.65E-04

Annotation Cluster 29 Enrichment Score: 5.94

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0043067~regulation of programmed cell death	18	7.24E-12	9.08	8.05E-09	7.38E-11
GO:0097190~apoptotic signaling pathway	8	9.10E-08	20.56	1.01E-04	5.68E-07
GO:0008284~positive regulation of cell proliferation	8	5.96E-07	15.78	6.62E-04	3.28E-06
GO:0043069~negative regulation of programmed cell death	9	3.12E-06	9.85	0.003456	1.55E-05
GO:0045610~regulation of hemocyte differentiation	6	5.18E-06	22.86	0.005734	2.50E-05
GO:0060548~negative regulation of cell death	9	1.06E-05	8.36	0.011711	4.93E-05
GO:2001234~negative regulation of apoptotic signaling pathway	4	5.62E-05	49.11	0.060484	2.34E-04
GO:2001233~regulation of apoptotic signaling pathway	4	7.10E-04	22.10	0.545516	0.002541

Annotation Cluster 30 Enrichment Score: 5.69

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007309~oocyte axis specification	12	6.60E-08	9.08	7.33E-05	4.24E-07
GO:0007308~oocyte construction	12	8.72E-08	8.84	9.68E-05	5.50E-07
GO:0048599~oocyte development	12	3.72E-07	7.66	4.14E-04	2.13E-06
GO:0010927~cellular component assembly involved in morphogenesis	13	7.41E-07	6.36	8.23E-04	4.02E-06
GO:0046843~dorsal appendage formation	7	8.01E-06	14.32	0.008855	3.78E-05
GO:0007306~eggshell chorion assembly	8	1.73E-05	9.61	0.019079	7.83E-05
GO:0007304~chorion-containing eggshell formation	9	3.29E-05	7.15	0.035886	1.42E-04
GO:0030703~eggshell formation	9	3.65E-05	7.05	0.039688	1.56E-04

Annotation Cluster 31 Enrichment Score: 5.486555472626155

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0032268~regulation of cellular protein metabolic process	24	7.27E-10	4.63	8.08E-07	5.81E-09
GO:0051246~regulation of protein metabolic process	24	2.45E-09	4.35	2.72E-06	1.84E-08
GO:0051174~regulation of phosphorus metabolic process	16	4.30E-08	6.03	4.78E-05	2.83E-07
GO:0006796~phosphate-containing compound metabolic process	26	3.93E-06	2.72	0.004354	1.92E-05
GO:0036211~protein modification process	29	1.41E-05	2.34	0.015585	6.44E-05
GO:0043412~macromolecule modification	30	2.07E-05	2.25	0.022698	9.26E-05
GO:0044267~cellular protein metabolic process	34	0.005516	1.55	0.997857	0.017115

GO:0019538~protein metabolic process	36	0.026412	1.37	1	0.071822

Annotation Cluster 32 Enrichment Score: 5.45

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048568~embryonic organ development	11	2.87E-11	22.93	3.19E-08	2.64E-10
GO:0048534~hematopoietic or lymphoid organ development	10	2.65E-07	10.94	2.95E-04	1.57E-06
GO:0002520~immune system development	10	2.65E-07	10.94	2.95E-04	1.57E-06
GO:0035162~embryonic hemopoiesis	6	2.93E-06	25.50	0.003248	1.47E-05
GO:0030097~hemopoiesis	8	1.11E-05	10.28	0.012264	5.14E-05
GO:0035099~hemocyte migration	5	2.11E-05	29.08	0.023135	9.40E-05
GO:0048542~lymph gland development	5	0.002003	9.21	0.892245	0.006668
GO:0009799~specification of symmetry	3	0.007721	22.10	0.999818	0.023128

Annotation Cluster 33 Enrichment Score: 5.28

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010453~regulation of cell fate commitment	11	5.77E-13	32.85	6.41E-10	6.54E-12
GO:0010454~negative regulation of cell fate commitment	9	1.99E-11	41.43	2.21E-08	1.87E-10
GO:0035051~cardiocyte differentiation	6	6.17E-06	22.10	0.006829	2.95E-05
GO:0010092~specification of organ identity	4	2.37E-05	63.14	0.025993	1.05E-04
GO:1905207~regulation of cardiocyte differentiation	4	5.62E-05	49.11	0.060484	2.34E-04
GO:2000736~regulation of stem cell differentiation	5	1.38E-04	18.41	0.142293	5.44E-04
GO:2000737~negative regulation of stem cell differentiation	4	2.36E-04	31.57	0.230283	9.02E-04
GO:2000044~negative regulation of cardiac cell fate specification	3	0.001618	47.35	0.83451	0.005486
GO:1905208~negative regulation of cardiocyte differentiation	3	0.001618	47.35	0.83451	0.005486
GO:0051892~negative regulation of cardioblast differentiation	3	0.001618	47.35	0.83451	0.005486

Annotation Cluster 34 Enrichment Score: 5.04

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060581~cell fate commitment involved in pattern specification	8	1.62E-08	26.00	1.80E-05	1.13E-07
GO:0008052~sensory organ boundary specification	5	5.58E-05	23.02	0.060126	2.33E-04
GO:0060582~cell fate deGO Category # and Term ination involved in pattern specification	4	8.22E-04	21.05	0.599136	0.002916

Annotation Cluster 35 Enrichment Score: 4.59

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0006109~regulation of carbohydrate metabolic process	11	7.18E-06	6.40	0.007943	3.42E-05
GO:0010675~regulation of cellular carbohydrate metabolic process	10	3.94E-05	5.97	0.042882	1.67E-04
GO:0044262~cellular carbohydrate metabolic process	11	5.81E-05	5.02	0.06248	2.41E-04

Annotation Cluster 36 Enrichment Score: 4.56

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0051130~positive regulation of cellular component organization	15	3.76E-08	6.68	4.17E-05	2.53E-07
GO:0031346~positive regulation of cell projection organization	8	2.68E-07	17.68	2.98E-04	1.58E-06
GO:0048639~positive regulation of developmental growth	10	5.11E-07	10.14	5.68E-04	2.87E-06
GO:0051963~regulation of synapse assembly	8	5.53E-05	8.04	0.059564	2.32E-04
GO:0050807~regulation of synapse organization	8	2.51E-04	6.31	0.24342	9.58E-04
GO:0044089~positive regulation of cellular component biogenesis	7	4.23E-04	7.10	0.375151	0.001582
GO:0097581~lamellipodium organization	4	0.001081	19.22	0.699325	0.003748
GO:0010591~regulation of lamellipodium assembly	3	0.008773	20.72	0.999944	0.026111

Annotation Cluster 37 Enrichment Score: 4.34

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0035162~embryonic hemopoiesis	6	2.93E-06	25.50	0.003248	1.47E-05
GO:0030031~cell projection assembly	10	3.94E-06	7.95	0.004371	1.92E-05
GO:0060491~regulation of cell projection assembly	5	2.27E-04	16.25	0.223336	8.74E-04
GO:0051489~regulation of filopodium assembly	4	0.001557	17.00	0.822847	0.005295

Annotation Cluster 38 Enrichment Score: 4.27

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:1903036~positive regulation of response to wounding	6	2.09E-07	41.43	2.32E-04	1.25E-06
GO:1903034~regulation of response to wounding	6	1.21E-06	30.13	0.001339	6.38E-06
GO:0090303~positive regulation of wound healing	5	2.82E-06	46.04	0.003132	1.42E-05

GO:0051017~actin filament bundle assembly	5	1.05E-04	19.73	0.109754	4.20E-04
GO:0000768~syncytium formation by plasma membrane fusion	6	1.06E-04	12.51	0.110694	4.22E-04
GO:0006949~syncytium formation	6	1.06E-04	12.51	0.110694	4.22E-04
GO:0061572~actin filament bundle organization	5	1.79E-04	17.26	0.180184	6.97E-04
GO:0019900~kinase binding	5	0.004108	7.52	0.30099	0.057936
GO:0034331~cell junction maintenance	3	0.005802	25.50	0.998443	0.017897

Annotation Cluster 39 Enrichment Score: 4.25

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051272~positive regulation of cellular component movement	8	5.61E-10	40.18	6.24E-07	4.52E-09
GO:0051129~negative regulation of cellular component organization	8	8.66E-04	5.14	0.618134	0.003061
GO:0030335~positive regulation of cell migration	3	0.004139	30.13	0.990032	0.013155
GO:2000147~positive regulation of cell motility	3	0.004938	27.62	0.995914	0.015461

Annotation Cluster 40 Enrichment Score: 3.86

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0035265~organ growth	8	2.52E-06	12.81	0.002799	1.29E-05
GO:0007398~ectoderm development	6	5.18E-06	22.86	0.005734	2.50E-05
GO:0007440~foregut morphogenesis	3	0.004139	30.13	0.990032	0.013155
GO:0007446~imaginal disc growth	4	0.006199	10.52	0.999001	0.019061

Annotation Cluster 41 Enrichment Score: 3.85

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045185~maintenance of protein location	6	2.34E-05	17.00	0.025644	1.04E-04
GO:0032507~maintenance of protein location in cell	5	2.27E-04	16.25	0.223336	8.74E-04
GO:0051651~maintenance of location in cell	5	5.20E-04	13.15	0.438787	0.001905

Annotation Cluster 42 Enrichment Score: 3.69

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007398~ectoderm development	6	5.18E-06	22.86	0.005734	2.50E-05
GO:0035161~imaginal disc lineage restriction	4	1.09E-04	40.18	0.113941	4.32E-04
GO:0030713~ovarian follicle cell stalk formation	3	0.014913	15.78	1	0.042759

Annotation Cluster 43 Enrichment Score: 3.60

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0008407~chaeta morphogenesis	6	6.53E-05	13.81	0.069943	2.69E-04
GO:0035285~appendage segmentation	5	1.05E-04	19.73	0.109754	4.20E-04
GO:0022416~chaeta development	6	0.002268	6.44	0.919712	0.007478

Annotation Cluster 44 Enrichment Score: 3.39

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007395~dorsal closure, spreading of leading edge cells	4	1.36E-05	73.66	0.015033	6.23E-05
GO:0046664~dorsal closure, amnioserosa morphology change	4	7.97E-05	44.20	0.084745	3.24E-04
GO:0000768~syncytium formation by plasma membrane fusion	6	1.06E-04	12.51	0.110694	4.22E-04
GO:0006949~syncytium formation	6	1.06E-04	12.51	0.110694	4.22E-04
GO:0016818~hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	5	0.897104	0.76	1	0.999343

Annotation Cluster 45 Enrichment Score: 3.28

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0016241~regulation of macroautophagy	6	1.53E-06	28.82	0.001699	8.02E-06
GO:0009267~cellular response to starvation	7	4.89E-04	6.91	0.419454	0.001805
GO:0031669~cellular response to nutrient levels	7	5.38E-04	6.78	0.449867	0.001964
GO:0040014~regulation of multicellular organism growth	6	5.44E-04	8.84	0.453675	0.00198
GO:0031668~cellular response to extracellular stimulus	7	5.90E-04	6.67	0.480798	0.00214
GO:0016242~negative regulation of macroautophagy	3	0.001162	55.24	0.725314	0.004005
GO:0031667~response to nutrient levels	8	0.001955	4.46	0.886332	0.006528
GO:0010507~negative regulation of autophagy	3	0.017761	14.41	1	0.050018

Annotation Cluster 46 Enrichment Score: 3.28

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010605~negative regulation of macromolecule metabolic process	20	1.51E-05	3.09	0.016659	6.86E-05
GO:0031324~negative regulation of cellular metabolic process	18	5.30E-05	3.07	0.057133	2.23E-04
GO:0010629~negative regulation of gene expression	16	1.95E-04	3.03	0.194949	7.58E-04

GO:0051253~negative regulation of RNA metabolic process	12	0.001251	3.16	0.751234	0.004298
GO:0010558~negative regulation of macromolecule biosynthetic process	13	0.00136	2.93	0.77944	0.004655
GO:0031327~negative regulation of cellular biosynthetic process	13	0.001698	2.86	0.848651	0.005723
GO:0009890~negative regulation of biosynthetic process	13	0.001698	2.86	0.848651	0.005723
GO:0045934~negative regulation of nucleobase-containing compound metabolic process	12	0.001816	3.02	0.867239	0.0061
GO:0051172~negative regulation of nitrogen compound metabolic process	13	0.002138	2.78	0.907214	0.007093

Annotation Cluster 47 Enrichment Score: 3.14

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0000768~syncytium formation by plasma membrane fusion	6	1.06E-04	12.51	0.110694	4.22E-04
GO:0006949~syncytium formation	6	1.06E-04	12.51	0.110694	4.22E-04
GO:0000281~mitotic cytokinesis	6	2.79E-04	10.20	0.266804	0.001062
GO:0061640~cytoskeleton-dependent cytokinesis	7	4.23E-04	7.10	0.375151	0.001582
GO:0000910~cytokinesis	7	4.66E-04	6.97	0.404493	0.001732
GO:0060142~regulation of syncytium formation by plasma membrane fusion	3	0.003407	33.15	0.977431	0.010992
GO:0051147~regulation of muscle cell differentiation	3	0.005802	25.50	0.998443	0.017897
GO:0051153~regulation of striated muscle cell differentiation	3	0.005802	25.50	0.998443	0.017897

Annotation Cluster 48 Enrichment Score: 3.02

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007392~initiation of dorsal closure	6	2.94E-07	39.00	3.26E-04	1.72E-06
GO:0045995~regulation of embryonic development	5	0.008242	6.21	0.999898	0.024611
GO:0009826~unidimensional cell growth	3	0.009885	19.50	0.999984	0.02931
GO:0016476~regulation of embryonic cell shape	3	0.03306	10.36	1	0.088516

Annotation Cluster 49 Enrichment Score: 3.02

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0055001~muscle cell development	8	7.62E-07	15.24	8.46E-04	4.11E-06
GO:0030239~myofibril assembly	4	0.006622	10.28	0.999377	0.020238
GO:0070925~organelle assembly	6	0.178085	1.99	1	0.388503

Annotation Cluster 50 Enrichment Score: 2.86

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0007314~oocyte anterior/posterior axis specification	8	2.99E-05	8.84	0.032669	1.31E-04
GO:0048139~female germ-line cyst encapsulation	3	0.005802	25.50	0.998443	0.017897
GO:0048138~germ-line cyst encapsulation	3	0.014913	15.78	1	0.042759

Annotation Cluster 51 Enrichment Score: 2.77

GO Category # and Term	**# of Genes**	***P*-Value**	**Fold Enrichment**	**Bonferroni**	**Benjamini**
GO:0090092~regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	6	2.07E-04	10.87	0.205382	8.01E-04
GO:0090100~positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	4	7.10E-04	22.10	0.545516	0.002541
GO:0071773~cellular response to BMP stimulus	3	0.03306	10.36	1	0.088516

Annotation Cluster 52 Enrichment Score: 2.60

GO Category # and Term	**# of Genes**	***P*-Value**	**Fold Enrichment**	**Bonferroni**	**Benjamini**
GO:0017145~stem cell division	7	2.13E-04	8.06	0.210406	8.20E-04
GO:0042078~germ-line stem cell division	4	0.007061	10.04	0.999619	0.021394
GO:0008354~germ cell migration	4	0.010054	8.84	0.999987	0.029651

Annotation Cluster 53 Enrichment Score: 2.59

GO Category # and Term	**# of Genes**	**PValue**	**Fold Enrichment**	**Bonferroni**	**Benjamini**
GO:0007310~oocyte dorsal/ventral axis specification	6	7.22E-05	13.53	0.077051	2.95E-04
GO:0040023~establishment of nucleus localization	5	2.85E-04	15.35	0.271436	0.00108
GO:0051647~nucleus localization	5	6.78E-04	12.28	0.529247	0.002435
GO:0030952~establishment or maintenance of cytoskeleton polarity	5	0.001013	11.05	0.675581	0.003523
GO:0030722~establishment of oocyte nucleus localization involved in oocyte dorsal/ventral axis specification	3	0.002741	36.83	0.952625	0.008929
GO:0000226~microtubule cytoskeleton organization	9	0.020636	2.61	1	0.057102
GO:0016325~oocyte microtubule cytoskeleton organization	3	0.034991	10.04	1	0.093306
GO:0051656~establishment of organelle localization	6	0.058675	2.83	1	0.149455

Annotation Cluster 54 Enrichment Score: 2.50

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0031324~negative regulation of cellular metabolic process	18	5.30E-05	3.07	0.057133	2.23E-04
GO:0010563~negative regulation of phosphorus metabolic process	5	0.007616	6.35	0.999795	0.02294
GO:0032269~negative regulation of cellular protein metabolic process	7	0.015442	3.44	1	0.044139
GO:0051248~negative regulation of protein metabolic process	7	0.015751	3.42	1	0.044896

Annotation Cluster 55 Enrichment Score: 2.47

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:1903036~positive regulation of response to wounding	6	2.09E-07	41.43	2.32E-04	1.25E-06
GO:1903034~regulation of response to wounding	6	1.21E-06	30.13	0.001339	6.38E-06
GO:0090303~positive regulation of wound healing	5	2.82E-06	46.04	0.003132	1.42E-05
GO:0016773~phosphotransferase activity, alcohol group as acceptor	11	0.003934	2.92	0.29034	0.066294
GO:0016301~kinase activity	11	0.01022	2.54	0.590865	0.1057
GO:0032549~ribonucleoside binding	17	0.046399	1.64	0.98397	0.272359
GO:0035639~purine ribonucleoside triphosphate binding	16	0.081663	1.55	0.999396	0.389884
GO:0001883~purine nucleoside binding	16	0.083465	1.54	0.999491	0.377435
GO:0032555~purine ribonucleotide binding	16	0.084681	1.54	0.999546	0.364171
GO:0017076~purine nucleotide binding	16	0.085294	1.54	0.999572	0.350078
GO:0032553~ribonucleotide binding	16	0.091578	1.52	0.999765	0.355828
GO:0000166~nucleotide binding	17	0.30344	1.22	1	0.730396

Annotation Cluster 56 Enrichment Score: 2.41

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051963~regulation of synapse assembly	8	5.53E-05	8.04	0.059564	2.32E-04
GO:0048640~negative regulation of developmental growth	7	8.88E-05	9.43	0.093929	3.59E-04
GO:0050807~regulation of synapse organization	8	2.51E-04	6.31	0.24342	9.58E-04
GO:0044089~positive regulation of cellular component biogenesis	7	4.23E-04	7.10	0.375151	0.001582
GO:0051124~synaptic growth at neuromuscular junction	7	5.13E-04	6.84	0.434585	0.001886
GO:0008582~regulation of synaptic growth at neuromuscular junction	6	0.002466	6.31	0.935664	0.008084
GO:0007528~neuromuscular junction development	7	0.004776	4.42	0.995104	0.014998

GO:0007416~synapse assembly	7	0.007626	4.01	0.999798	0.022909
GO:1904398~positive regulation of neuromuscular junction development	3	0.029329	11.05	1	0.079165
GO:0045887~positive regulation of synaptic growth at neuromuscular junction	3	0.029329	11.05	1	0.079165
GO:0051965~positive regulation of synapse assembly	3	0.034991	10.04	1	0.093306
GO:0051964~negative regulation of synapse assembly	3	0.061262	7.37	1	0.155011
GO:1904397~negative regulation of neuromuscular junction development	3	0.061262	7.37	1	0.155011
GO:0045886~negative regulation of synaptic growth at neuromuscular junction	3	0.061262	7.37	1	0.155011

Annotation Cluster 57 Enrichment Score: 2.14

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0014070~response to organic cyclic compound	8	6.30E-04	5.42	0.503553	0.002271
GO:0097305~response to alcohol	7	8.74E-04	6.19	0.621597	0.00308
GO:0033993~response to lipid	5	0.002398	8.77	0.930545	0.007883
GO:0036314~response to sterol	4	0.006199	10.52	0.999001	0.019061
GO:1901654~response to ketone	4	0.007061	10.04	0.999619	0.021394
GO:0060033~anatomical structure regression	4	0.085687	3.81	1	0.210982
GO:0035070~salivary gland histolysis	3	0.205731	3.53	1	0.438055

Annotation Cluster 58 Enrichment Score: 2.11

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0034613~cellular protein localization	16	7.50E-06	4.02	0.008293	3.56E-05
GO:0051169~nuclear transport	8	2.51E-04	6.31	0.24342	9.58E-04
GO:0032386~regulation of intracellular transport	6	2.79E-04	10.20	0.266804	0.001062
GO:1903827~regulation of cellular protein localization	6	3.95E-04	9.47	0.355354	0.001482
GO:0017038~protein import	7	8.03E-04	6.29	0.59058	0.002858
GO:0051223~regulation of protein transport	6	9.13E-04	7.89	0.637722	0.003198
GO:0070201~regulation of establishment of protein localization	6	0.001127	7.53	0.714338	0.003896
GO:0045184~establishment of protein localization	12	0.003297	2.80	0.974485	0.010669
GO:0046907~intracellular transport	12	0.005156	2.64	0.996794	0.016091
GO:0006886~intracellular protein transport	9	0.006387	3.22	0.99919	0.019578
GO:1902582~single-organism intracellular transport	7	0.012844	3.58	0.999999	0.037185
GO:0080135~regulation of cellular response to stress	5	0.016415	5.07	1	0.046521
GO:0072594~establishment of protein	6	0.022114	3.70	1	0.060961

localization to organelle					
GO:0046883~regulation of hormone secretion	3	0.022423	12.75	1	0.061644
GO:0009914~hormone transport	3	0.022423	12.75	1	0.061644
GO:0051046~regulation of secretion	4	0.040554	5.20	1	0.106611
GO:0051051~negative regulation of transport	3	0.041039	9.21	1	0.107591
GO:0015031~protein transport	9	0.049712	2.20	1	0.128758
GO:0044765~single-organism transport	20	0.054227	1.52	1	0.138962
GO:0071702~organic substance transport	11	0.13956	1.60	1	0.320006
GO:0023061~signal release	4	0.151088	2.95	1	0.340606
GO:0046903~secretion	5	0.171507	2.28	1	0.377489
GO:0071705~nitrogen compound transport	4	0.324872	1.97	1	0.616821

Annotation Cluster 59 Enrichment Score: 2.08

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0061640~cytoskeleton-dependent cytokinesis	7	4.23E-04	7.10	0.375151	0.001582
GO:0000910~cytokinesis	7	4.66E-04	6.97	0.404493	0.001732
GO:0000912~assembly of actomyosin apparatus involved in cytokinesis	3	0.019264	13.81	1	0.053637
GO:0032506~cytokinetic process	3	0.054197	7.89	1	0.139201
GO:0070925~organelle assembly	6	0.178085	1.99	1	0.388503

Annotation Cluster 60 Enrichment Score: 1.98

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010243~response to organonitrogen compound	6	0.004541	5.48	0.993633	0.014385
GO:1901699~cellular response to nitrogen compound	5	0.007315	6.42	0.999713	0.0221
GO:0043434~response to peptide hormone	4	0.010054	8.84	0.999987	0.029651
GO:1901652~response to peptide	4	0.010615	8.67	0.999993	0.031203
GO:0032870~cellular response to hormone stimulus	5	0.010697	5.75	0.999994	0.03136
GO:0009725~response to hormone	5	0.011082	5.70	0.999996	0.032392
GO:1901701~cellular response to oxygen-containing compound	5	0.017961	4.93	1	0.050445
GO:0071417~cellular response to organonitrogen compound	4	0.017969	7.13	1	0.050341

Annotation Cluster 61 Enrichment Score: 1.95

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0044843~cell cycle G1/S phase transition	6	1.17E-05	19.50	0.012922	5.40E-05
GO:0048872~homeostasis of number of cells	4	0.001081	19.22	0.699325	0.003748
GO:0044772~mitotic cell cycle phase transition	7	0.003478	4.72	0.979157	0.011189
GO:0007346~regulation of mitotic cell cycle	8	0.003991	3.93	0.988235	0.012758
GO:1901987~regulation of cell cycle phase transition	6	0.009903	4.54	0.999984	0.029285
GO:0045787~positive regulation of cell cycle	4	0.011788	8.34	0.999998	0.034343
GO:0010564~regulation of cell cycle process	6	0.036696	3.23	1	0.097476
GO:0045931~positive regulation of mitotic cell cycle	3	0.038982	9.47	1	0.102857
GO:1901988~negative regulation of cell cycle phase transition	4	0.070977	4.13	1	0.177339
GO:0010948~negative regulation of cell cycle process	4	0.096158	3.62	1	0.231771
GO:0045930~negative regulation of mitotic cell cycle	4	0.096158	3.62	1	0.231771
GO:0045786~negative regulation of cell cycle	4	0.159633	2.87	1	0.35541

Annotation Cluster 62 Enrichment Score: 1.797

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0035126~post-embryonic genitalia morphogenesis	3	0.009885	19.50	0.999984	0.02931
GO:0090598~male anatomical structure morphogenesis	3	0.009885	19.50	0.999984	0.02931
GO:0048808~male genitalia morphogenesis	3	0.009885	19.50	0.999984	0.02931
GO:0035112~genitalia morphogenesis	3	0.012285	17.45	0.999999	0.03568
GO:0030539~male genitalia development	3	0.014913	15.78	1	0.042759
GO:0007484~imaginal disc-derived genitalia development	3	0.024078	12.28	1	0.065932
GO:0046661~male sex differentiation	3	0.029329	11.05	1	0.079165
GO:0048806~genitalia development	3	0.03306	10.36	1	0.088516

Annotation Cluster 63 Enrichment Score: 1.79

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0001704~formation of primary germ layer	4	0.004007	12.28	0.988452	0.012774
GO:0001707~mesoderm formation	3	0.029329	11.05	1	0.079165
GO:0048332~mesoderm morphogenesis	3	0.034991	10.04	1	0.093306

Annotation Cluster 64 Enrichment Score: 1.77

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045476~nurse cell apoptotic process	4	7.10E-04	22.10	0.545516	0.002541
GO:0043085~positive regulation of catalytic activity	7	0.004646	4.44	0.994334	0.014672
GO:0070997~neuron death	4	0.004674	11.63	0.994512	0.01472
GO:1901214~regulation of neuron death	4	0.005404	11.05	0.997572	0.016816
GO:0010212~response to ionizing radiation	4	0.005794	10.78	0.998428	0.01792
GO:0071478~cellular response to radiation	4	0.02036	6.80	1	0.056492
GO:1901215~negative regulation of neuron death	3	0.025781	11.84	1	0.070316
GO:0051336~regulation of hydrolase activity	5	0.086751	2.95	1	0.212961
GO:0044389~ubiquitin-like protein ligase binding	3	0.155394	4.23	1	0.487197
GO:0006508~proteolysis	9	0.550775	1.12	1	0.8516

Annotation Cluster 65 Enrichment Score: 1.23

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0043085~positive regulation of catalytic activity	7	0.004646	4.44	0.994334	0.014672
GO:0035006~melanization defense response	3	0.061262	7.37	1	0.155011
GO:0006582~melanin metabolic process	3	0.103445	5.43	1	0.246819
GO:0018958~phenol-containing compound metabolic process	3	0.148148	4.36	1	0.336656
GO:0045087~innate immune response	5	0.149324	2.41	1	0.338368

Annotation Cluster 66 Enrichment Score: 0.45

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0048285~organelle fission	8	0.221875	1.61	1	0.465447
GO:0007067~mitotic nuclear division	5	0.234253	2.00	1	0.484891
GO:0000070~mitotic sister chromatid segregation	3	0.281922	2.83	1	0.558527
GO:0000819~sister chromatid segregation	3	0.334825	2.49	1	0.628855
GO:0098813~nuclear chromosome segregation	3	0.579781	1.53	1	0.871748
GO:0051276~chromosome organization	7	0.679085	1.02	1	0.930723

Annotation Cluster 67 Enrichment Score: 0.37

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048285~organelle fission	8	0.221875	1.61	1	0.465447
GO:0007126~meiotic nuclear division	4	0.50398	1.48	1	0.814754
GO:1903046~meiotic cell cycle process	4	0.521812	1.44	1	0.828414
GO:0051321~meiotic cell cycle	4	0.554308	1.37	1	0.853766

Annotation Cluster 68 Enrichment Score: 0.32

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060179~male mating behavior	3	0.347904	2.42	1	0.644741
GO:0007619~courtship behavior	3	0.377011	2.27	1	0.680329
GO:0007617~mating behavior	3	0.515754	1.73	1	0.824493
GO:0019098~reproductive behavior	3	0.564361	1.58	1	0.860905
GO:0007618~mating	3	0.58984	1.51	1	0.878357

Annotation Cluster 69 Enrichment Score: 0.12

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0019693~ribose phosphate metabolic process	3	0.666093	1.31	1	0.924782
GO:0072521~purine-containing compound metabolic process	3	0.716038	1.19	1	0.947699
GO:0006753~nucleoside phosphate metabolic process	3	0.749729	1.12	1	0.960834
GO:0019637~organophosphate metabolic process	4	0.766001	1.00	1	0.966052
GO:0055086~nucleobase-containing small molecule metabolic process	3	0.822539	0.96	1	0.98172

Annotation Cluster 70 Enrichment Score: 0.11

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0071822~protein complex subunit organization	5	0.676497	1.08	1	0.929799
GO:0006461~protein complex assembly	4	0.763303	1.00	1	0.965379
GO:0065003~macromolecular complex assembly	4	0.924633	0.71	1	0.997354

Annotation Cluster 71 Enrichment Score: 0.058

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0030163~protein catabolic process	3	0.808344	0.99	1	0.978329
GO:0044265~cellular macromolecule catabolic process	3	0.887204	0.81	1	0.993392
GO:0009057~macromolecule catabolic process	3	0.934785	0.69	1	0.998077

Appendix 3.3. Enriched gene ontology categories for candidate climbing speed genes independent of age. Overrepresented gene ontology categories among candidate genes identified in the climbing speed GWA analysis (data combined across both ages). Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8.

Annotation Cluster 1 Enrichment Score: 5.35

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0035295~tube development	9	8.00E-08	9.93	1.83E-05	1.83E-05
GO:0007389~pattern specification process	8	1.48E-07	13.27	3.40E-05	1.70E-05
GO:0007444~imaginal disc development	8	6.28E-07	10.76	1.44E-04	4.79E-05
GO:0035120~post-embryonic appendage morphogenesis	7	9.37E-07	15.23	2.14E-04	5.36E-05
GO:0035114~imaginal disc-derived appendage morphogenesis	7	1.07E-06	14.89	2.45E-04	4.91E-05
GO:0035107~appendage morphogenesis	7	1.11E-06	14.79	2.55E-04	4.25E-05
GO:0048737~imaginal disc-derived appendage development	7	1.14E-06	14.72	2.62E-04	3.74E-05
GO:0048736~appendage development	7	1.22E-06	14.56	2.80E-04	3.49E-05
GO:0048563~post-embryonic organ morphogenesis	7	2.20E-06	13.17	5.04E-04	4.58E-05
GO:0007560~imaginal disc morphogenesis	7	2.20E-06	13.17	5.04E-04	4.58E-05
GO:0009880~embryonic pattern specification	6	2.28E-06	21.54	5.21E-04	4.35E-05
GO:0035220~wing disc development	7	2.44E-06	12.93	5.59E-04	4.00E-05
GO:0003002~regionalization	7	2.99E-06	12.49	6.85E-04	4.57E-05
GO:0060562~epithelial tube morphogenesis	7	5.49E-06	11.26	0.00125642	6.62E-05
GO:0048569~post-embryonic organ development	7	5.60E-06	11.22	0.00128153	6.41E-05
GO:0048707~instar larval or pupal morphogenesis	7	6.30E-06	11.00	0.00144102	6.87E-05
GO:0009886~post-embryonic morphogenesis	7	7.06E-06	10.78	0.00161642	6.74E-05
GO:0035239~tube morphogenesis	7	7.55E-06	10.66	0.00172657	6.91E-05
GO:0007552~metamorphosis	7	7.98E-06	10.56	0.00182582	7.03E-05
GO:0048513~animal organ development	9	8.13E-06	5.49	0.00186117	6.90E-05
GO:0009790~embryo development	7	1.01E-05	10.14	0.00231147	7.98E-05
GO:0002165~instar larval or pupal development	7	1.67E-05	9.30	0.0038124	1.19E-04
GO:0002009~morphogenesis of an epithelium	7	1.81E-05	9.17	0.00413174	1.25E-04
GO:0060429~epithelium development	8	2.01E-05	6.45	0.00458695	1.35E-04
GO:0048729~tissue morphogenesis	7	2.07E-05	8.96	0.00472452	1.35E-04
GO:0009791~post-embryonic development	7	3.31E-05	8.25	0.00755603	2.11E-04
GO:0009887~organ morphogenesis	7	1.15E-04	6.64	0.02591727	6.56E-04
GO:0048731~system development	9	4.44E-04	3.24	0.09672326	0.002117

Annotation Cluster 2 Enrichment Score: 4.64

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051252~regulation of RNA metabolic process	9	1.39E-06	6.90	3.17E-04	3.53E-05
GO:0019219~regulation of nucleobase-containing compound metabolic process	9	1.99E-06	6.58	4.57E-04	4.57E-05

GO:0010556~regulation of macromolecule biosynthetic process	9	2.38E-06	6.43	5.46E-04	4.20E-05
GO:0003002~regionalization	7	2.99E-06	12.49	6.85E-04	4.57E-05
GO:0031326~regulation of cellular biosynthetic process	9	3.27E-06	6.18	7.49E-04	4.68E-05
GO:0034654~nucleobase-containing compound biosynthetic process	9	4.70E-06	5.89	0.00107634	6.33E-05
GO:0010468~regulation of gene expression	9	5.15E-06	5.82	0.00117844	6.55E-05
GO:0018130~heterocycle biosynthetic process	9	6.55E-06	5.65	0.00149773	6.81E-05
GO:0019438~aromatic compound biosynthetic process	9	6.85E-06	5.61	0.00156825	6.82E-05
GO:1901362~organic cyclic compound biosynthetic process	9	8.46E-06	5.46	0.00193658	6.92E-05
GO:0010467~gene expression	10	3.83E-05	3.53	0.00874005	2.37E-04
GO:0016070~RNA metabolic process	9	4.11E-05	4.44	0.00936916	2.48E-04
GO:0090304~nucleic acid metabolic process	9	9.20E-05	4.00	0.02085716	5.40E-04
GO:0044271~cellular nitrogen compound biosynthetic process	9	1.92E-04	3.63	0.04292201	0.00102
GO:0034645~cellular macromolecule biosynthetic process	9	2.11E-04	3.58	0.04726751	0.0011
GO:0009059~macromolecule biosynthetic process	9	2.18E-04	3.56	0.04874454	0.00111
GO:0006139~nucleobase-containing compound metabolic process	9	2.94E-04	3.43	0.0651472	0.001432
GO:0044260~cellular macromolecule metabolic process	9	0.01104	2.08	0.92130065	0.040173

Appendix 3.4. Enriched gene ontology categories for candidate endurance genes at one week of age. Overrepresented gene ontology categories among candidate genes identified in the week one endurance GWA analysis. Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8.

Annotation Cluster 1 Enrichment Score: 5.68

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048666~neuron development	8	8.98E-08	11.99	3.56E-05	3.56E-05
GO:0000902~cell morphogenesis	8	1.73E-07	10.91	6.85E-05	3.42E-05
GO:0032989~cellular component morphogenesis	8	4.53E-07	9.50	1.79E-04	5.98E-05
GO:0000904~cell morphogenesis involved in differentiation	7	6.04E-07	14.45	2.39E-04	5.98E-05
GO:0031175~neuron projection development	7	1.16E-06	12.95	4.59E-04	9.18E-05
GO:0060429~epithelium development	8	1.64E-06	7.88	6.51E-04	1.09E-04
GO:0030154~cell differentiation	9	1.36E-05	4.05	0.005370268	7.69E-04
GO:0048731~system development	9	1.64E-05	3.96	0.006460942	8.10E-04
GO:0048468~cell development	8	2.76E-05	5.23	0.010877558	0.0012145
GO:0007399~nervous system development	8	2.78E-05	5.22	0.010961647	0.0011016

Annotation Cluster 2 Enrichment Score: 3.60

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0000904~cell morphogenesis involved in differentiation	7	6.04E-07	14.45	2.39E-04	5.98E-05
GO:0048858~cell projection morphogenesis	6	5.08E-05	10.40	0.01993506	0.0018289
GO:0032990~cell part morphogenesis	6	5.54E-05	10.21	0.02171683	0.001828
GO:0030707~ovarian follicle cell development	5	8.80E-05	16.07	0.034254433	0.0024865
GO:0048477~oogenesis	6	1.22E-04	8.68	0.047064258	0.0032087
GO:0009791~post-embryonic development	6	1.24E-04	8.64	0.047900218	0.0030631
GO:0007292~female gamete generation	6	1.28E-04	8.59	0.049318207	0.0029706
GO:0002064~epithelial cell development	5	1.29E-04	14.57	0.049800271	0.0028339
GO:0007281~germ cell development	6	2.48E-04	7.48	0.093638357	0.0046708

GO:0022412~cellular process involved in reproduction in multicellular organism	6	3.50E-04	6.96	0.129284209	0.006001
GO:0007276~gamete generation	6	5.72E-04	6.27	0.202693011	0.0093937
GO:0019953~sexual reproduction	6	0.001326	5.24	0.408771415	0.0153386
GO:0001654~eye development	4	0.003844	10.26	0.782386796	0.0348444
GO:0007423~sensory organ development	4	0.007372	8.14	0.946619325	0.0569219
GO:0048732~gland development	3	0.014018	14.19	0.996266805	0.0875732

Appendix 3.5. Enriched gene ontology categories for candidate endurance genes at five weeks of age. Overrepresented gene ontology categories among candidate genes identified in the week five endurance GWA analysis. Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8.

Annotation Cluster 1 Enrichment Score: 3.85

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060429~epithelium development	10	7.59E-06	5.54	0.00368397	0.0012295
GO:0035295~tube development	9	7.85E-06	6.82	0.0038098	9.54E-04
GO:0030154~cell differentiation	13	1.05E-05	3.29	0.00508185	0.00101844
GO:0035114~imaginal disc-derived appendage morphogenesis	7	2.12E-05	10.23	0.01027387	0.00171969
GO:0035107~appendage morphogenesis	7	2.21E-05	10.17	0.01067321	0.00153177
GO:0048737~imaginal disc-derived appendage development	7	2.26E-05	10.12	0.01094632	0.00137489
GO:0048736~appendage development	7	2.41E-05	10.01	0.01165378	0.00130162
GO:0009887~organ morphogenesis	9	2.42E-05	5.87	0.01169988	0.00117619
GO:0007444~imaginal disc development	8	2.49E-05	7.40	0.012038	0.0011004
GO:0002009~morphogenesis of an epithelium	8	2.97E-05	7.20	0.01431248	0.0011083
GO:0048729~tissue morphogenesis	8	3.45E-05	7.04	0.01663732	0.00111786
GO:0007560~imaginal disc morphogenesis	7	4.26E-05	9.05	0.02050139	0.00129382
GO:0048563~post-embryonic organ morphogenesis	7	4.26E-05	9.05	0.02050139	0.00129382
GO:0048513~animal organ development	10	7.57E-05	4.19	0.03610668	0.00204095
GO:0060562~epithelial tube morphogenesis	7	1.02E-04	7.74	0.04858842	0.00248733
GO:0048569~post-embryonic organ development	7	1.04E-04	7.71	0.0494945	0.00241429
GO:0048707~instar larval or pupal morphogenesis	7	1.17E-04	7.56	0.05520831	0.00257807
GO:0048731~system development	12	1.25E-04	2.97	0.05913563	0.00264676
GO:0009886~post-embryonic morphogenesis	7	1.30E-04	7.41	0.06141601	0.00263747
GO:0035239~tube morphogenesis	7	1.39E-04	7.33	0.06527536	0.00269649
GO:0007552~metamorphosis	7	1.46E-04	7.26	0.06872781	0.00273486
GO:0007267~cell-cell signaling	7	2.28E-04	6.70	0.10469104	0.00408742
GO:0002165~instar larval or pupal development	7	2.95E-04	6.39	0.13367355	0.00511166

GO:0009791~post-embryonic development	7	5.64E-04	5.67	0.23974164	0.00880286
GO:0007423~sensory organ development	6	9.05E-04	6.87	0.35600549	0.01214962
GO:0035120~post-embryonic appendage morphogenesis	5	0.002842	7.48	0.74927044	0.02962578
GO:0060541~respiratory system development	4	0.005466	10.10	0.93030007	0.04645008

Annotation Cluster 2 Enrichment Score: 3.51

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009966~regulation of signal transduction	8	3.13E-05	7.14	0.01510027	0.00108623
GO:0009967~positive regulation of signal transduction	5	7.99E-04	10.54	0.32193693	0.01136187
GO:0010647~positive regulation of cell communication	5	0.001131	9.60	0.42314968	0.01475952

Annotation Cluster 3 Enrichment Score: 2.61

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048732~gland development	5	3.31E-04	13.30	0.14853377	0.00552933
GO:0097485~neuron projection guidance	5	5.07E-04	11.89	0.21843861	0.00818173
GO:0006935~chemotaxis	5	5.97E-04	11.39	0.25175265	0.00902223
GO:0000902~cell morphogenesis	7	7.56E-04	5.37	0.30764224	0.01107915
GO:0000904~cell morphogenesis involved in differentiation	6	8.48E-04	6.97	0.33800482	0.01171645
GO:0031175~neuron projection development	6	0.001396	6.24	0.49278698	0.01770518
GO:0032989~cellular component morphogenesis	7	0.001578	4.67	0.53584943	0.01948827
GO:0048858~cell projection morphogenesis	6	0.001869	5.85	0.5970567	0.02140939
GO:0032990~cell part morphogenesis	6	0.002023	5.74	0.62629339	0.02263034
GO:0035272~exocrine system development	4	0.002297	13.74	0.67288305	0.02452617
GO:0007431~salivary gland development	4	0.002297	13.74	0.67288305	0.02452617
GO:0048666~neuron development	6	0.003545	5.06	0.82202646	0.03393331
GO:0060541~respiratory system development	4	0.005466	10.10	0.93030007	0.04645008

Appendix 3.6. Enriched gene ontology categories for candidate endurance genes independent of age. Overrepresented gene ontology categories among candidate genes identified in the endurance GWA analysis (data combined across both ages). Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8.

Annotation Cluster 1 Enrichment Score: 18.35

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009887~organ morphogenesis	33	1.08E-23	7.65	9.13E-21	9.13E-21
GO:0007423~sensory organ development	24	3.80E-18	9.77	3.20E-15	1.28E-16
GO:0001654~eye development	22	1.14E-17	11.28	9.59E-15	3.09E-16
GO:0090596~sensory organ morphogenesis	20	8.49E-17	12.68	9.36E-14	2.66E-15

Annotation Cluster 2 Enrichment Score: 18.00

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0009887~organ morphogenesis	33	1.08E-23	7.65	9.13E-21	9.13E-21
GO:0060429~epithelium development	34	7.45E-23	6.70	6.28E-20	3.14E-20
GO:0002009~morphogenesis of an epithelium	28	7.00E-21	8.96	5.90E-18	1.47E-18
GO:0048729~tissue morphogenesis	28	1.29E-20	8.76	1.09E-17	2.17E-18
GO:0048513~animal organ development	35	2.92E-20	5.22	2.46E-17	3.52E-18
GO:0009886~post-embryonic morphogenesis	26	5.48E-20	9.79	4.62E-17	5.77E-18
GO:0007560~imaginal disc morphogenesis	24	2.50E-19	11.03	2.11E-16	1.62E-17
GO:0048563~post-embryonic organ morphogenesis	24	2.50E-19	11.03	2.11E-16	1.62E-17
GO:0048569~post-embryonic organ development	25	4.48E-19	9.80	3.78E-16	2.52E-17
GO:0035295~tube development	28	6.13E-19	7.55	5.17E-16	3.23E-17
GO:0048707~instar larval or pupal morphogenesis	25	7.17E-19	9.60	6.04E-16	3.55E-17
GO:0048731~system development	40	1.01E-18	3.52	8.53E-16	4.49E-17
GO:0035239~tube morphogenesis	25	1.48E-18	9.31	1.24E-15	6.22E-17
GO:0007552~metamorphosis	25	1.85E-18	9.22	1.56E-15	7.41E-17
GO:0002165~instar larval or pupal development	26	1.98E-18	8.44	1.67E-15	7.57E-17

GO:0009791~post-embryonic development	27	2.03E-18	7.78	1.71E-15	7.42E-17
GO:0060562~epithelial tube morphogenesis	24	8.21E-18	9.44	6.92E-15	2.56E-16
GO:0035114~imaginal disc-derived appendage morphogenesis	22	8.65E-18	11.44	7.29E-15	2.60E-16
GO:0035107~appendage morphogenesis	22	9.92E-18	11.36	8.37E-15	2.88E-16
GO:0048737~imaginal disc-derived appendage development	22	1.09E-17	11.31	9.17E-15	3.06E-16
GO:0048736~appendage development	22	1.36E-17	11.18	1.15E-14	3.59E-16
GO:0007444~imaginal disc development	25	2.59E-17	8.22	2.19E-14	6.62E-16
GO:0035120~post-embryonic appendage morphogenesis	20	2.29E-15	10.64	1.97E-12	4.92E-14
GO:0035220~wing disc development	18	8.90E-12	8.13	7.50E-09	1.23E-10

Annotation Cluster 3 Enrichment Score: 15.88

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048477~oogenesis	28	9.92E-20	8.10	8.36E-17	9.29E-18
GO:0048468~cell development	36	1.18E-19	4.71	9.98E-17	9.98E-18
GO:0007292~female gamete generation	28	1.29E-19	8.02	1.08E-16	9.86E-18
GO:0002064~epithelial cell development	22	8.24E-19	12.82	6.95E-16	3.86E-17
GO:0030707~ovarian follicle cell development	21	2.79E-18	13.50	2.35E-15	9.80E-17
GO:0007281~germ cell development	28	4.66E-18	6.98	3.93E-15	1.51E-16
GO:0022412~cellular process involved in reproduction in multicellular organism	28	2.94E-17	6.49	2.48E-14	7.30E-16
GO:0007276~gamete generation	28	4.14E-16	5.85	3.74E-13	1.04E-14
GO:0019953~sexual reproduction	28	4.05E-14	4.89	3.42E-11	7.43E-13
GO:0019538~protein metabolic process	25	1.78E-04	1.99	0.1392772	7.81E-04

Annotation Cluster 4 Enrichment Score: 12.67

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060284~regulation of cell development	20	2.89E-19	17.16	2.44E-16	1.74E-17
GO:0051960~regulation of nervous system development	17	6.15E-14	12.54	5.18E-11	1.08E-12
GO:0050767~regulation of neurogenesis	15	1.08E-13	16.18	9.13E-11	1.82E-12
GO:0031344~regulation of cell projection organization	8	1.07E-06	14.20	8.99E-04	8.10E-06

Annotation Cluster 5 Enrichment Score: 11.17

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007399~nervous system development	33	4.48E-16	4.31	3.74E-13	1.01E-14
GO:0000902~cell morphogenesis	25	1.92E-15	6.82	1.59E-12	4.07E-14
GO:0032989~cellular component morphogenesis	26	3.50E-15	6.17	2.90E-12	6.91E-14
GO:0000904~cell morphogenesis involved in differentiation	21	1.57E-14	8.67	1.32E-11	2.93E-13
GO:0048666~neuron development	22	5.63E-13	6.59	4.75E-10	8.63E-12
GO:0031175~neuron projection development	19	1.93E-11	7.03	1.63E-08	2.63E-10
GO:0048858~cell projection morphogenesis	18	5.74E-10	6.24	4.84E-07	6.81E-09
GO:0032990~cell part morphogenesis	18	7.56E-10	6.13	6.38E-07	8.86E-09
GO:0097485~neuron projection guidance	13	8.52E-10	10.99	7.19E-07	9.84E-09
GO:0006935~chemotaxis	13	1.39E-09	10.53	1.18E-06	1.57E-08
GO:0048813~dendrite morphogenesis	9	4.14E-06	9.15	0.0034836	2.66E-05

Annotation Cluster 6 Enrichment Score: 9.52

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007389~pattern specification process	19	4.11E-12	7.71	3.47E-09	5.98E-11
GO:0035220~wing disc development	18	8.90E-12	8.13	7.50E-09	1.23E-10
GO:0003002~regionalization	17	1.81E-10	7.42	1.53E-07	2.25E-09
GO:0009880~embryonic pattern specification	10	1.22E-06	8.78	0.0010267	9.17E-06

Annotation Cluster 7 Enrichment Score: 9.38

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0002064~epithelial cell development	22	8.24E-19	12.82	6.95E-16	3.86E-17
GO:0030707~ovarian follicle cell development	21	2.79E-18	13.50	2.35E-15	9.80E-17
GO:0016477~cell migration	18	2.67E-15	13.36	2.25E-12	5.47E-14
GO:0048870~cell motility	18	1.12E-14	12.25	9.45E-12	2.20E-13
GO:0048598~embryonic morphogenesis	16	9.04E-14	14.04	7.62E-11	1.55E-12
GO:0090132~epithelium migration	14	2.68E-13	17.95	2.26E-10	4.26E-12
GO:0007297~ovarian follicle cell migration	13	5.41E-13	20.55	4.56E-10	8.45E-12
GO:0001667~ameboidal-type cell migration	14	8.58E-13	16.40	7.23E-10	1.29E-11
GO:0009790~embryo development	19	4.01E-11	6.73	3.38E-08	5.37E-10
GO:0016331~morphogenesis of embryonic epithelium	11	1.07E-10	19.68	8.99E-08	1.38E-09
GO:0000165~MAPK cascade	9	9.39E-08	15.05	7.91E-05	8.24E-07

GO:0023014~signal transduction by protein phosphorylation	9	9.39E-08	15.05	7.91E-05	8.24E-07
GO:1902531~regulation of intracellular signal transduction	12	1.02E-07	8.24	8.57E-05	8.84E-07
GO:0051174~regulation of phosphorus metabolic process	11	2.91E-07	8.66	2.45E-04	2.40E-06
GO:0006796~phosphate-containing compound metabolic process	17	3.13E-06	3.71	0.0026364	2.08E-05
GO:0036211~protein modification process	19	4.36E-06	3.21	0.0036682	2.76E-05
GO:0043412~macromolecule modification	19	1.29E-05	2.97	0.0108315	7.16E-05
GO:0044267~cellular protein metabolic process	24	2.99E-05	2.28	0.024903	1.54E-04
GO:0019538~protein metabolic process	25	1.78E-04	1.99	0.1392772	7.81E-04
GO:0000166~nucleotide binding	13	0.025165	1.92	0.8362718	0.1136439

Annotation Cluster 8 Enrichment Score: 8.66

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007166~cell surface receptor signaling pathway	21	1.79E-12	6.74	1.51E-09	2.64E-11
GO:0007164~establishment of tissue polarity	11	4.35E-12	27.01	3.67E-09	6.22E-11
GO:0001736~establishment of planar polarity	11	4.35E-12	27.01	3.67E-09	6.22E-11
GO:0042067~establishment of ommatidial planar polarity	9	1.51E-10	33.50	1.28E-07	1.91E-09
GO:0010648~negative regulation of cell communication	13	2.50E-09	10.00	2.11E-06	2.70E-08
GO:0009968~negative regulation of signal transduction	12	1.56E-08	9.89	1.32E-05	1.52E-07
GO:0016318~ommatidial rotation	6	1.19E-07	47.75	1.00E-04	1.02E-06
GO:0010454~negative regulation of cell fate commitment	5	2.80E-06	48.08	0.0023567	1.87E-05
GO:0010453~regulation of cell fate commitment	5	1.67E-05	31.19	0.0139901	9.15E-05

Annotation Cluster 9 Enrichment Score: 7.811

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010604~positive regulation of macromolecule metabolic process	21	1.34E-13	7.74	1.13E-10	2.21E-12
GO:0031325~positive regulation of cellular metabolic process	21	2.15E-13	7.55	1.81E-10	3.49E-12
GO:0032270~positive regulation of cellular protein metabolic process	12	2.39E-09	11.84	2.02E-06	2.62E-08
GO:0051247~positive regulation of protein metabolic process	12	3.88E-09	11.30	3.27E-06	4.09E-08
GO:0032268~regulation of cellular protein metabolic process	16	5.76E-09	6.44	4.86E-06	5.92E-08
GO:0051246~regulation of protein metabolic process	16	1.35E-08	6.05	1.13E-05	1.32E-07
GO:0051174~regulation of phosphorus	11	2.91E-07	8.66	2.45E-04	2.40E-06

metabolic process					
GO:0051338~regulation of transferase activity	7	4.78E-06	15.39	0.0040214	2.98E-05
GO:0043085~positive regulation of catalytic activity	7	8.44E-05	9.29	0.0687092	3.91E-04
GO:0010562~positive regulation of phosphorus metabolic process	6	3.15E-04	9.75	0.233152	0.0013133

Annotation Cluster 10 Enrichment Score: 7.05

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048732~gland development	13	2.34E-10	12.30	1.97E-07	2.86E-09
GO:0060541~respiratory system development	13	4.26E-10	11.67	3.59E-07	5.13E-09
GO:0035272~exocrine system development	9	1.05E-06	10.99	8.84E-04	8.04E-06
GO:0007431~salivary gland development	9	1.05E-06	10.99	8.84E-04	8.04E-06
GO:0022612~gland morphogenesis	7	4.93E-05	10.23	0.0407408	2.39E-04

Annotation Cluster 11 Enrichment Score: 7.01

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045596~negative regulation of cell differentiation	13	5.53E-14	24.80	4.66E-11	9.92E-13
GO:0010721~negative regulation of cell development	10	4.31E-11	28.15	3.63E-08	5.67E-10
GO:0043067~regulation of programmed cell death	12	1.19E-09	12.65	1.00E-06	1.35E-08
GO:2000027~regulation of organ morphogenesis	9	4.51E-09	22.10	3.81E-06	4.70E-08
GO:0035214~eye-antennal disc development	8	1.32E-08	26.76	1.11E-05	1.31E-07
GO:0012501~programmed cell death	13	2.97E-08	8.02	2.50E-05	2.84E-07
GO:0010623~programmed cell death involved in cell development	9	2.67E-07	13.15	2.25E-04	2.25E-06
GO:0097190~apoptotic signaling pathway	6	9.22E-07	32.20	7.77E-04	7.13E-06
GO:0046668~regulation of retinal cell programmed cell death	5	1.29E-06	57.70	0.0010886	9.55E-06
GO:0043069~negative regulation of programmed cell death	7	3.81E-06	16.00	0.0032083	2.49E-05
GO:0046673~negative regulation of compound eye retinal cell programmed cell death	4	3.91E-06	115.40	0.0032947	2.54E-05
GO:0046671~negative regulation of retinal cell programmed cell death	4	3.91E-06	115.40	0.0032947	2.54E-05
GO:0060548~negative regulation of cell death	7	9.86E-06	13.58	0.0082795	5.66E-05
GO:0043068~positive regulation of programmed cell death	6	2.18E-05	17.10	0.0182048	1.16E-04
GO:0010942~positive regulation of cell death	6	3.44E-05	15.56	0.0286102	1.75E-04

Annotation Cluster 12 Enrichment Score: 6.57

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009967~positive regulation of signal transduction	13	3.37E-09	9.74	2.84E-06	3.59E-08
GO:0010647~positive regulation of cell communication	13	9.60E-09	8.88	8.09E-06	9.75E-08
GO:1902533~positive regulation of intracellular signal transduction	6	5.77E-04	8.55	0.3850794	0.0023019

Annotation Cluster 13 Enrichment Score: 5.66

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007164~establishment of tissue polarity	11	4.35E-12	27.01	3.67E-09	6.22E-11
GO:0001736~establishment of planar polarity	11	4.35E-12	27.01	3.67E-09	6.22E-11
GO:0198738~cell-cell signaling by wnt	6	2.58E-04	10.18	0.1952986	0.0011024
GO:1905114~cell surface receptor signaling pathway involved in cell-cell signaling	6	2.76E-04	10.03	0.2074774	0.001162
GO:0030111~regulation of Wnt signaling pathway	4	0.007061	9.93	0.9974538	0.021642
GO:0030178~negative regulation of Wnt signaling pathway	3	0.011195	18.22	0.9999244	0.0328641

Annotation Cluster 14 Enrichment Score: 5.02

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0001708~cell fate specification	11	6.67E-12	25.91	5.62E-09	9.36E-11
GO:0007422~peripheral nervous system development	10	1.51E-10	24.55	1.27E-07	1.93E-09
GO:0042067~establishment of ommatidial planar polarity	9	1.51E-10	33.50	1.28E-07	1.91E-09
GO:0035218~leg disc development	9	1.09E-08	19.78	9.21E-06	1.10E-07
GO:0072001~renal system development	8	3.44E-08	23.37	2.90E-05	3.18E-07
GO:0001655~urogenital system development	8	3.44E-08	23.37	2.90E-05	3.18E-07
GO:0055123~digestive system development	8	4.11E-07	16.34	3.47E-04	3.37E-06
GO:0048565~digestive tract development	8	4.11E-07	16.34	3.47E-04	3.37E-06
GO:0072002~Malpighian tubule development	7	4.35E-07	23.08	3.66E-04	3.52E-06
GO:0061326~renal tubule development	7	4.35E-07	23.08	3.66E-04	3.52E-06
GO:0048546~digestive tract morphogenesis	7	5.15E-07	22.44	4.34E-04	4.13E-06
GO:0008284~positive regulation of cell proliferation	6	3.51E-06	24.73	0.0029579	2.31E-05
GO:0007548~sex differentiation	7	4.27E-06	15.69	0.0035962	2.73E-05
GO:0061525~hindgut development	6	5.39E-06	22.70	0.0045315	3.24E-05
GO:0007442~hindgut morphogenesis	6	5.39E-06	22.70	0.0045315	3.24E-05

GO:2001013~epithelial cell proliferation involved in renal tubule morphogenesis	4	1.14E-05	83.93	0.0095912	6.47E-05
GO:0035215~genital disc development	5	4.76E-05	24.04	0.0393313	2.32E-04
GO:0007443~Malpighian tubule morphogenesis	5	5.17E-05	23.55	0.0426231	2.49E-04
GO:0048619~embryonic hindgut morphogenesis	5	7.59E-05	21.37	0.0620168	3.54E-04
GO:0042692~muscle cell differentiation	6	1.67E-04	11.17	0.1316109	7.46E-04
GO:0048608~reproductive structure development	5	4.04E-04	13.90	0.2884556	0.0016587
GO:0061458~reproductive system development	5	4.04E-04	13.90	0.2884556	0.0016587
GO:0060249~anatomical structure homeostasis	6	4.71E-04	8.93	0.3279235	0.0019087
GO:2001234~negative regulation of apoptotic signaling pathway	3	6.20E-04	76.93	0.4069944	0.0024618
GO:0016337~single organismal cell-cell adhesion	5	7.87E-04	11.66	0.4851226	0.0030828
GO:0007447~imaginal disc pattern formation	5	8.80E-04	11.31	0.5241018	0.0034318
GO:0007445~deGO Category # and Term ination of imaginal disc primordium	3	9.42E-04	62.95	0.5480481	0.0036034
GO:2001233~regulation of apoptotic signaling pathway	3	0.003175	34.62	0.9314976	0.0104992
GO:0007517~muscle organ development	5	0.00371	7.64	0.9564453	0.0121665
GO:0009798~axis specification	5	0.017089	4.91	0.9999995	0.0490366

Annotation Cluster 15 Enrichment Score: 4.80

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045610~regulation of hemocyte differentiation	7	1.79E-09	55.71	1.51E-06	1.99E-08
GO:0050890~cognition	8	7.18E-06	10.67	0.0060361	4.23E-05
GO:0048139~female germ-line cyst encapsulation	3	0.001328	53.26	0.6738694	0.0048179
GO:0048138~germ-line cyst encapsulation	3	0.0035	32.97	0.9479534	0.0115237

Annotation Cluster 16 Enrichment Score: 4.69

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010604~positive regulation of macromolecule metabolic process	21	1.34E-13	7.74	1.13E-10	2.21E-12
GO:0031325~positive regulation of cellular metabolic process	21	2.15E-13	7.55	1.81E-10	3.49E-12
GO:0010557~positive regulation of macromolecule biosynthetic process	12	5.59E-07	6.96	4.71E-04	4.44E-06
GO:0010628~positive regulation of gene expression	12	8.06E-07	6.71	6.79E-04	6.35E-06
GO:0031328~positive regulation of cellular biosynthetic process	12	1.64E-06	6.24	0.0013851	1.19E-05
GO:0009891~positive regulation of	12	1.64E-06	6.24	0.0013851	1.19E-05

biosynthetic process					
GO:0051173~positive regulation of nitrogen compound metabolic process	12	1.83E-06	6.17	0.0015453	1.31E-05
GO:0019219~regulation of nucleobase-containing compound metabolic process	19	1.89E-06	3.40	0.0015956	1.34E-05
GO:0051254~positive regulation of RNA metabolic process	11	2.39E-06	6.88	0.0020135	1.65E-05
GO:0010468~regulation of gene expression	20	2.40E-06	3.16	0.0020181	1.64E-05
GO:0010556~regulation of macromolecule biosynthetic process	19	2.65E-06	3.32	0.0022328	1.80E-05
GO:0045935~positive regulation of nucleobase-containing compound metabolic process	11	4.71E-06	6.38	0.003959	2.96E-05
GO:0031326~regulation of cellular biosynthetic process	19	4.79E-06	3.19	0.004032	2.97E-05
GO:0051252~regulation of RNA metabolic process	18	4.85E-06	3.37	0.0040825	2.99E-05
GO:0010605~negative regulation of macromolecule metabolic process	14	5.01E-06	4.51	0.004214	3.06E-05
GO:0034654~nucleobase-containing compound biosynthetic process	19	9.38E-06	3.04	0.0078724	5.41E-05
GO:0018130~heterocycle biosynthetic process	19	1.71E-05	2.91	0.0143531	9.27E-05
GO:0019438~aromatic compound biosynthetic process	19	1.86E-05	2.90	0.0155936	1.00E-04
GO:0010629~negative regulation of gene expression	12	2.26E-05	4.74	0.0189011	1.19E-04
GO:1901362~organic cyclic compound biosynthetic process	19	2.73E-05	2.82	0.0227448	1.41E-04
GO:0031324~negative regulation of cellular metabolic process	12	5.90E-05	4.27	0.0485606	2.83E-04
GO:0051253~negative regulation of RNA metabolic process	9	3.24E-04	4.96	0.2389591	0.0013443
GO:0045934~negative regulation of nucleobase-containing compound metabolic process	9	4.43E-04	4.73	0.3117062	0.0018117
GO:0010558~negative regulation of macromolecule biosynthetic process	9	9.15E-04	4.24	0.5378163	0.003518
GO:0009890~negative regulation of biosynthetic process	9	0.001085	4.13	0.5994437	0.0040943
GO:0031327~negative regulation of cellular biosynthetic process	9	0.001085	4.13	0.5994437	0.0040943
GO:0051172~negative regulation of nitrogen compound metabolic process	9	0.001295	4.02	0.6645189	0.0047374
GO:0016070~RNA metabolic process	18	0.001309	2.17	0.6684548	0.0047678
GO:0090304~nucleic acid metabolic process	19	0.001569	2.06	0.7338934	0.0055939
GO:0044271~cellular nitrogen compound biosynthetic process	19	0.004848	1.87	0.9833704	0.0155731
GO:0034645~cellular macromolecule biosynthetic process	19	0.005614	1.85	0.9913119	0.0175524
GO:0009059~macromolecule biosynthetic process	19	0.005882	1.84	0.9930772	0.0182497
GO:0003677~DNA binding	12	0.00747	2.39	0.4127732	0.0732294
GO:0010467~gene expression	20	0.008932	1.72	0.9994808	0.0268391
GO:0006139~nucleobase-containing compound metabolic process	19	0.009084	1.77	0.9995439	0.0271004

Annotation Cluster 17 Enrichment Score: 4.50

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0008407~chaeta morphogenesis	6	1.62E-06	28.85	0.0013607	1.18E-05
GO:0022416~chaeta development	6	6.95E-05	13.44	0.0569107	3.25E-04
GO:0035051~cardiocyte differentiation	4	2.66E-04	30.77	0.2008461	0.001126

Annotation Cluster 18 Enrichment Score: 4.37

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045610~regulation of hemocyte differentiation	7	1.79E-09	55.71	1.51E-06	1.99E-08
GO:0048872~homeostasis of number of cells	6	3.43E-08	60.21	2.89E-05	3.22E-07
GO:0072001~renal system development	8	3.44E-08	23.37	2.90E-05	3.18E-07
GO:0001655~urogenital system development	8	3.44E-08	23.37	2.90E-05	3.18E-07
GO:0044843~cell cycle G1/S phase transition	6	2.74E-07	40.73	2.31E-04	2.29E-06
GO:0072002~Malpighian tubule development	7	4.35E-07	23.08	3.66E-04	3.52E-06
GO:0061326~renal tubule development	7	4.35E-07	23.08	3.66E-04	3.52E-06
GO:0001709~cell fate deGO Category # and Term ination	8	2.22E-06	12.73	0.0018734	1.55E-05
GO:0008284~positive regulation of cell proliferation	6	3.51E-06	24.73	0.0029579	2.31E-05
GO:0044772~mitotic cell cycle phase transition	8	5.05E-06	11.26	0.0042466	3.06E-05
GO:2001234~negative regulation of apoptotic signaling pathway	3	6.20E-04	76.93	0.4069944	0.0024618
GO:2001233~regulation of apoptotic signaling pathway	3	0.003175	34.62	0.9314976	0.0104992
GO:0009798~axis specification	5	0.017089	4.91	0.9999995	0.0490366
GO:0009994~oocyte differentiation	4	0.049208	4.73	1	0.1301803
GO:0007309~oocyte axis specification	3	0.126845	4.74	1	0.2989062
GO:0007308~oocyte construction	3	0.132537	4.62	1	0.3100125
GO:0048599~oocyte development	3	0.166303	4.00	1	0.375207
GO:0048469~cell maturation	3	0.22477	3.28	1	0.4771247

Annotation Cluster 19 Enrichment Score: 4.359

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0016331~morphogenesis of embryonic epithelium	11	1.07E-10	19.68	8.99E-08	1.38E-09
GO:0045610~regulation of hemocyte differentiation	7	1.79E-09	55.71	1.51E-06	1.99E-08
GO:0048646~anatomical structure formation involved in morphogenesis	16	4.03E-08	5.58	3.40E-05	3.65E-07

GO:0000165~MAPK cascade	9	9.39E-08	15.05	7.91E-05	8.24E-07
GO:0023014~signal transduction by protein phosphorylation	9	9.39E-08	15.05	7.91E-05	8.24E-07
GO:0022604~regulation of cell morphogenesis	10	1.83E-07	10.99	1.54E-04	1.56E-06
GO:0051174~regulation of phosphorus metabolic process	11	2.91E-07	8.66	2.45E-04	2.40E-06
GO:0031344~regulation of cell projection organization	8	1.07E-06	14.20	8.99E-04	8.10E-06
GO:0030036~actin cytoskeleton organization	10	1.26E-06	8.74	0.0010597	9.38E-06
GO:0030031~cell projection assembly	8	1.68E-06	13.28	0.0014114	1.21E-05
GO:0060560~developmental growth involved in morphogenesis	7	2.07E-06	17.75	0.0017426	1.45E-05
GO:0048568~embryonic organ development	6	2.66E-06	26.13	0.0022439	1.80E-05
GO:0051130~positive regulation of cellular component organization	9	7.95E-06	8.38	0.0066822	4.62E-05
GO:0060491~regulation of cell projection assembly	5	1.18E-05	33.94	0.0099352	6.66E-05
GO:0031098~stress-activated protein kinase signaling cascade	6	1.70E-05	17.98	0.014244	9.26E-05
GO:0007304~chorion-containing eggshell formation	7	2.40E-05	11.62	0.0199986	1.25E-04
GO:0030703~eggshell formation	7	2.60E-05	11.46	0.0216673	1.35E-04
GO:0042060~wound healing	6	3.63E-05	15.39	0.0301742	1.83E-04
GO:0007306~eggshell chorion assembly	6	4.04E-05	15.05	0.0334955	2.00E-04
GO:1990138~neuron projection extension	5	7.59E-05	21.37	0.0620168	3.54E-04
GO:0046843~dorsal appendage formation	5	7.59E-05	21.37	0.0620168	3.54E-04
GO:0097581~lamellipodium organization	4	1.18E-04	40.14	0.0950149	5.42E-04
GO:0035162~embryonic hemopoiesis	4	1.72E-04	35.51	0.135219	7.64E-04
GO:0051489~regulation of filopodium assembly	4	1.72E-04	35.51	0.135219	7.64E-04
GO:0008544~epidermis development	5	1.87E-04	16.97	0.1460845	8.18E-04
GO:0019900~kinase binding	5	2.56E-04	15.56	0.018007	0.018007
GO:0048588~developmental cell growth	5	2.60E-04	15.59	0.1967211	0.0011057
GO:0007010~cytoskeleton organization	11	3.02E-04	3.91	0.2248383	0.0012663
GO:0010927~cellular component assembly involved in morphogenesis	7	3.51E-04	7.15	0.2562755	0.0014503
GO:0030097~hemopoiesis	5	4.62E-04	13.42	0.3227215	0.0018807
GO:0010769~regulation of cell morphogenesis involved in differentiation	5	5.26E-04	12.97	0.3584969	0.0021118
GO:0030381~chorion-containing eggshell pattern formation	3	9.42E-04	62.95	0.5480481	0.0036034
GO:0090303~positive regulation of wound healing	3	0.001127	57.70	0.6134677	0.0042345
GO:0031346~positive regulation of cell projection organization	4	0.00121	18.46	0.639684	0.0044867
GO:0035316~non-sensory hair organization	4	0.001282	18.10	0.6608857	0.0047112
GO:1903036~positive regulation of response to wounding	3	0.002027	43.28	0.8192276	0.0070433
GO:1902589~single-organism organelle organization	11	0.003147	2.89	0.9298312	0.0104464

GO:1903034~regulation of response to wounding	3	0.00384	31.47	0.9609537	0.0125394
GO:0001737~establishment of imaginal disc-derived wing hair orientation	3	0.006183	24.73	0.9946364	0.0191071
GO:0080135~regulation of cellular response to stress	4	0.0109	8.47	0.9999028	0.032119
GO:0070302~regulation of stress-activated protein kinase signaling cascade	3	0.023373	12.36	1	0.0657829
GO:0032535~regulation of cellular component size	4	0.028561	5.88	1	0.0792129
GO:0032956~regulation of actin cytoskeleton organization	3	0.042995	8.88	1	0.1154456

Annotation Cluster 20 Enrichment Score: 4.24

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0042067~establishment of ommatidial planar polarity	9	1.51E-10	33.50	1.28E-07	1.91E-09
GO:0010454~negative regulation of cell fate commitment	5	2.80E-06	48.08	0.0023567	1.87E-05
GO:0050768~negative regulation of neurogenesis	6	1.22E-05	19.23	0.0102668	6.83E-05
GO:0010453~regulation of cell fate commitment	5	1.67E-05	31.19	0.0139901	9.15E-05
GO:0035215~genital disc development	5	4.76E-05	24.04	0.0393313	2.32E-04
GO:0051961~negative regulation of nervous system development	6	1.17E-04	12.04	0.0942029	5.41E-04
GO:2000736~regulation of stem cell differentiation	4	2.66E-04	30.77	0.2008461	0.001126
GO:0016337~single organismal cell-cell adhesion	5	7.87E-04	11.66	0.4851226	0.0030828
GO:0022408~negative regulation of cell-cell adhesion	3	9.42E-04	62.95	0.5480481	0.0036034
GO:2000737~negative regulation of stem cell differentiation	3	0.001545	49.46	0.7285093	0.0055328
GO:0007162~negative regulation of cell adhesion	3	0.001545	49.46	0.7285093	0.0055328
GO:0022407~regulation of cell-cell adhesion	3	0.005756	25.64	0.9923011	0.0179291

Annotation Cluster 21 Enrichment Score: 4.06

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048871~multicellular organismal homeostasis	6	1.14E-05	19.50	0.0095871	6.51E-05
GO:0001894~tissue homeostasis	5	1.23E-04	18.92	0.0982308	5.56E-04
GO:0060249~anatomical structure homeostasis	6	4.71E-04	8.93	0.3279235	0.0019087

Annotation Cluster 22 Enrichment Score: 3.98

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0000278~mitotic cell cycle	15	3.26E-08	6.26	2.75E-05	3.09E-07
GO:0048872~homeostasis of number of cells	6	3.43E-08	60.21	2.89E-05	3.22E-07
GO:0044843~cell cycle G1/S phase transition	6	2.74E-07	40.73	2.31E-04	2.29E-06
GO:0044772~mitotic cell cycle phase transition	8	5.05E-06	11.26	0.0042466	3.06E-05
GO:0022402~cell cycle process	15	5.46E-06	4.11	0.0045904	3.26E-05
GO:0010564~regulation of cell cycle process	8	2.17E-05	9.01	0.0181472	1.16E-04
GO:1901987~regulation of cell cycle phase transition	7	3.16E-05	11.07	0.0263246	1.62E-04
GO:0007346~regulation of mitotic cell cycle	8	3.95E-05	8.21	0.032756	1.97E-04
GO:0010948~negative regulation of cell cycle process	5	0.001711	9.46	0.7640207	0.0060237
GO:0045930~negative regulation of mitotic cell cycle	5	0.001711	9.46	0.7640207	0.0060237
GO:0045786~negative regulation of cell cycle	5	0.003981	7.49	0.9653484	0.012948
GO:1901988~negative regulation of cell cycle phase transition	4	0.010366	8.63	0.9998468	0.0306684
GO:0045787~positive regulation of cell cycle	3	0.021082	13.06	1	0.0600598
GO:0010563~negative regulation of phosphorus metabolic process	3	0.052303	7.96	1	0.1375644
GO:0044839~cell cycle G2/M phase transition	3	0.062261	7.21	1	0.1608571

Annotation Cluster 23 Enrichment Score: 3.90

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0043153~entrainment of circadian clock by photoperiod	4	5.85E-06	102.58	0.0049234	3.48E-05
GO:0009649~entrainment of circadian clock	4	4.63E-05	54.31	0.0382678	2.28E-04
GO:0071478~cellular response to radiation	5	1.57E-04	17.75	0.1241015	7.05E-04
GO:0009416~response to light stimulus	5	0.005419	6.87	0.9897546	0.0170741

Annotation Cluster 24 Enrichment Score: 3.87

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060541~respiratory system development	13	4.26E-10	11.67	3.59E-07	5.13E-09
GO:0001763~morphogenesis of a branching structure	7	8.96E-07	20.45	7.55E-04	6.99E-06
GO:0048568~embryonic organ development	6	2.66E-06	26.13	0.0022439	1.80E-05
GO:0048754~branching morphogenesis of an epithelial tube	6	1.60E-05	18.22	0.0133668	8.80E-05
GO:0061138~morphogenesis of a branching epithelium	6	1.60E-05	18.22	0.0133668	8.80E-05

GO:0031098~stress-activated protein kinase signaling cascade	6	1.70E-05	17.98	0.014244	9.26E-05
GO:0002520~immune system development	6	6.33E-05	13.71	0.0519519	3.00E-04
GO:0048534~hematopoietic or lymphoid organ development	6	6.33E-05	13.71	0.0519519	3.00E-04
GO:0035162~embryonic hemopoiesis	4	1.72E-04	35.51	0.135219	7.64E-04
GO:0051489~regulation of filopodium assembly	4	1.72E-04	35.51	0.135219	7.64E-04
GO:0008544~epidermis development	5	1.87E-04	16.97	0.1460845	8.18E-04
GO:0030097~hemopoiesis	5	4.62E-04	13.42	0.3227215	0.0018807
GO:0046664~dorsal closure, amnioserosa morphology change	3	7.73E-04	69.24	0.4787269	0.0030397
GO:0035316~non-sensory hair organization	4	0.001282	18.10	0.6608857	0.0047112
GO:0035099~hemocyte migration	3	0.002865	36.44	0.9109819	0.0095908
GO:0048542~lymph gland development	3	0.02658	11.54	1	0.0740945
GO:0007293~germarium-derived egg chamber formation	3	0.100729	5.45	1	0.2453145
GO:0016818~hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	4	0.607765	1.26	1	0.9639348

Annotation Cluster 25 Enrichment Score: 3.71

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009880~embryonic pattern specification	10	1.22E-06	8.78	0.0010267	9.17E-06
GO:0010454~negative regulation of cell fate commitment	5	2.80E-06	48.08	0.0023567	1.87E-05
GO:0010453~regulation of cell fate commitment	5	1.67E-05	31.19	0.0139901	9.15E-05
GO:2000736~regulation of stem cell differentiation	4	2.66E-04	30.77	0.2008461	0.001126
GO:0035051~cardiocyte differentiation	4	2.66E-04	30.77	0.2008461	0.001126
GO:1905207~regulation of cardiocyte differentiation	3	6.20E-04	76.93	0.4069944	0.0024618
GO:0007507~heart development	5	0.001128	10.59	0.6138575	0.0042202
GO:0072359~circulatory system development	5	0.001167	10.49	0.6263552	0.0043465
GO:0072358~cardiovascular system development	5	0.001167	10.49	0.6263552	0.0043465
GO:0009798~axis specification	5	0.017089	4.91	0.9999995	0.0490366

Annotation Cluster 26 Enrichment Score: 3.58

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045597~positive regulation of cell differentiation	6	4.72E-05	14.58	0.0389849	2.31E-04
GO:0051962~positive regulation of nervous system development	6	6.03E-05	13.85	0.0495959	2.87E-04

GO:0010720~positive regulation of cell development	5	5.26E-04	12.97	0.3584969	0.0021118
GO:0050769~positive regulation of neurogenesis	4	0.003059	13.38	0.9244226	0.0101961

Annotation Cluster 27 Enrichment Score: 3.58

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0001709~cell fate deGO Category # and Term ination	8	2.22E-06	12.73	0.0018734	1.55E-05
GO:0007447~imaginal disc pattern formation	5	8.80E-04	11.31	0.5241018	0.0034318
GO:0060581~cell fate commitment involved in pattern specification	3	0.009029	20.36	0.9995222	0.0270343

Annotation Cluster 28 Enrichment Score: 3.50

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051338~regulation of transferase activity	7	4.78E-06	15.39	0.0040214	2.98E-05
GO:0042060~wound healing	6	3.63E-05	15.39	0.0301742	1.83E-04
GO:0043085~positive regulation of catalytic activity	7	8.44E-05	9.29	0.0687092	3.91E-04
GO:0010562~positive regulation of phosphorus metabolic process	6	3.15E-04	9.75	0.233152	0.0013133
GO:0035006~melanization defense response	4	8.89E-04	20.52	0.5275762	0.0034339
GO:0006582~melanin metabolic process	4	0.002151	15.13	0.8372351	0.0074431
GO:0045087~innate immune response	6	0.002689	6.05	0.8966368	0.0091095
GO:0018958~phenol-containing compound metabolic process	4	0.004021	12.15	0.9665022	0.0130274

Annotation Cluster 29 Enrichment Score: 3.34

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010605~negative regulation of macromolecule metabolic process	14	5.01E-06	4.51	0.004214	3.06E-05
GO:0031324~negative regulation of cellular metabolic process	12	5.90E-05	4.27	0.0485606	2.83E-04
GO:0032269~negative regulation of cellular protein metabolic process	6	0.002491	6.15	0.8778331	0.0085444
GO:0051248~negative regulation of protein metabolic process	6	0.002539	6.13	0.8827302	0.0086747
GO:0008356~asymmetric cell division	4	0.009594	8.88	0.9997045	0.028507

Annotation Cluster 30 Enrichment Score: 3.34

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010675~regulation of cellular carbohydrate metabolic process	7	1.18E-04	8.73	0.0950263	5.40E-04
GO:0006109~regulation of carbohydrate metabolic process	7	1.37E-04	8.50	0.1091654	6.18E-04
GO:0044262~cellular carbohydrate metabolic process	7	5.06E-04	6.68	0.3470879	0.0020377
GO:0071310~cellular response to organic substance	6	0.004937	5.25	0.9845797	0.0157978

Annotation Cluster 31 Enrichment Score: 3.02

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0006796~phosphate-containing compound metabolic process	17	3.13E-06	3.71	0.0026364	2.08E-05
GO:0036211~protein modification process	19	4.36E-06	3.21	0.0036682	2.76E-05
GO:0043412~macromolecule modification	19	1.29E-05	2.97	0.0108315	7.16E-05
GO:0044267~cellular protein metabolic process	24	2.99E-05	2.28	0.024903	1.54E-04
GO:0007369~gastrulation	6	3.83E-05	15.22	0.0318021	1.92E-04
GO:0019538~protein metabolic process	25	1.78E-04	1.99	0.1392772	7.81E-04
GO:0016773~phosphotransferase activity, alcohol group as acceptor	8	0.001757	4.39	0.1173539	0.0605075
GO:0016301~kinase activity	8	0.003848	3.82	0.2394447	0.066138
GO:0035639~purine ribonucleoside triphosphate binding	11	0.019872	2.20	0.7595229	0.1328238
GO:0032549~ribonucleoside binding	11	0.020292	2.19	0.7667198	0.123939
GO:0001883~purine nucleoside binding	11	0.020292	2.19	0.7667198	0.123939
GO:0032555~purine ribonucleotide binding	11	0.020575	2.19	0.7714599	0.1157396
GO:0017076~purine nucleotide binding	11	0.020717	2.19	0.7738124	0.1080433
GO:0032553~ribonucleotide binding	11	0.022186	2.16	0.7966687	0.1075456
GO:0000166~nucleotide binding	13	0.025165	1.92	0.8362718	0.1136439

Annotation Cluster 32 Enrichment Score: 2.89

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0031344~regulation of cell projection organization	8	1.07E-06	14.20	8.99E-04	8.10E-06
GO:0051130~positive regulation of cellular component organization	9	7.95E-06	8.38	0.0066822	4.62E-05
GO:0010769~regulation of cell morphogenesis involved in differentiation	5	5.26E-04	12.97	0.3584969	0.0021118
GO:0008582~regulation of synaptic growth at neuromuscular junction	5	9.81E-04	10.99	0.5629289	0.0037381

GO:0044089~positive regulation of cellular component biogenesis	5	0.001128	10.59	0.6138575	0.0042202
GO:0051963~regulation of synapse assembly	5	0.001167	10.49	0.6263552	0.0043465
GO:0031346~positive regulation of cell projection organization	4	0.00121	18.46	0.639684	0.0044867
GO:0007416~synapse assembly	6	0.001269	7.18	0.6570931	0.0046833
GO:0016773~phosphotransferase activity, alcohol group as acceptor	8	0.001757	4.39	0.1173539	0.0605075
GO:0050807~regulation of synapse organization	5	0.002827	8.24	0.90803	0.0094998
GO:0016301~kinase activity	8	0.003848	3.82	0.2394447	0.066138
GO:0007528~neuromuscular junction development	5	0.006255	6.59	0.9949541	0.0192577
GO:0051124~synaptic growth at neuromuscular junction	3	0.082632	6.13	1	0.2072823
GO:0099536~synaptic signaling	3	0.367515	2.29	1	0.6831538

Annotation Cluster 33 Enrichment Score: 2.77

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0050890~cognition	8	7.18E-06	10.67	0.0060361	4.23E-05
GO:0097305~response to alcohol	6	1.74E-04	11.08	0.1363055	7.67E-04
GO:0048149~behavioral response to ethanol	4	0.00177	16.20	0.7753409	0.0062022
GO:0007612~learning	3	0.058873	7.45	1	0.1530166
GO:0042048~olfactory behavior	3	0.100729	5.45	1	0.2453145

Appendix 3.7. Enriched gene ontology categories for candidate climbing speed and endurance genes independent of age. Overrepresented gene ontology categories among candidate genes identified in the climbing speed and endurance GWA analysis (data combined across both ages). Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8

Annotation Cluster 1 Enrichment Score: 15.43

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009887~organ morphogenesis	25	3.92E-21	8.99	2.28E-18	2.28E-18
GO:0048736~appendage development	20	4.84E-20	15.78	2.81E-17	1.40E-17
GO:0048513~animal organ development	27	7.38E-20	6.25	4.28E-17	1.43E-17
GO:0035295~tube development	23	2.52E-19	9.62	1.46E-16	3.65E-17
GO:0035114~imaginal disc-derived appendage morphogenesis	19	1.40E-18	15.33	8.14E-16	1.63E-16
GO:0035107~appendage morphogenesis	19	1.58E-18	15.22	9.19E-16	1.53E-16
GO:0048737~imaginal disc-derived appendage development	19	1.72E-18	15.16	9.95E-16	1.42E-16
GO:0007444~imaginal disc development	20	1.74E-16	10.20	1.29E-13	1.61E-14
GO:0060562~epithelial tube morphogenesis	19	2.01E-16	11.59	1.29E-13	1.43E-14
GO:0060429~epithelium development	23	2.19E-16	7.03	1.29E-13	1.29E-14
GO:0048707~instar larval or pupal morphogenesis	19	3.05E-16	11.32	1.93E-13	1.75E-14
GO:0048563~post-embryonic organ morphogenesis	18	4.19E-16	12.84	2.58E-13	2.14E-14
GO:0007560~imaginal disc morphogenesis	18	4.19E-16	12.84	2.58E-13	2.14E-14
GO:0009886~post-embryonic morphogenesis	19	4.33E-16	11.10	2.58E-13	1.98E-14
GO:0035239~tube morphogenesis	19	5.29E-16	10.98	3.22E-13	2.30E-14
GO:0007552~metamorphosis	19	6.27E-16	10.87	3.86E-13	2.58E-14
GO:0035120~post-embryonic appendage morphogenesis	17	1.23E-15	14.03	7.08E-13	4.43E-14
GO:0002165~instar larval or pupal development	19	6.07E-15	9.57	3.54E-12	2.08E-13
GO:0048569~post-embryonic organ development	18	6.21E-15	10.94	3.61E-12	2.00E-13
GO:0002009~morphogenesis of an epithelium	19	7.80E-15	9.44	4.51E-12	2.37E-13
GO:0048729~tissue morphogenesis	19	1.17E-14	9.22	6.76E-12	3.38E-13
GO:0009791~post-embryonic development	19	4.88E-14	8.50	2.83E-11	1.35E-12
GO:0048731~system development	27	5.68E-14	3.69	3.30E-11	1.50E-12
GO:0035220~wing disc development	16	4.06E-13	11.21	2.35E-10	1.02E-11
GO:0007165~signal transduction	16	2.98E-06	3.59	0.00172437	3.08E-05

Annotation Cluster 2 Enrichment Score: 9.58

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0035120~post-embryonic appendage morphogenesis	17	1.23E-15	14.03	7.08E-13	4.43E-14
GO:0009966~regulation of signal transduction	14	1.22E-08	6.90	7.08E-06	1.65E-07
GO:0007166~cell surface receptor signaling pathway	12	1.17E-06	5.98	6.79E-04	1.33E-05

Annotation Cluster 3 Enrichment Score: 8.59

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0000904~cell morphogenesis involved in differentiation	14	4.92E-10	8.97	2.85E-07	1.10E-08
GO:0097485~neuron projection guidance	11	1.15E-09	14.43	6.70E-07	2.31E-08
GO:0006935~chemotaxis	11	1.75E-09	13.82	1.02E-06	3.39E-08
GO:0031175~neuron projection development	14	1.90E-09	8.04	1.10E-06	3.56E-08
GO:0048666~neuron development	15	2.03E-09	6.98	1.18E-06	3.69E-08
GO:0030154~cell differentiation	23	3.01E-09	3.22	1.74E-06	5.29E-08
GO:0048468~cell development	20	3.16E-09	4.06	1.83E-06	5.39E-08
GO:0007399~nervous system development	20	3.22E-09	4.05	1.87E-06	5.34E-08
GO:0032989~cellular component morphogenesis	16	3.88E-09	5.90	2.25E-06	6.08E-08
GO:0048858~cell projection morphogenesis	14	4.22E-09	7.53	2.45E-06	6.44E-08
GO:0032990~cell part morphogenesis	14	5.24E-09	7.40	3.04E-06	7.79E-08
GO:0000902~cell morphogenesis	15	6.93E-09	6.35	4.02E-06	1.01E-07

Annotation Cluster 4 Enrichment Score: 8.32

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0007423~sensory organ development	14	5.87E-10	8.84	3.40E-07	1.26E-08
GO:0001654~eye development	12	9.85E-09	9.55	5.71E-06	1.36E-07
GO:0090596~sensory organ morphogenesis	11	1.85E-08	10.82	1.07E-05	2.43E-07

Annotation Cluster 5 Enrichment Score: 4.92

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048732~gland development	9	1.71E-07	13.21	9.94E-05	2.11E-06
GO:0007431~salivary gland development	7	9.05E-06	13.26	0.00523748	8.61E-05
GO:0035272~exocrine system development	7	9.05E-06	13.26	0.00523748	8.61E-05
GO:0022612~gland morphogenesis	6	5.66E-05	13.60	0.03231783	4.44E-04
GO:0048813~dendrite morphogenesis	6	3.11E-04	9.47	0.1650826	0.0020027

Annotation Cluster 6 Enrichment Score: 4.32

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0060284~regulation of cell development	10	2.06E-08	13.31	1.19E-05	2.65E-07
GO:0050767~regulation of neurogenesis	9	6.22E-08	15.06	3.60E-05	7.84E-07
GO:0051960~regulation of nervous system	9	1.14E-06	10.30	6.63E-04	1.33E-05

development					
GO:0009968~negative regulation of signal transduction	8	6.99E-06	10.23	0.00404782	6.76E-05
GO:0010648~negative regulation of cell communication	8	1.10E-05	9.55	0.00635375	1.03E-04
GO:0031344~regulation of cell projection organization	6	2.22E-05	16.53	0.01278497	1.92E-04
GO:0010769~regulation of cell morphogenesis involved in differentiation	4	0.001707	16.10	0.62868093	0.0078942
GO:0060560~developmental growth involved in morphogenesis	4	0.001819	15.74	0.65217109	0.0082808
GO:0022604~regulation of cell morphogenesis	5	0.002272	8.53	0.73272777	0.0097986
GO:1990138~neuron projection extension	3	0.009198	19.90	0.99529796	0.0351009
GO:0048588~developmental cell growth	3	0.016796	14.52	0.99994589	0.0588431

Annotation Cluster 7 Enrichment Score: 4.21

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009966~regulation of signal transduction	14	1.22E-08	6.90	7.08E-06	1.65E-07
GO:0009967~positive regulation of signal transduction	7	1.41E-04	8.14	0.07830443	0.0010448
GO:1902531~regulation of intracellular signal transduction	7	2.26E-04	7.46	0.1228399	0.0016168
GO:0010647~positive regulation of cell communication	7	2.33E-04	7.42	0.12659282	0.0016294
GO:1902533~positive regulation of intracellular signal transduction	4	0.009167	8.84	0.99521199	0.0352149

Annotation Cluster 8 Enrichment Score: 4.06

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045165~cell fate commitment	11	3.46E-07	7.93	2.01E-04	4.18E-06
GO:0045168~cell-cell signaling involved in cell fate commitment	6	2.27E-04	10.14	0.12312282	0.001601
GO:0007267~cell-cell signaling	7	0.008271	3.70	0.99190817	0.032237

Annotation Cluster 9 Enrichment Score: 3.86

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010604~positive regulation of macromolecule metabolic process	11	2.87E-06	6.29	0.00166227	3.02E-05
GO:0031325~positive regulation of cellular metabolic process	11	3.60E-06	6.14	0.00208694	3.60E-05
GO:0034654~nucleobase-containing compound biosynthetic process	14	3.30E-05	3.48	0.01897733	2.82E-04
GO:0018130~heterocycle biosynthetic process	14	5.24E-05	3.33	0.02995813	4.28E-04

GO:0019438~aromatic compound biosynthetic process	14	5.59E-05	3.31	0.03190804	4.44E-04
GO:1901362~organic cyclic compound biosynthetic process	14	7.49E-05	3.22	0.04252686	5.72E-04
GO:0051254~positive regulation of RNA metabolic process	7	3.74E-04	6.79	0.19526548	0.0023585
GO:0045935~positive regulation of nucleobase-containing compound metabolic process	7	5.61E-04	6.30	0.27761809	0.0033129
GO:0010557~positive regulation of macromolecule biosynthetic process	7	5.61E-04	6.30	0.27761809	0.0033129
GO:0010628~positive regulation of gene expression	7	6.82E-04	6.07	0.32664826	0.0036893
GO:0031328~positive regulation of cellular biosynthetic process	7	9.96E-04	5.65	0.43902653	0.0049287
GO:0009891~positive regulation of biosynthetic process	7	9.96E-04	5.65	0.43902653	0.0049287
GO:0051173~positive regulation of nitrogen compound metabolic process	7	0.001056	5.58	0.45817322	0.0051798

Annotation Cluster 10 Enrichment Score: 3.54

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051252~regulation of RNA metabolic process	15	8.12E-07	4.36	4.71E-04	9.62E-06
GO:0019219~regulation of nucleobase-containing compound metabolic process	15	1.44E-06	4.16	8.37E-04	1.58E-05
GO:0010556~regulation of macromolecule biosynthetic process	15	1.91E-06	4.07	0.00110856	2.05E-05
GO:0031326~regulation of cellular biosynthetic process	15	3.14E-06	3.90	0.00181946	3.19E-05
GO:0010468~regulation of gene expression	15	6.36E-06	3.68	0.00367993	6.25E-05
GO:0034654~nucleobase-containing compound biosynthetic process	14	3.30E-05	3.48	0.01897733	2.82E-04
GO:0018130~heterocycle biosynthetic process	14	5.24E-05	3.33	0.02995813	4.28E-04
GO:0019438~aromatic compound biosynthetic process	14	5.59E-05	3.31	0.03190804	4.44E-04
GO:1901362~organic cyclic compound biosynthetic process	14	7.49E-05	3.22	0.04252686	5.72E-04
GO:0010629~negative regulation of gene expression	9	1.09E-04	5.52	0.06101423	8.17E-04
GO:0016070~RNA metabolic process	15	1.46E-04	2.81	0.0814474	0.0010748
GO:0010558~negative regulation of macromolecule biosynthetic process	8	2.48E-04	5.85	0.13416196	0.0016934
GO:0009890~negative regulation of biosynthetic process	8	2.92E-04	5.70	0.15564625	0.0019207
GO:0031327~negative regulation of cellular biosynthetic process	8	2.92E-04	5.70	0.15564625	0.0019207
GO:0051172~negative regulation of nitrogen compound metabolic process	8	3.45E-04	5.54	0.18135231	0.0021965
GO:0010605~negative regulation of macromolecule metabolic process	9	4.42E-04	4.50	0.22603306	0.0026655
GO:0090304~nucleic acid metabolic process	15	4.70E-04	2.53	0.23884185	0.0028096
GO:0051253~negative regulation of RNA metabolic process	7	7.35E-04	5.98	0.34734866	0.0039071

GO:0045934~negative regulation of nucleobase-containing compound metabolic process	7	9.39E-04	5.71	0.42010175	0.0047684
GO:0044271~cellular nitrogen compound biosynthetic process	15	0.001318	2.29	0.53457843	0.0063532
GO:0031324~negative regulation of cellular metabolic process	8	0.00133	4.42	0.53794646	0.0063605
GO:0034645~cellular macromolecule biosynthetic process	15	0.00151	2.26	0.58374322	0.0071583
GO:0010467~gene expression	16	0.00155	2.14	0.59341878	0.0072901
GO:0009059~macromolecule biosynthetic process	15	0.001577	2.25	0.59961833	0.0073546
GO:0006139~nucleobase-containing compound metabolic process	15	0.00237	2.17	0.74747059	0.0101425
GO:0044260~cellular macromolecule metabolic process	19	0.006695	1.66	0.97967496	0.0268763
GO:0007447~imaginal disc pattern formation	3	0.030585	10.53	0.99999999	0.093759
GO:0006325~chromatin organization	5	0.054379	3.33	1	0.1561575
GO:0051276~chromosome organization	5	0.142665	2.37	1	0.3586414

Annotation Cluster 11 Enrichment Score: 3.51

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0009880~embryonic pattern specification	7	5.87E-05	9.53	0.03347482	4.54E-04
GO:0007379~segment specification	4	2.63E-04	30.48	0.14141164	0.0017509
GO:0035287~head segmentation	3	0.001865	44.77	0.66128829	0.0084222

Annotation Cluster 12 Enrichment Score: 3.50

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048859~formation of anatomical boundary	5	1.41E-05	31.98	0.00814242	1.28E-04
GO:0010160~formation of organ boundary	4	2.47E-04	31.14	0.13323759	0.0017008
GO:0048646~anatomical structure formation involved in morphogenesis	9	2.59E-04	4.87	0.13947801	0.0017452
GO:0048645~organ formation	4	4.19E-04	26.05	0.21585916	0.0025564
GO:0016477~cell migration	6	0.0013	6.91	0.52965133	0.0063185
GO:0048870~cell motility	6	0.001903	6.34	0.66880027	0.0085296

Annotation Cluster 13 Enrichment Score: 3.00

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0031344~regulation of cell projection organization	6	2.22E-05	16.53	0.01278497	1.92E-04
GO:0042067~establishment of ommatidial planar polarity	4	5.97E-04	23.11	0.29261489	0.0034217

GO:0008544~epidermis development	4	7.82E-04	21.07	0.3648143	0.0041173
GO:0007164~establishment of tissue polarity	4	0.001996	15.24	0.68621448	0.0088761
GO:0001736~establishment of planar polarity	4	0.001996	15.24	0.68621448	0.0088761
GO:0001737~establishment of imaginal disc-derived wing hair orientation	3	0.002537	38.37	0.77084402	0.0106968
GO:0035316~non-sensory hair organization	3	0.008237	21.07	0.99174526	0.032322

Annotation Cluster 14 Enrichment Score: 2.96

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0008347~glial cell migration	5	1.26E-06	57.76	7.33E-04	1.41E-05
GO:0055123~digestive system development	6	1.12E-05	19.02	0.0064906	1.03E-04
GO:0048565~digestive tract development	6	1.12E-05	19.02	0.0064906	1.03E-04
GO:0072002~Malpighian tubule development	5	3.43E-05	25.58	0.01969133	2.88E-04
GO:0061326~renal tubule development	5	3.43E-05	25.58	0.01969133	2.88E-04
GO:0048546~digestive tract morphogenesis	5	3.83E-05	24.87	0.02198709	3.18E-04
GO:0072001~renal system development	5	5.53E-05	22.67	0.03154344	4.45E-04
GO:0001655~urogenital system development	5	5.53E-05	22.67	0.03154344	4.45E-04
GO:0007443~Malpighian tubule morphogenesis	4	2.98E-04	29.24	0.15853892	0.0019376
GO:0048619~embryonic hindgut morphogenesis	4	3.97E-04	26.53	0.20571694	0.0024472
GO:0061525~hindgut development	4	5.69E-04	23.48	0.28109006	0.003328
GO:0007442~hindgut morphogenesis	4	5.69E-04	23.48	0.28109006	0.003328
GO:0001709~cell fate deGO Category # and Term ination	5	5.75E-04	12.35	0.28366345	0.0033305
GO:0042067~establishment of ommatidial planar polarity	4	5.97E-04	23.11	0.29261489	0.0034217
GO:0048598~embryonic morphogenesis	6	6.11E-04	8.17	0.29845709	0.0034692
GO:0016477~cell migration	6	0.0013	6.91	0.52965133	0.0063185
GO:0048870~cell motility	6	0.001903	6.34	0.66880027	0.0085296
GO:0001736~establishment of planar polarity	4	0.001996	15.24	0.68621448	0.0088761
GO:0007164~establishment of tissue polarity	4	0.001996	15.24	0.68621448	0.0088761
GO:0007422~peripheral nervous system development	4	0.001996	15.24	0.68621448	0.0088761
GO:0048568~embryonic organ development	3	0.008872	20.27	0.99430956	0.0343226
GO:0090132~epithelium migration	4	0.012199	7.96	0.99919036	0.0451746
GO:0030707~ovarian follicle cell development	5	0.014912	4.99	0.99983579	0.0530083
GO:0001667~ameboidal-type cell migration	4	0.015535	7.27	0.99988621	0.0548434
GO:0002064~epithelial cell development	5	0.020652	4.52	0.99999446	0.0703196
GO:0002520~immune system development	3	0.030035	10.64	0.99999998	0.0926096
GO:0048534~hematopoietic or lymphoid organ development	3	0.030035	10.64	0.99999998	0.0926096
GO:0007297~ovarian follicle cell migration	3	0.058472	7.36	1	0.1648407

GO:0016192~vesicle-mediated transport	4	0.23596	2.29	1	0.5382658
GO:0045184~establishment of protein localization	3	0.367717	2.27	1	0.7266458

Annotation Cluster 15 Enrichment Score: 2.35

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0042692~muscle cell differentiation	6	1.76E-05	17.33	0.0101821	1.55E-04
GO:0007417~central nervous system development	6	9.65E-04	7.38	0.42889175	0.0048593
GO:0001708~cell fate specification	4	0.002249	14.62	0.72906314	0.0097705
GO:0007419~ventral cord development	3	0.021322	12.79	0.99999628	0.0721221
GO:0007507~heart development	3	0.034549	9.86	1	0.1038421
GO:0072359~circulatory system development	3	0.035131	9.77	1	0.104992
GO:0072358~cardiovascular system development	3	0.035131	9.77	1	0.104992

Annotation Cluster 16 Enrichment Score: 2.26

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048598~embryonic morphogenesis	6	6.11E-04	8.17	0.29845709	0.0034692
GO:0048477~oogenesis	9	9.11E-04	4.04	0.4106318	0.0047094
GO:0007292~female gamete generation	9	9.73E-04	4.00	0.43143861	0.0048558
GO:0007281~germ cell development	9	0.002388	3.48	0.75013016	0.0101454
GO:0022412~cellular process involved in reproduction in multicellular organism	9	0.003751	3.24	0.88695294	0.0154505
GO:0007276~gamete generation	9	0.007081	2.92	0.98378092	0.0280244
GO:0030707~ovarian follicle cell development	5	0.014912	4.99	0.99983579	0.0530083
GO:0019953~sexual reproduction	9	0.02003	2.44	0.99999199	0.0690573
GO:0002064~epithelial cell development	5	0.020652	4.52	0.99999446	0.0703196
GO:0010927~cellular component assembly involved in morphogenesis	4	0.022314	6.34	0.99999793	0.0745259
GO:0042060~wound healing	3	0.024256	11.94	0.99999935	0.0794652

Annotation Cluster 17 Enrichment Score: 1.90

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0035556~intracellular signal transduction	9	1.90E-04	5.09	0.10446329	0.0013782
GO:0032268~regulation of cellular protein metabolic process	8	6.42E-04	5.00	0.31111115	0.003543
GO:0051246~regulation of protein metabolic process	8	9.32E-04	4.70	0.41784211	0.0047763

GO:0009798~axis specification	5	0.003413	7.62	0.86232738	0.014164
GO:0000165~MAPK cascade	4	0.005897	10.38	0.96762466	0.023868
GO:0023014~signal transduction by protein phosphorylation	4	0.005897	10.38	0.96762466	0.023868
GO:0043412~macromolecule modification	10	0.012481	2.43	0.99931406	0.0459109
GO:0036211~protein modification process	9	0.024202	2.36	0.99999933	0.0797398
GO:0032270~positive regulation of cellular protein metabolic process	4	0.024429	6.12	0.99999941	0.0795722
GO:0051247~positive regulation of protein metabolic process	4	0.027509	5.85	0.99999991	0.0868844
GO:0051174~regulation of phosphorus metabolic process	4	0.043284	4.89	1	0.1276054
GO:0010562~positive regulation of phosphorus metabolic process	3	0.055664	7.57	1	0.1588743
GO:0044267~cellular protein metabolic process	11	0.097302	1.62	1	0.2602065
GO:0019538~protein metabolic process	12	0.130681	1.48	1	0.3337778
GO:0006796~phosphate-containing compound metabolic process	5	0.31696	1.69	1	0.6616951

Annotation Cluster 18 Enrichment Score: 1.88

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010623~programmed cell death involved in cell development	5	7.94E-04	11.33	0.36920535	0.0041425
GO:0012501~programmed cell death	6	0.002923	5.75	0.81687899	0.0122262
GO:0043068~positive regulation of programmed cell death	3	0.019916	13.26	0.99999144	0.0690809
GO:0043067~regulation of programmed cell death	4	0.020551	6.54	0.99999412	0.0703928
GO:0010942~positive regulation of cell death	3	0.023756	12.07	0.99999912	0.0787532
GO:2000027~regulation of organ morphogenesis	3	0.026298	11.43	0.99999981	0.0845378
GO:0007548~sex differentiation	3	0.03114	10.43	0.99999999	0.0949074
GO:0097305~response to alcohol	3	0.04431	8.60	1	0.1298435

Annotation Cluster 19 Enrichment Score: 1.78

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0030036~actin cytoskeleton organization	6	6.22E-04	8.14	0.30277472	0.0034953
GO:0098742~cell-cell adhesion via plasma-membrane adhesion molecules	4	6.25E-04	22.74	0.30430202	0.0034828
GO:0007517~muscle organ development	5	6.70E-04	11.86	0.32204851	0.0036601
GO:0007010~cytoskeleton organization	7	0.00671	3.86	0.97985259	0.0267515
GO:0010927~cellular component assembly involved in morphogenesis	4	0.022314	6.34	0.99999793	0.0745259
GO:1902589~single-organism organelle organization	7	0.027229	2.85	0.99999989	0.0864926

GO:0016337~single organismal cell-cell adhesion	3	0.028947	10.85	0.99999996	0.0903077
GO:0016331~morphogenesis of embryonic epithelium	3	0.046894	8.33	1	0.1363744
GO:0033043~regulation of organelle organization	4	0.081621	3.76	1	0.2227242
GO:0022402~cell cycle process	4	0.399015	1.70	1	0.761558
GO:0000278~mitotic cell cycle	3	0.443811	1.94	1	0.8067449

Annotation Cluster 20 Enrichment Score: 1.76

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010675~regulation of cellular carbohydrate metabolic process	4	0.013132	7.74	0.99953198	0.0479592
GO:0006109~regulation of carbohydrate metabolic process	4	0.014105	7.54	0.9997359	0.0508104
GO:0030707~ovarian follicle cell development	5	0.014912	4.99	0.99983579	0.0530083
GO:0002064~epithelial cell development	5	0.020652	4.52	0.99999446	0.0703196
GO:0044262~cellular carbohydrate metabolic process	4	0.026649	5.92	0.99999984	0.085166

Annotation Cluster 21 Enrichment Score: 0.74

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0032269~negative regulation of cellular protein metabolic process	3	0.122435	4.78	1	0.3178985
GO:0051248~negative regulation of protein metabolic process	3	0.123329	4.75	1	0.318613
GO:0022402~cell cycle process	4	0.399015	1.70	1	0.761558

Annotation Cluster 22 Enrichment Score: 0.11

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0000166~nucleotide binding	4	0.736942	1.04	1	0.9999957
GO:0035639~purine ribonucleoside triphosphate binding	3	0.774908	1.06	1	0.9999839
GO:0001883~purine nucleoside binding	3	0.776543	1.05	1	0.999903
GO:0032549~ribonucleoside binding	3	0.776543	1.05	1	0.999903
GO:0032555~purine ribonucleotide binding	3	0.777627	1.05	1	0.9996461
GO:0017076~purine nucleotide binding	3	0.778168	1.05	1	0.9990551
GO:0032553~ribonucleotide binding	3	0.783514	1.04	1	0.998147

Appendix 3.8A. Enriched gene ontology categories for human orthologs: climbing speed. Overrepresented gene ontology categories among human orthologs of candidate genes identified in the climbing speed GWA analysis (data combined across both ages). Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8

Annotation Cluster 1 Enrichment Score: 11.22

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0000904~cell morphogenesis involved in differentiation	14	3.45E-16	19.07453519	1.82E-13	1.82E-13
GO:0000902~cell morphogenesis	14	2.24E-13	11.58316532	1.22E-10	4.07E-11
GO:0032989~cellular component morphogenesis	14	5.06E-13	10.87291824	2.76E-10	6.91E-11
GO:0048468~cell development	15	1.62E-12	7.819645579	8.86E-10	1.48E-10
GO:0030154~cell differentiation	15	8.49E-09	4.213872536	4.64E-06	2.90E-07
GO:0048731~system development	15	7.77E-08	3.586358075	4.24E-05	1.77E-06

Annotation Cluster 2 Enrichment Score: 6.79

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048870~cell motility	12	9.56E-10	9.256578947	5.22E-07	4.01E-08
GO:0016477~cell migration	11	7.90E-09	9.531513936	4.31E-06	2.87E-07
GO:0048513~animal organ development	10	5.38E-04	3.278803132	0.25446115	0.00465023

Annotation Cluster 3 Enrichment Score: 5.382

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0031175~neuron projection development	13	8.47E-14	16.3245869	4.63E-11	2.31E-11
GO:0048666~neuron development	13	6.16E-13	13.82091969	3.36E-10	6.73E-11
GO:0048858~cell projection morphogenesis	12	3.77E-12	15.44698871	2.06E-09	2.94E-10

GO:0032990~cell part morphogenesis	12	4.94E-12	15.06884945	2.70E-09	3.37E-10
GO:0022604~regulation of cell morphogenesis	11	5.85E-12	19.86850792	3.19E-09	3.55E-10
GO:0010769~regulation of cell morphogenesis involved in differentiation	9	1.73E-10	27.23727876	9.44E-08	8.58E-09
GO:0006935~chemotaxis	10	2.15E-10	18.58582428	1.18E-07	9.80E-09
GO:0007399~nervous system development	13	8.56E-09	6.146169355	4.68E-06	2.75E-07
GO:0048588~developmental cell growth	7	9.57E-09	38.19980053	5.23E-06	2.90E-07
GO:0060284~regulation of cell development	10	9.80E-09	12.0556698	5.35E-06	2.81E-07
GO:0031344~regulation of cell projection organization	9	1.07E-08	16.14237325	5.86E-06	2.93E-07
GO:0060560~developmental growth involved in morphogenesis	7	1.61E-08	35.0320122	8.77E-06	4.18E-07
GO:0050767~regulation of neurogenesis	9	4.23E-08	13.53876466	2.31E-05	1.05E-06
GO:0051960~regulation of nervous system development	9	1.07E-07	12.00707087	5.87E-05	2.35E-06
GO:1990138~neuron projection extension	6	1.22E-07	43.65691489	6.66E-05	2.56E-06
GO:0008361~regulation of cell size	6	3.38E-07	35.5816474	1.85E-04	6.60E-06
GO:0032535~regulation of cellular component size	7	4.10E-07	20.28689972	2.24E-04	7.45E-06
GO:0030334~regulation of cell migration	8	1.03E-06	11.98175182	5.60E-04	1.75E-05
GO:0061387~regulation of extent of cell growth	5	1.97E-06	49.32391827	0.00107338	3.25E-05
GO:0010771~negative regulation of cell morphogenesis involved in differentiation	5	3.37E-06	43.10661765	0.00183807	5.26E-05
GO:0032956~regulation of actin cytoskeleton organization	6	4.62E-06	20.9375	0.00251797	6.63E-05
GO:1902667~regulation of axon guidance	4	5.98E-06	102.59375	0.00325796	8.16E-05
GO:0050919~negative chemotaxis	4	6.44E-06	100.0914634	0.00351279	8.58E-05
GO:0051496~positive regulation of stress fiber assembly	4	6.94E-06	97.70833333	0.00378042	9.02E-05
GO:0031345~negative regulation of cell projection organization	5	9.14E-06	33.52736928	0.00498036	1.16E-04
GO:0051129~negative regulation of cellular component organization	7	1.10E-05	11.50891426	0.00598363	1.36E-04
GO:0032233~positive regulation of actin filament bundle assembly	4	1.18E-05	82.075	0.00641762	1.43E-04
GO:0010770~positive regulation of cell morphogenesis involved in differentiation	5	1.20E-05	31.27858232	0.0065481	1.43E-04
GO:0050920~regulation of chemotaxis	5	1.98E-05	27.57896505	0.01073084	2.30E-04
GO:0001558~regulation of cell growth	6	2.13E-05	15.27450372	0.01157549	2.43E-04
GO:0033043~regulation of organelle organization	8	3.21E-05	7.124565972	0.01737589	3.58E-04
GO:0051130~positive regulation of cellular component organization	8	3.81E-05	6.937869822	0.02061078	4.16E-04
GO:0050768~negative regulation of neurogenesis	5	5.81E-05	20.9375	0.03121149	6.22E-04
GO:0030516~regulation of axon extension	4	6.91E-05	45.59722222	0.03704953	7.26E-04
GO:0051961~negative regulation of nervous system development	5	7.76E-05	19.43063447	0.04150314	7.99E-04
GO:0007417~central nervous system development	7	8.94E-05	7.944206305	0.04761946	8.87E-04

GO:0031346~positive regulation of cell projection organization	5	1.34E-04	16.87397204	0.07054621	0.00130554
GO:0010721~negative regulation of cell development	5	1.37E-04	16.76368464	0.07228543	0.00131547
GO:0007420~brain development	6	2.62E-04	8.999451754	0.13345855	0.0024667
GO:1902668~negative regulation of axon guidance	3	2.91E-04	109.921875	0.14673902	0.00264134
GO:0050769~positive regulation of neurogenesis	5	3.38E-04	13.25500646	0.16847778	0.00301998
GO:0030308~negative regulation of cell growth	4	4.61E-04	23.99853801	0.22254404	0.0040519
GO:0048513~animal organ development	10	5.38E-04	3.278803132	0.25446115	0.00465023
GO:0051962~positive regulation of nervous system development	5	5.93E-04	11.42469376	0.27673297	0.00497186
GO:0010720~positive regulation of cell development	5	7.81E-04	10.62047101	0.34710004	0.00634295
GO:0035556~intracellular signal transduction	9	0.001024	3.475136432	0.42837571	0.00807265
GO:0050922~negative regulation of chemotaxis	3	0.001085	56.99652778	0.44722425	0.00831458
GO:0051271~negative regulation of cellular component movement	4	0.001535	15.84459459	0.56778314	0.01112206
GO:0045596~negative regulation of cell differentiation	5	0.002216	8.015136719	0.70213781	0.01448591
GO:0001667~ameboidal-type cell migration	4	0.002806	12.82421875	0.78437608	0.01768158
GO:0048640~negative regulation of developmental growth	3	0.002851	34.97514205	0.78964821	0.01775965
GO:0045597~positive regulation of cell differentiation	5	0.005932	6.099509512	0.96116797	0.03293522
GO:0030336~negative regulation of cell migration	3	0.015886	14.3823014	0.99984047	0.06970954
GO:2000146~negative regulation of cell motility	3	0.017758	13.55864537	0.99994358	0.07527763
GO:0032102~negative regulation of response to external stimulus	3	0.024072	11.52738764	0.99999833	0.09799328
GO:0004888~transmembrane signaling receptor activity	4	0.148557	2.762796028	0.99839221	0.41495982

Annotation Cluster 4 Enrichment Score: 5.27

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:2000145~regulation of cell motility	9	7.65E-08	12.54543139	4.18E-05	1.82E-06
GO:0030334~regulation of cell migration	8	1.03E-06	11.98175182	5.60E-04	1.75E-05
GO:0060429~epithelium development	6	0.001935	5.807193396	0.6526968	0.0129714

Annotation Cluster 5 Enrichment Score: 3.08

GO Category # and Term	# of Genes	PValue	Fold Enrichment	Bonferroni	Benjamini
GO:0050920~regulation of chemotaxis	5	1.98E-05	27.57896505	0.01073084	2.30E-04
GO:0007420~brain development	6	2.62E-04	8.999451754	0.13345855	0.0024667
GO:0021537~telencephalon development	4	0.001104	17.76515152	0.45303958	0.00834525
GO:0021872~forebrain generation of neurons	3	0.001665	45.9375	0.59744687	0.01190135
GO:0030900~forebrain development	4	0.003943	11.36772853	0.88433929	0.02394566
GO:0021543~pallium development	3	0.008796	19.60390127	0.99196222	0.04489984

Appendix 3.8B. Enriched gene ontology categories for human orthologs: endurance. Overrepresented gene ontology categories among human orthologs of candidate genes identified in the endurance GWA analysis (data combined across both ages). Statistical significance determined by the Holm-Bonferroni test and the Benjamini-Hochberg test. GO terms are ranked by Benjamini-Hochberg significance. Results aquired by DAVID 6.8

Annotation Cluster 1 Enrichment Score: 7.53

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045597~positive regulation of cell differentiation	16	5.42E-12	9.76	5.18E-09	1.30E-09
GO:0000904~cell morphogenesis involved in differentiation	14	3.45E-10	9.54	3.30E-07	4.71E-08
GO:0048468~cell development	19	1.07E-09	4.95	1.02E-06	1.02E-07
GO:0000902~cell morphogenesis	16	1.30E-09	6.62	1.24E-06	1.03E-07
GO:0032989~cellular component morphogenesis	16	3.11E-09	6.21	2.98E-06	2.29E-07
GO:0048858~cell projection morphogenesis	13	9.50E-09	8.37	9.09E-06	6.06E-07
GO:0032990~cell part morphogenesis	13	1.25E-08	8.16	1.20E-05	7.50E-07
GO:0031175~neuron projection development	13	1.25E-08	8.16	1.20E-05	7.50E-07
GO:0007399~nervous system development	18	4.55E-08	4.26	4.35E-05	2.56E-06
GO:0048666~neuron development	13	7.93E-08	6.91	7.59E-05	3.99E-06
GO:0030154~cell differentiation	21	5.34E-07	2.95	5.11E-04	1.76E-05
GO:0006935~chemotaxis	10	5.36E-07	9.29	5.13E-04	1.71E-05
GO:0048731~system development	21	8.01E-06	2.51	0.0076355	1.67E-04
GO:0060429~epithelium development	8	0.00306756	3.87	0.9471428	0.0311201

Annotation Cluster 2 Enrichment Score: 6.45

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0012501~programmed cell death	18	4.06E-09	4.99	3.88E-06	2.77E-07
GO:0005089~Rho guanyl-nucleotide exchange factor activity	6	2.94E-07	39.74	1.88E-05	1.88E-05

GO:0043068~positive regulation of programmed cell death	10	7.76E-07	8.89	7.43E-04	2.06E-05
GO:0010942~positive regulation of cell death	10	1.18E-06	8.45	0.0011326	2.98E-05
GO:0043067~regulation of programmed cell death	13	4.73E-06	4.72	0.0045205	1.03E-04

Annotation Cluster 3 Enrichment Score: 6.23

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0045597~positive regulation of cell differentiation	16	5.42E-12	9.76	5.18E-09	1.30E-09
GO:0000904~cell morphogenesis involved in differentiation	14	3.45E-10	9.54	3.30E-07	4.71E-08
GO:0010770~positive regulation of cell morphogenesis involved in differentiation	9	5.43E-10	28.15	5.19E-07	6.49E-08
GO:0010720~positive regulation of cell development	12	6.36E-10	12.74	6.08E-07	6.76E-08
GO:0048468~cell development	19	1.07E-09	4.95	1.02E-06	1.02E-07
GO:0000902~cell morphogenesis	16	1.30E-09	6.62	1.24E-06	1.03E-07
GO:0032989~cellular component morphogenesis	16	3.11E-09	6.21	2.98E-06	2.29E-07
GO:0031346~positive regulation of cell projection organization	9	6.87E-08	15.19	6.58E-05	3.66E-06
GO:0051130~positive regulation of cellular component organization	14	8.02E-08	6.07	7.67E-05	3.84E-06
GO:0010769~regulation of cell morphogenesis involved in differentiation	9	1.59E-07	13.62	1.52E-04	7.24E-06
GO:0060284~regulation of cell development	12	2.22E-07	7.23	2.12E-04	9.23E-06
GO:0048870~cell motility	14	3.16E-07	5.40	3.02E-04	1.16E-05
GO:0050769~positive regulation of neurogenesis	9	4.36E-07	11.93	4.17E-04	1.49E-05
GO:0022604~regulation of cell morphogenesis	10	6.81E-07	9.03	6.51E-04	1.97E-05
GO:0051962~positive regulation of nervous system development	9	1.34E-06	10.28	0.0012774	3.28E-05
GO:0031344~regulation of cell projection organization	9	8.03E-06	8.07	0.0076583	1.64E-04
GO:0050767~regulation of neurogenesis	9	2.87E-05	6.77	0.0271283	5.39E-04
GO:0051960~regulation of nervous system development	9	6.75E-05	6.00	0.0625459	0.0011527
GO:0009887~organ morphogenesis	9	3.91E-04	4.65	0.3125178	0.0056615
GO:0048513~animal organ development	15	8.09E-04	2.46	0.5392257	0.0101436
GO:0060560~developmental growth involved in morphogenesis	4	0.00666942	10.01	0.9983451	0.0537108
GO:0044765~single-organism transport	12	0.04232108	1.80	1	0.2138443
GO:0030031~cell projection assembly	4	0.04378553	4.90	1	0.2182746

Annotation Cluster 4 Enrichment Score: 4.47

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010770~positive regulation of cell morphogenesis involved in differentiation	9	5.43E-10	28.15	5.19E-07	6.49E-08
GO:0010769~regulation of cell morphogenesis involved in differentiation	9	1.59E-07	13.62	1.52E-04	7.24E-06
GO:0048870~cell motility	14	3.16E-07	5.40	3.02E-04	1.16E-05
GO:2000145~regulation of cell motility	11	5.81E-07	7.67	5.56E-04	1.79E-05
GO:2000147~positive regulation of cell motility	9	6.62E-07	11.29	6.33E-04	1.98E-05
GO:0016477~cell migration	13	7.30E-07	5.63	6.98E-04	2.00E-05
GO:0051272~positive regulation of cellular component movement	9	8.09E-07	10.99	7.74E-04	2.09E-05
GO:0030334~regulation of cell migration	10	3.21E-06	7.49	0.0030698	7.32E-05
GO:0030335~positive regulation of cell migration	8	7.07E-06	10.39	0.0067449	1.50E-04
GO:0008284~positive regulation of cell proliferation	10	1.83E-05	6.04	0.0173209	3.57E-04
GO:0002009~morphogenesis of an epithelium	7	3.72E-04	6.89	0.2994734	0.0054608
GO:0042060~wound healing	7	4.59E-04	6.63	0.3555163	0.0065354
GO:0048729~tissue morphogenesis	7	9.46E-04	5.77	0.5958944	0.0116983
GO:0007267~cell-cell signaling	10	0.00148028	3.38	0.7577228	0.0165402
GO:0060429~epithelium development	8	0.00306756	3.87	0.9471428	0.0311201
GO:0010243~response to organonitrogen compound	5	0.05956415	3.25	1	0.2746883
GO:0051046~regulation of secretion	4	0.1368563	3.00	1	0.4564976
GO:0046903~secretion	5	0.15004176	2.34	1	0.4770347

Annotation Cluster 5 Enrichment Score: 3.63

GO Category # and Term	# of Genes	P-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0051147~regulation of muscle cell differentiation	6	1.37E-05	18.54	0.0130021	2.73E-04
GO:0051149~positive regulation of muscle cell differentiation	5	2.48E-05	28.19	0.0234531	4.75E-04
GO:0042692~muscle cell differentiation	7	5.10E-05	9.92	0.0475994	8.86E-04
GO:0045661~regulation of myoblast differentiation	3	0.00368846	32.06	0.9708815	0.0347458
GO:0045165~cell fate commitment	4	0.01060983	8.44	0.9999631	0.0760808

Annotation Cluster 6 Enrichment Score: 3.08

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0048870~cell motility	14	3.16E-07	5.40	3.02E-04	1.16E-05
GO:0016477~cell migration	13	7.30E-07	5.63	6.98E-04	2.00E-05
GO:0051338~regulation of transferase activity	10	4.63E-05	5.37	0.0433478	8.36E-04
GO:0002764~immune response-regulating signaling pathway	8	4.77E-05	7.73	0.044593	8.44E-04
GO:0042060~wound healing	7	4.59E-04	6.63	0.3555163	0.0065354
GO:0030036~actin cytoskeleton organization	7	5.11E-04	6.49	0.3865833	0.0071612
GO:0002429~immune response-activating cell surface receptor signaling pathway	6	5.72E-04	8.39	0.4217498	0.0075788
GO:0007010~cytoskeleton organization	9	0.00100939	4.04	0.6195772	0.0123142
GO:0016192~vesicle-mediated transport	10	0.00132831	3.43	0.7197399	0.0150292
GO:0051017~actin filament bundle assembly	4	0.00173533	16.16	0.8102701	0.0189239
GO:0038096~Fc-gamma receptor signaling pathway involved in phagocytosis	4	0.00173533	16.16	0.8102701	0.0189239
GO:0061572~actin filament bundle organization	4	0.00189622	15.66	0.8373909	0.0204294
GO:0002253~activation of immune response	6	0.00342356	5.60	0.9624457	0.0332687
GO:0050778~positive regulation of immune response	6	0.00855407	4.50	0.9997312	0.0646562
GO:0044089~positive regulation of cellular component biogenesis	5	0.00910593	5.82	0.9998422	0.0681646
GO:0006909~phagocytosis	4	0.0150761	7.41	0.9999995	0.0986311
GO:0000278~mitotic cell cycle	6	0.03343622	3.18	1	0.1790117
GO:0016818~hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	3	0.46142915	1.88	1	0.915866

Annotation Cluster 7 Enrichment Score: 2.86

GO Category # and Term	# of Genes	*P*-Value	Fold Enrichment	Bonferroni	Benjamini
GO:0010770~positive regulation of cell morphogenesis involved in differentiation	9	5.43E-10	28.15	5.19E-07	6.49E-08
GO:0010769~regulation of cell morphogenesis involved in differentiation	9	1.59E-07	13.62	1.52E-04	7.24E-06
GO:1900026~positive regulation of substrate adhesion-dependent cell spreading	3	0.00165263	48.09	0.7946166	0.0182372
GO:1900024~regulation of substrate adhesion-dependent cell spreading	3	0.00339231	33.45	0.9613017	0.0336519
GO:0010810~regulation of cell-substrate adhesion	4	0.00457932	11.46	0.9876299	0.04022
GO:0034446~substrate adhesion-dependent cell spreading	3	0.01019291	19.00	0.9999448	0.0737387
GO:0007030~Golgi organization	3	0.01354374	16.37	0.9999978	0.0914955

GO:0048534~hematopoietic or lymphoid organ development	6	0.01439798	3.96	0.9999991	0.0956806
GO:0010811~positive regulation of cell-substrate adhesion	3	0.0170037	14.52	0.9999999	0.1084311
GO:0002520~immune system development	6	0.01780702	3.75	1	0.111824
GO:0001667~ameboidal-type cell migration	4	0.02204586	6.41	1	0.1301525
GO:0045785~positive regulation of cell adhesion	4	0.0354292	5.33	1	0.1867464
GO:0030097~hemopoiesis	5	0.04890021	3.47	1	0.2362792

Appendix A. GWA results. Output from the DGRP Freeze 2.0 analysis pipeline (http://dgrp.gnets.ncsu.edu/). Candidate SNPs are associated with (1) age-specific climbing speed and (2) age-specific endurance at $P < 10^{-5}$. Age is measured in weeks, Trt = treatment, C = control, untreated food, L = Lisinopril-treated food, SNP = Single Nucleotide Polymorphism, Chrom/Pos is chromosome and position of SNP, FBgn = FlyBase gene. Information for each SNP is described in Mackay et al. (2012).

1. Climbing speed GWA results

Age	Trt	Chrom/Pos	FlyBase ID	Gene Symbol	Site Class	SNP *P*-value
1	C	3R_1289892_SNP	FBgn0037327	*PEK*	INTRON	1.336E-07
1	C	3L_13506826_SNP	FBgn0036376	*Liprin-beta*	SYNONYMOUS_CODING	3.969E-07
1	C	3R_1297119_SNP	FBgn0011715	*Snr1*	INTRON	3.849E-07
1	C	2L_17806199_SNP	FBgn0262018	*CadN2*	INTRON	4.234E-08
1	C	3R_1292660_SNP	FBgn0037328	*RpL35A*	INTRON	6.684E-07
1	C	X_7995298_SNP	FBgn0029997	*CG2258*	UTR_5_PRIME	0.000004618
1	C	3R_1295688_SNP	FBgn0037330	*mRpL44*	SYNONYMOUS_CODING	0.000001243
1	C	3R_1297920_SNP	FBgn0011715	*Snr1*	NON_SYNONYMOUS_CODING	0.000001286
1	C	3L_2260229_SNP	FBgn0015360	*oxt*	SYNONYMOUS_CODING	0.000002834
1	C	3R_25913089_INS	FBgn0015542	*sima*	INTRON	0.000002844
1	C	3R_1287165_SNP	FBgn0037327	*PEK*	NON_SYNONYMOUS_CODING	0.000001833
1	C	3L_8332925_SNP	FBgn0035876	*Pex2*	INTRON	0.000005126
1	C	3L_8332928_SNP	FBgn0035876	*Pex2*	INTRON	0.000005126
1	C	2R_18434571_SNP	FBgn0003175	*px*	INTRON	0.000001031
1	C	3L_9203774_SNP	|||			0.000005527
1	C	3R_24529177_SNP	FBgn0027655	*htt*	INTRON	0.000024
1	C	2L_12333262_SNP	|||			0.000001596
1	C	2L_6446129_SNP	FBgn0031813	*CG9527*	SYNONYMOUS_CODING	0.00004627
1	C	3L_5711860_SNP	FBgn0085447	*sif*	INTRON	0.000003548
1	C	3L_2000594_SNP	|||			0.000001074
1	C	3R_1293685_SNP	FBgn0037329	*CG12162*	UTR_5_PRIME	0.00001142
1	C	X_1797431_DEL	FBgn0264446	*CR43864*	EXON	0.000009476
1	C	2R_4356187_SNP	FBgn0033296	*Mal-A7*	DOWNSTREAM	2.628E-07
1	C	2R_19525497_SNP	FBgn0004795	*retn*	INTRON	4.386E-07
1	C	2L_9264520_SNP	FBgn0263984	*CG43733*	INTRON	0.00001291
1	C	3L_9203776_SNP	|||			0.000007911
1	C	3R_24518766_SNP	FBgn0039594	*CG9990*	INTRON	0.000005946
1	C	3R_26469967_SNP	FBgn0051013	*CG31013*	INTRON	0.000006496
1	C	2R_3841980_SNP	FBgn0033236	*CG14764*	INTRON	0.000008452
1	C	2R_17693199_SNP	|||			0.00001049
1	C	3L_8332939_SNP	FBgn0035876	*Pex2*	INTRON	0.00001204
1	C	3R_26468899_SNP	FBgn0051013	*CG31013*	DOWNSTREAM	0.00001268
1	C	3R_7632221_SNP	FBgn0051116	*ClC-a*	INTRON	0.00000306
1	C	3R_1284830_SNP	FBgn0037327	*PEK*	UTR_3_PRIME	0.000007981
1	C	3R_15188715_DEL	|||			0.000007664
1	C	2L_6839820_SNP	FBgn0051632	*sens-2*	DOWNSTREAM	0.000004721
1	C	3R_7114662_SNP	FBgn0051386	*CR31386*	INTRON	0.00001392
1	C	3R_24503465_INS	FBgn0039594	*CG9990*	INTRON	0.00003219
1	C	3R_26470169_SNP	FBgn0051013	*CG31013*	INTRON	0.00001974
1	C	2L_17799501_SNP	FBgn0262018	*CadN2*	SYNONYMOUS_CODING	0.000004706
1	C	3R_26470279_SNP	FBgn0051013	*CG31013*	SYNONYMOUS_CODING	0.000009692
1	C	3R_5450762_SNP	FBgn0037720	*CG8312*	UTR_3_PRIME	0.00001806
1	C	3L_15504392_SNP	FBgn0000565	*Eip71CD*	INTRON	0.000001762
1	C	3R_12658248_SNP	FBgn0264857	*iab-8*	INTRON	0.00001405
1	C	3R_2248710_SNP	FBgn0264495	*gpp*	INTRON	0.00001229
1	C	3L_10004732_SNP	FBgn0040823	*dpr6*	INTRON	0.00005773
1	C	X_16278780_SNP	FBgn0030744	*CG9992*	UPSTREAM	0.0000249

1	C	X_7995294_DEL	FBgn0029997	*CG2258*	UTR_5_PRIME	0.00007103
1	C	3L_1092199_SNP	FBgn0004870	*bab1*	INTRON	0.00003302
1	C	2L_17537887_SNP	|||		0.00003009
1	C	3R_1283377_INS	FBgn0037326	*CG14669*	DOWNSTREAM	0.00002371
1	C	3R_1283380_INS	FBgn0037326	*CG14669*	DOWNSTREAM	0.00002371
1	C	2R_3842016_SNP	FBgn0033236	*CG14764*	INTRON	0.00002939
1	C	3L_3683003_SNP	|||		0.00006119
1	C	3L_3682976_SNP	|||		0.00006565
1	C	3R_1279608_SNP	FBgn0037326	*CG14669*	INTRON	0.0000163
1	C	3L_8331646_SNP	FBgn0035875	*Cpr66Cb*	UTR_3_PRIME	0.00003299
1	C	3L_8331638_SNP	FBgn0035875	*Cpr66Cb*	UTR_3_PRIME	0.00003702
1	C	3R_4583622_SNP	FBgn0083971	*CG34135*	INTRON	0.000007833
1	C	2L_17014954_SNP	|||		0.000004178
1	C	3L_15504415_DEL	FBgn0000565	*Eip71CD*	INTRON	0.000002932
1	C	2R_17126498_SNP	FBgn0034606	*ASPP*	INTRON	0.000008875
1	C	3R_26872843_SNP	FBgn0010015	*CanA1*	DOWNSTREAM	0.000009101
1	C	2R_1687881_SNP	|||		0.000007965
1	C	3R_1252838_SNP	FBgn0037325	*CG12147*	NON_SYNONYMOUS_CODING	0.000004118
1	C	2R_18899287_SNP	FBgn0261705	*CG42741*	UTR_3_PRIME	0.000005097
1	C	2R_5115029_INS	FBgn0010114	*hig*	DOWNSTREAM	0.00000196
1	C	3R_10582196_SNP	FBgn0263929	*jvl*	INTRON	0.000008947
1	C	2R_1409381_SNP	FBgn0050438	*CG30438*	INTRON	0.000001243
1	C	2R_20438711_SNP	FBgn0035021	*CG4622*	INTRON	0.000004364
1	C	3L_3335646_SNP	FBgn0052274	*Drsl1*	NON_SYNONYMOUS_CODING	0.000005363
1	C	2L_17798166_SNP	FBgn0262018	*CadN2*	SYNONYMOUS_CODING	0.000003556
1	C	X_1801269_SNP	FBgn0023511	*Edem1*	SYNONYMOUS_CODING	0.000008868
1	C	2L_17784657_SNP	|||		0.000009624
1	C	2R_2547377_SNP	FBgn0013732	*sced*	SYNONYMOUS_CODING	0.000009918
1	C	2R_17789946_SNP	FBgn0085397	*Fili*	INTRON	0.000009827
5	C	2L_18865715_SNP	FBgn0003896	*tup*	INTRON	1.17E-07
5	C	X_6402103_SNP	FBgn0259242	*CG42340*	INTRON	5.32E-08
5	C	X_6402098_DEL	FBgn0259242	*CG42340*	INTRON	7.45E-08
5	C	3L_5177435_SNP	FBgn0052423	*shep*	INTRON	5.60E-08
5	C	3L_5178242_SNP	FBgn0052423	*shep*	INTRON	5.05E-08
5	C	3L_12411005_SNP	FBgn0020655	*Gap69C*	UPSTREAM	2.33E-07
5	C	3L_21253627_SNP	FBgn0004865	*Eip78C*	INTRON	1.20E-07
5	C	3L_20162751_SNP	FBgn0261556	*CG42674*	INTRON	1.11E-07
5	C	X_20969348_SNP	FBgn0064123	*stg1*	INTRON	3.51E-07
5	C	2L_15124366_SNP	|||		1.24E-07
5	C	2L_9467050_SNP	|||		2.37E-07
5	C	3R_2706221_SNP	|||		1.73E-07
5	C	2L_1061418_SNP	FBgn0003310	*S*	SYNONYMOUS_CODING	7.73E-07
5	C	2R_10529867_SNP	|||		3.22E-07
5	C	2L_9668716_SNP	|||		7.79E-07
5	C	3L_15996950_SNP	FBgn0036556	*CG5830*	INTRON	6.31E-07
5	C	3L_3892819_SNP	FBgn0026592	*Fie*	START_GAINED	4.59E-07
5	C	3L_4417369_SNP	FBgn0035542	*DOR*	INTRON	3.95E-07
5	C	2L_15100020_SNP	|||		1.61E-06
5	C	2L_15124338_SNP	|||		1.01E-06
5	C	3L_7002277_SNP	FBgn0260660	*mp*	INTRON	8.30E-07
5	C	2L_13959098_SNP	FBgn0261514	*nimA*	INTRON	1.09E-06

5	C	3L_20244293_SNP	FBgn0036960	*CG13814*	INTRON	7.52E-07
5	C	2L_5926729_SNP	FBgn0015381	*dsf*	INTRON	4.37E-06
5	C	3L_394542_SNP	FBgn0264700	*CR43969*	DOWNSTREAM	1.13E-06
5	C	2R_17242255_SNP	FBgn0000395	*cv-2*	DOWNSTREAM	1.07E-06
5	C	2R_17242258_SNP	FBgn0000395	*cv-2*	DOWNSTREAM	1.08E-06
5	C	3L_6604653_SNP	FBgn0035708	*CG8398*	INTRON	1.35E-06
5	C	2L_13901197_SNP	FBgn0019890	*Smg5*	SYNONYMOUS_CODING	1.30E-06
5	C	3L_19682448_SNP	FBgn0004623	*Gbeta76C*	SYNONYMOUS_CODING	1.15E-06
5	C	2R_3074376_SNP	FBgn0003090	*pk*	INTRON	7.26E-07
5	C	3L_4394183_SNP	FBgn0035539	*slow*	INTRON	8.93E-07
5	C	3R_9197801_DEL	FBgn0023495	*Lip3*	UPSTREAM	1.85E-06
5	C	3R_1967895_SNP	|||		8.79E-07
5	C	X_20938898_SNP	FBgn0031150	*bves*	INTRON	7.19E-07
5	C	2R_10301344_SNP	FBgn0033935	*Sin1*	UTR_3_PRIME	1.67E-06
5	C	3L_18155807_SNP	FBgn0036781	*CG13699*	UPSTREAM	1.02E-06
5	C	2L_1321151_SNP	|||		2.07E-06
5	C	3L_4416900_SNP	FBgn0035542	*DOR*	INTRON	8.40E-07
5	C	3L_7319042_SNP	FBgn0035762	*CG8605*	INTRON	1.82E-06
5	C	2L_1150585_SNP	FBgn0031309	*Tfb4*	INTRON	1.63E-06
5	C	2R_3068502_SNP	FBgn0003090	*pk*	INTRON	1.11E-06
5	C	3L_1003196_DEL	FBgn0024277	*trio*	INTRON	1.53E-06
5	C	3L_21962842_SNP	FBgn0029091	*CS-2*	UTR_3_PRIME	1.81E-06
5	C	2L_14642375_SNP	FBgn0003016	*osp*	INTRON	2.40E-06
5	C	3L_21901406_SNP	FBgn0262737	*mub*	INTRON	2.26E-06
5	C	3L_623347_SNP	|||		2.92E-06
5	C	2L_13955322_SNP	FBgn0032536	*Ance-3*	SYNONYMOUS_CODING	2.78E-06
5	C	3L_19618371_SNP	FBgn0036896	*wnd*	DOWNSTREAM	2.19E-06
5	C	2L_9453764_SNP	FBgn0002973	*numb*	INTRON	2.24E-06
5	C	3L_10974208_SNP	FBgn0013469	*klu*	DOWNSTREAM	2.79E-06
5	C	3L_14152650_SNP	|||		2.52E-06
5	C	X_18066571_SNP	FBgn0030897	*Frq1*	UTR_3_PRIME	2.32E-06
5	C	3L_16003440_INS	FBgn0263601	*mib1*	INTRON	2.70E-06
5	C	X_20945383_SNP	FBgn0031150	*bves*	INTRON	3.46E-06
5	C	2R_16811758_DEL	FBgn0020617	*Rx*	INTRON	3.63E-06
5	C	2R_4165908_SNP	FBgn0265307	*CR44280*	DOWNSTREAM	3.22E-06
5	C	X_18065958_SNP	FBgn0030897	*Frq1*	UTR_3_PRIME	2.86E-06
5	C	2R_7679294_SNP	FBgn0033652	*ths*	INTRON	2.63E-06
5	C	3L_8848125_SNP	FBgn0263930	*dally*	INTRON	3.37E-06
5	C	3L_22009991_SNP	|||		4.46E-06
5	C	3L_4416208_SNP	FBgn0035542	*DOR*	INTRON	1.91E-06
5	C	2L_10026729_SNP	FBgn0032176	*CG13127*	UPSTREAM	3.40E-06
5	C	2R_3074307_SNP	FBgn0003090	*pk*	INTRON	1.80E-06
5	C	3L_21853842_SNP	FBgn0262737	*mub*	INTRON	3.10E-06
5	C	2L_10026720_DEL	FBgn0032176	*CG13127*	UPSTREAM	3.26E-06
5	C	3L_387476_SNP	FBgn0262139	*trh*	INTRON	4.80E-06
5	C	X_20641766_SNP	FBgn0085387	*shakB*	DOWNSTREAM	3.17E-06
5	C	2R_7328911_SNP	FBgn0033635	*CG7777*	SYNONYMOUS_CODING	5.22E-06
5	C	X_16867710_SNP	FBgn0030810	*CG9059*	SYNONYMOUS_CODING	2.63E-06
5	C	3L_18851520_SNP	|||		5.36E-06
5	C	3L_387478_MNP	FBgn0262139	*trh*	INTRON	4.64E-06
5	C	3L_1364528_SNP	FBgn0003138	*Ptp61F*	INTRON	6.78E-06

5	C	2L_14436795_SNP	|||		3.41E-06
5	C	2R_7679336_SNP	FBgn0033652	*ths*	INTRON	3.59E-06
5	C	3L_9230633_SNP	FBgn0035970	*CG4483*	UPSTREAM	2.91E-06
5	C	2R_6832569_SNP	|||		6.14E-06
5	C	3L_14092589_SNP	|||		1.06E-05
5	C	2L_3251421_SNP	|||		4.89E-06
5	C	3L_4412294_SNP	FBgn0035542	*DOR*	INTRON	2.98E-06
5	C	2L_10602733_SNP	FBgn0051721	*Trim9*	INTRON	4.70E-06
5	C	3R_2811760_SNP	FBgn0260642	*Antp*	INTRON	6.03E-06
5	C	2L_14790095_DEL	|||		1.71E-05
5	C	2R_2117044_SNP	FBgn0263144	*bin3*	INTRON	5.65E-06
5	C	2R_2117176_SNP	FBgn0263144	*bin3*	INTRON	5.65E-06
5	C	2R_16810295_SNP	FBgn0020617	*Rx*	SYNONYMOUS_CODING	7.15E-06
5	C	2R_3009959_SNP	FBgn0263934	*esn*	INTRON	4.51E-06
5	C	3L_22009967_SNP	|||		5.66E-06
5	C	3L_18410489_SNP	|||		5.01E-06
5	C	X_13124674_SNP	FBgn0004456	*mew*	INTRON	4.71E-06
5	C	3L_19180874_SNP	FBgn0016797	*fz2*	INTRON	5.99E-06
5	C	3L_18155959_SNP	FBgn0036781	*CG13699*	UPSTREAM	9.80E-06
5	C	2R_6135265_SNP	FBgn0033499	*CG12914*	UPSTREAM	2.89E-06
5	C	3L_19180875_SNP	FBgn0016797	*fz2*	INTRON	5.29E-06
5	C	3L_19180885_SNP	FBgn0016797	*fz2*	INTRON	5.29E-06
5	C	2R_10216807_SNP	FBgn0085408	*Shroom*	SYNONYMOUS_CODING	1.10E-05
5	C	3L_22041217_SNP	FBgn0004514	*Oct-TyrR*	INTRON	5.56E-06
5	C	2L_19676638_SNP	FBgn0000464	*Lar*	INTRON	5.04E-06
5	C	2R_2116377_SNP	FBgn0263144	*bin3*	INTRON	6.56E-06
5	C	3L_22003667_SNP	|||		5.18E-06
5	C	2L_17460592_SNP	FBgn0000183	*BicD*	DOWNSTREAM	7.99E-06
5	C	X_13124649_SNP	FBgn0004456	*mew*	INTRON	6.01E-06
5	C	3R_2844723_SNP	|||		6.53E-06
5	C	3L_4401363_SNP	FBgn0035539	*slow*	INTRON	5.53E-06
5	C	2L_5113111_SNP	FBgn0261836	*Msp-300*	INTRON	6.30E-06
5	C	2L_17460588_INS	FBgn0000183	*BicD*	DOWNSTREAM	8.22E-06
5	C	2L_19678287_SNP	FBgn0000464	*Lar*	INTRON	4.99E-06
5	C	2L_19682939_SNP	FBgn0000464	*Lar*	INTRON	4.99E-06
5	C	3L_4411648_SNP	FBgn0035542	*DOR*	INTRON	3.84E-06
5	C	3L_7445106_SNP	FBgn0259935	*CG42458*	INTRON	6.79E-06
5	C	2L_18665482_SNP	FBgn0086200	*CG42490*	INTRON	6.44E-06
5	C	2R_3018330_SNP	FBgn0015039	*Cyp9b2*	SYNONYMOUS_CODING	7.46E-06
5	C	2L_10529499_SNP	FBgn0032264	*Lip4*	INTRON	6.62E-06
5	C	2L_10547036_SNP	FBgn0051721	*Trim9*	INTRON	7.24E-06
5	C	2L_12080933_SNP				7.30E-06
5	C	2L_9354241_SNP				1.31E-05
5	C	2L_4077009_SNP	FBgn0000547	*ed*	INTRON	8.20E-06
5	C	2L_11687269_SNP				8.19E-06
5	C	3L_21991927_SNP				7.00E-06
5	C	3L_12520476_SNP	FBgn0036298	*nst*	UTR_3_PRIME	8.22E-06
5	C	3L_728265_SNP				8.44E-06
5	C	X_20936141_SNP	FBgn0031150	*bves*	INTRON	3.80E-06
5	C	X_20561220_SNP				8.23E-06
5	C	2R_3009182_SNP	FBgn0263934	*esn*	INTRON	6.86E-06

5	C	3L_9239522_DEL				5.00E-06
5	C	3L_3001675_SNP	FBgn0035385	*FR*	UPSTREAM	9.22E-06
5	C	3L_12960532_SNP	FBgn0041622	*Or69a*	NON_SYNONYMOUS_CODING	1.36E-05
5	C	3L_18399692_SNP				9.17E-06
5	C	3L_730116_SNP				7.63E-06
5	C	3L_3001816_SNP	FBgn0035385	*FR*	UPSTREAM	8.44E-06
5	C	3L_9535396_SNP	FBgn0036010	*Ir67a*	NON_SYNONYMOUS_CODING	7.84E-06
5	C	3L_3677793_SNP				4.65E-06
5	C	2R_14092703_SNP				9.12E-06
5	C	2L_2067860_SNP	FBgn0053516	*dpr3*	INTRON	8.92E-06
5	C	3L_12206632_SNP	FBgn0260941	*app*	INTRON	7.31E-06
5	C	3L_12207964_SNP	FBgn0260941	*app*	INTRON	7.31E-06
5	C	3L_12211029_SNP	FBgn0260941	*app*	INTRON	7.31E-06
5	C	3R_26047809_SNP				8.13E-06
5	C	3L_1035032_SNP	FBgn0035181	*CG9205*	INTRON	7.99E-06
5	C	3L_12207965_SNP	FBgn0260941	*app*	INTRON	8.19E-06
5	C	2R_12509045_SNP	FBgn0034145	*CG5065*	NON_SYNONYMOUS_CODING	8.37E-06
5	C	3L_15996133_SNP	FBgn0036556	*CG5830*	INTRON	7.83E-06
5	C	2R_3010214_SNP	FBgn0263934	*esn*	INTRON	8.25E-06
5	C	3R_2707658_SNP				7.22E-06
5	C	2L_5928407_SNP	FBgn0015381	*dsf*	INTRON	1.05E-05
5	C	2L_16846383_SNP	FBgn0032614	*CG13284*	INTRON	9.46E-06
5	C	2L_18923274_SNP	FBgn0032723	*ssp3*	INTRON	9.74E-06
5	C	3R_13793867_SNP	FBgn0263995	*cpo*	INTRON	5.30E-06
5	C	2R_17085986_SNP	FBgn0034602	*Lapsyn*	INTRON	1.12E-05
5	C	3L_13007243_SNP	FBgn0036333	*MICAL-like*	NON_SYNONYMOUS_CODING	9.22E-06
5	C	2L_1058162_DEL	FBgn0003310	*S*	INTRON	1.48E-05
5	C	3L_12203246_SNP	FBgn0260941	*app*	INTRON	1.03E-05
5	C	3R_13793879_SNP	FBgn0263995	*cpo*	INTRON	5.53E-06
5	C	2R_9550693_SNP	FBgn0000633	*fas*	INTRON	8.22E-06
5	C	2L_19595369_SNP	FBgn0000464	*Lar*	INTRON	1.23E-05
5	C	3R_25854927_SNP	FBgn0026597	*Axn*	INTRON	7.60E-06
5	C	X_19461316_SNP	FBgn0000257	*car*	SYNONYMOUS_CODING	8.79E-06
5	C	2R_5551232_SNP	FBgn0033438	*Mmp2*	INTRON	1.16E-05
5	C	X_20561258_SNP				1.15E-05
5	C	3L_728191_SNP				1.03E-05
5	C	3L_9239519_SNP				7.57E-06
5	C	3L_15177321_SNP				9.31E-06
5	C	3R_25916441_SNP	FBgn0015542	*sima*	INTRON	8.55E-06
5	C	2R_3074979_SNP	FBgn0003090	*pk*	INTRON	7.78E-06
5	C	2R_3072386_SNP	FBgn0003090	*pk*	INTRON	6.30E-06
5	C	2L_18834177_SNP	FBgn0032717	*CG10600*	DOWNSTREAM	1.15E-05
5	C	3L_19616069_SNP	FBgn0036895	*CG9392*	SYNONYMOUS_CODING	1.17E-05
5	C	X_20936725_SNP	FBgn0031150	*bves*	INTRON	1.11E-05
5	C	3L_5491280_SNP	FBgn0035608	*blanks*	SYNONYMOUS_CODING	1.04E-05
5	C	3L_7183392_SNP	FBgn0265296	*Dscam2*	UPSTREAM	1.07E-05
5	C	X_20561285_SNP				1.28E-05
5	C	X_2585016_SNP	FBgn0003068	*per*	SYNONYMOUS_CODING	1.44E-05
5	C	3L_429310_SNP				1.06E-05
5	C	3L_729542_SNP				1.10E-05
5	C	2R_10524993_INS				9.93E-06

5	C	X_19980120_SNP				1.08E-05
5	C	3L_4408282_SNP	FBgn0035542	*DOR*	INTRON	9.43E-06
5	C	2L_3670446_SNP	FBgn0261054	*Sfp24Bc*	INTRON	1.24E-05
5	C	2R_17694342_SNP				1.63E-05
5	C	2R_17694350_SNP				1.63E-05
5	C	3R_2709189_SNP				8.84E-06
5	C	X_20561262_SNP				1.28E-05
5	C	2R_4165612_SNP	FBgn0265307	*CR44280*	DOWNSTREAM	1.33E-05
5	C	3L_14152529_SNP				1.14E-05
5	C	3L_729804_SNP				1.18E-05
5	C	2R_10529834_SNP				7.48E-06
5	C	3L_10367363_SNP				1.38E-05
5	C	3R_2709431_SNP				9.86E-06
5	C	3R_2850776_SNP				1.29E-05
5	C	3L_12335667_SNP	FBgn0036278	*GRHRII*	SYNONYMOUS_CODING	1.12E-05
5	C	3L_8955283_SNP	FBgn0035942	*CG5660*	INTRON	1.20E-05
5	C	2L_7448917_SNP	FBgn0025697	*santa-maria*	INTRON	1.50E-05
5	C	3L_747102_SNP	FBgn0035160	*CG13897*	INTRON	1.03E-05
5	C	X_20561193_SNP				1.14E-05
5	C	3L_4413195_SNP	FBgn0035542	*DOR*	INTRON	9.60E-06
5	C	2R_15484679_SNP	FBgn0003435	*sm*	INTRON	1.19E-05
5	C	2R_15484702_SNP	FBgn0003435	*sm*	INTRON	1.19E-05
5	C	2R_15484703_SNP	FBgn0003435	*sm*	INTRON	1.19E-05
5	C	3L_5184658_SNP	FBgn0052423	*shep*	INTRON	1.15E-05
5	C	3L_5184891_SNP	FBgn0052423	*shep*	INTRON	1.15E-05
5	C	3L_5184906_SNP	FBgn0052423	*shep*	INTRON	1.15E-05
5	C	3L_1035369_SNP	FBgn0035181	*CG9205*	UTR_5_PRIME	1.37E-05
5	C	3R_25857064_SNP	FBgn0026597	*Axn*	SYNONYMOUS_CODING	1.37E-05
5	C	3R_25860575_SNP	FBgn0264837	*CR44045*	EXON	1.37E-05
5	C	3L_8955313_SNP	FBgn0035942	*CG5660*	NON_SYNONYMOUS_CODING	1.35E-05
5	C	3L_8955326_SNP	FBgn0035942	*CG5660*	NON_SYNONYMOUS_CODING	1.35E-05
5	C	3L_14544617_SNP	FBgn0036428	*CG9238*	UPSTREAM	1.20E-05
5	C	2R_7550884_SNP	FBgn0044020	*Roc2*	INTRON	1.62E-05
5	C	3R_25850094_SNP	FBgn0026597	*Axn*	INTRON	1.30E-05
5	C	3R_13793877_SNP	FBgn0263995	*cpo*	INTRON	7.96E-06
5	C	3L_4411315_SNP	FBgn0035542	*DOR*	INTRON	9.15E-06
5	C	3R_9494312_SNP	FBgn0038165	*Task6*	UTR_3_PRIME	8.42E-06
1	L	3R_1289892_SNP	FBgn0037327	*PEK*	INTRON	3.03E-09
1	L	3R_1295688_SNP	FBgn0037330	*mRpL44*	SYNONYMOUS_CODING	9.30E-09
1	L	X_1797431_DEL	FBgn0264446	*CR43864*	EXON	1.25E-07
1	L	3R_1287165_SNP	FBgn0037327	*PEK*	NON_SYNONYMOUS_CODING	2.22E-08
1	L	3R_24518766_SNP	FBgn0039594	*CG9990*	INTRON	1.14E-07
1	L	3R_1297920_SNP	FBgn0011715	*Snr1*	NON_SYNONYMOUS_CODING	8.20E-08
1	L	3R_1291334_SNP	FBgn0037328	*RpL35A*	DOWNSTREAM	1.10E-07
1	L	3R_1297119_SNP	FBgn0011715	*Snr1*	INTRON	5.44E-08
1	L	3R_1252838_SNP	FBgn0037325	*CG12147*	NON_SYNONYMOUS_CODING	2.94E-08
1	L	3R_1293685_SNP	FBgn0037329	*CG12162*	UTR_5_PRIME	6.35E-07
1	L	3R_24522280_SNP	FBgn0027655	*htt*	INTRON	3.43E-07
1	L	3R_24822787_SNP	FBgn0039620	*CG1443*	INTRON	5.45E-08
1	L	3R_24822508_SNP	FBgn0039620	*CG1443*	INTRON	4.59E-08

1	L	3R_24518538_SNP	FBgn0039594	*CG9990*	INTRON	8.71E-07
1	L	3R_24518539_SNP	FBgn0039594	*CG9990*	INTRON	8.71E-07
1	L	3R_1284830_SNP	FBgn0037327	*PEK*	UTR_3_PRIME	4.49E-07
1	L	3R_1292660_SNP	FBgn0037328	*RpL35A*	INTRON	1.99E-07
1	L	X_1801269_SNP	FBgn0023511	*Edem1*	SYNONYMOUS_CODING	2.17E-07
1	L	3R_1300822_SNP	FBgn0037332	*Hcs*	UTR_5_PRIME	2.79E-07
1	L	3R_1279608_SNP	FBgn0037326	*CG14669*	INTRON	5.44E-07
1	L	3L_16252542_SNP	FBgn0040801	*CG13053*	UTR_5_PRIME	1.54E-07
1	L	X_1799900_SNP	FBgn0023511	*Edem1*	SYNONYMOUS_CODING	3.06E-07
1	L	3R_10987515_SNP	FBgn0038295	*Gyc88E*	INTRON	5.06E-07
1	L	X_7995298_SNP	FBgn0029997	*CG2258*	UTR_5_PRIME	3.42E-06
1	L	2R_17126498_SNP	FBgn0034606	*ASPP*	INTRON	9.62E-07
1	L	3L_10343750_SNP	FBgn0265415	*CR44327*	INTRON	1.24E-06
1	L	3L_13506826_SNP	FBgn0036376	*Liprin-beta*	SYNONYMOUS_CODING	9.62E-07
1	L	2R_18855643_SNP				1.15E-06
1	L	X_1797459_DEL	FBgn0264446	*CR43864*	EXON	4.45E-06
1	L	X_1798372_SNP	FBgn0023511	*Edem1*	INTRON	5.85E-07
1	L	3R_1306351_SNP	FBgn0037332	*Hcs*	INTRON	2.73E-06
1	L	3L_16300831_SNP	FBgn0036602	*CG13042*	DOWNSTREAM	1.75E-06
1	L	3R_2260376_SNP	FBgn0264495	*gpp*	INTRON	2.93E-07
1	L	2R_17693199_SNP				3.73E-06
1	L	3L_6097019_SNP	FBgn0005658	*Ets65A*	INTRON	2.03E-06
1	L	3R_2248710_SNP	FBgn0264495	*gpp*	INTRON	2.42E-06
1	L	3R_7256343_SNP	FBgn0037898	*CG18643*	INTRON	3.63E-07
1	L	3L_15504392_SNP	FBgn0000565	*Eip71CD*	INTRON	2.93E-07
1	L	3R_1283377_INS	FBgn0037326	*CG14669*	DOWNSTREAM	3.06E-06
1	L	3R_1283380_INS	FBgn0037326	*CG14669*	DOWNSTREAM	3.06E-06
1	L	3R_1279701_SNP	FBgn0037326	*CG14669*	INTRON	4.96E-06
1	L	3L_15504415_DEL	FBgn0000565	*Eip71CD*	INTRON	3.12E-07
1	L	2L_6446129_SNP	FBgn0031813	*CG9527*	SYNONYMOUS_CODING	2.59E-05
1	L	3R_10947861_SNP	FBgn0261859	*CG42788*	INTRON	2.81E-06
1	L	3R_7256598_SNP	FBgn0037899	*RpL24-like*	NON_SYNONYMOUS_CODING	1.68E-06
1	L	3L_16300822_SNP	FBgn0036602	*CG13042*	DOWNSTREAM	3.34E-06
1	L	3R_1296823_SNP	FBgn0011715	*Snr1*	SYNONYMOUS_CODING	3.61E-06
1	L	3R_1726662_SNP	FBgn0083949	*CG34113*	INTRON	1.81E-06
1	L	3R_5450762_SNP	FBgn0037720	*CG8312*	UTR_3_PRIME	3.69E-06
1	L	2R_17693230_SNP				2.77E-06
1	L	3L_11252214_SNP				1.81E-06
1	L	3R_1310115_SNP	FBgn0037332	*Hcs*	SYNONYMOUS_CODING	4.00E-06
1	L	2R_18860769_SNP				2.67E-06
1	L	2L_16864941_SNP				1.98E-05
1	L	3R_12658248_SNP	FBgn0264857	*iab-8*	INTRON	4.63E-06
1	L	3R_15192839_SNP				5.75E-05
1	L	3R_1260224_SNP	FBgn0037326	*CG14669*	INTRON	2.48E-06
1	L	2R_18867093_SNP				1.32E-05
1	L	3L_10991399_SNP	FBgn0013469	*klu*	INTRON	2.69E-06
1	L	3R_1285579_SNP	FBgn0037327	*PEK*	SYNONYMOUS_CODING	3.00E-06
1	L	3R_24534116_SNP	FBgn0027655	*htt*	INTRON	4.37E-06
1	L	3R_24519663_SNP	FBgn0039594	*CG9990*	INTRON	7.13E-06
1	L	3L_8915654_SNP	FBgn0035936	*Tsp66E*	INTRON	8.90E-06
1	L	3R_1264677_SNP	FBgn0037326	*CG14669*	INTRON	2.15E-06

1	L	3R_1264680_SNP	FBgn0037326	*CG14669*	INTRON	2.23E-06
1	L	3R_1705342_SNP	FBgn0083949	*CG34113*	INTRON	1.99E-06
1	L	2L_16493569_SNP	FBgn0032586	*Tpr2*	INTRON	2.94E-05
1	L	3R_1287839_SNP	FBgn0037327	*PEK*	NON_SYNONYMOUS_CODING	1.50E-05
1	L	2R_17709432_SNP	FBgn0034662	*CG13492*	SYNONYMOUS_CODING	2.01E-05
1	L	3R_24820754_SNP	FBgn0039620	*CG1443*	SYNONYMOUS_CODING	9.88E-07
1	L	3L_9204262_SNP	FBgn0035969	*CG4476*	UPSTREAM	3.29E-06
1	L	3R_13902257_SNP				4.95E-06
1	L	3R_1120202_SNP	FBgn0013576	*mtd*	SYNONYMOUS_CODING	2.53E-06
1	L	3R_1645087_SNP	FBgn0037382	*Hpr1*	SYNONYMOUS_CODING	9.47E-06
1	L	3R_2248807_SNP	FBgn0264495	*gpp*	INTRON	1.98E-06
1	L	3L_1752750_SNP	FBgn0022702	*Cht2*	SYNONYMOUS_CODING	2.09E-06
1	L	3R_1067910_SNP	FBgn0260462	*CG12163*	DOWNSTREAM	3.13E-06
1	L	2R_3841980_SNP	FBgn0033236	*CG14764*	INTRON	7.06E-06
1	L	2R_18863015_SNP				1.10E-05
1	L	3R_1060052_SNP	FBgn0037301	*Mms19*	NON_SYNONYMOUS_CODING	2.67E-06
1	L	3R_7303305_SNP				2.45E-05
1	L	3R_24519016_SNP	FBgn0039594	*CG9990*	INTRON	1.07E-05
1	L	2L_4232146_SNP				1.38E-05
1	L	2L_4232148_SNP				1.38E-05
1	L	3L_21409054_SNP	FBgn0261258	*rgn*	INTRON	0.0001421
1	L	3R_15192779_SNP				4.47E-05
1	L	2R_11879749_SNP	FBgn0034058	*Pex11*	NON_SYNONYMOUS_CODING	9.26E-07
1	L	2R_18855712_DEL				5.90E-06
1	L	2R_18434571_SNP	FBgn0003175	*px*	INTRON	1.41E-05
1	L	3R_1121849_SNP	FBgn0013576	*mtd*	INTRON	3.00E-06
1	L	3R_1129679_SNP	FBgn0013576	*mtd*	INTRON	3.00E-06
1	L	3L_16299771_SNP	FBgn0036601	*CG13063*	INTRON	1.74E-05
1	L	3L_15591491_SNP	FBgn0036518	*RhoGAP71E*	INTRON	5.52E-05
1	L	2R_15129720_SNP	FBgn0010434	*cora*	SYNONYMOUS_CODING	2.86E-06
1	L	3R_1251661_SNP	FBgn0037325	*CG12147*	SYNONYMOUS_CODING	9.99E-06
1	L	3L_1090136_SNP	FBgn0004870	*bab1*	INTRON	2.19E-05
1	L	3L_13482978_SNP	FBgn0036373	*CG10741*	INTRON	1.18E-05
1	L	3R_2130834_SNP	FBgn0051561	*Osi16*	UPSTREAM	1.14E-05
1	L	3L_15766850_SNP				3.40E-06
1	L	3R_22303962_SNP	FBgn0039431	*CG6490*	INTRON	7.43E-06
1	L	2R_13496603_SNP	FBgn0034253	*CG10936*	SYNONYMOUS_CODING	4.79E-06
1	L	3L_16300839_SNP	FBgn0036602	*CG13042*	DOWNSTREAM	8.18E-06
1	L	3R_26870330_SNP	FBgn0053920	*CG33920*	NON_SYNONYMOUS_CODING	4.53E-06
1	L	3R_2248695_SNP	FBgn0264495	*gpp*	INTRON	2.62E-06
1	L	3R_2206262_SNP	FBgn0010282	*TfIIFalpha*	UTR_5_PRIME	1.50E-06
1	L	2R_5115029_INS	FBgn0010114	*hig*	DOWNSTREAM	1.77E-06
1	L	2R_18899287_SNP	FBgn0261705	*CG42741*	UTR_3_PRIME	3.74E-06
1	L	2R_19525497_SNP	FBgn0004795	*retn*	INTRON	8.32E-06
1	L	3R_26872843_SNP	FBgn0010015	*CanA1*	DOWNSTREAM	5.53E-06
1	L	3R_2222911_SNP	FBgn0037443	*CG1021*	INTRON	6.65E-06
1	L	3R_26871365_SNP	FBgn0010015	*CanA1*	INTRON	7.62E-06
1	L	3R_26870500_SNP	FBgn0053920	*CG33920*	NON_SYNONYMOUS_CODING	6.87E-06
1	L	3L_7145947_SNP	FBgn0259173	*corn*	INTRON	5.50E-06
1	L	2L_16610446_SNP	FBgn0259735	*CG42389*	INTRON	9.37E-06
1	L	3R_7261004_SNP	FBgn0037901	*CG6744*	NON_SYNONYMOUS_CODING	8.78E-06

1	L	3R_26871176_SNP	FBgn0010015	*CanA1*	INTRON	8.57E-06
1	L	2L_17806199_SNP	FBgn0262018	*CadN2*	INTRON	7.93E-06
1	L	3L_2572860_SNP	FBgn0010909	*msn*	INTRON	7.38E-06
1	L	3R_1293243_SNP	FBgn0037329	*CG12162*	UPSTREAM	8.30E-06
1	L	2R_1687881_SNP				7.30E-06
1	L	3R_24822865_SNP	FBgn0039620	*CG1443*	INTRON	4.02E-06
1	L	2R_1687822_SNP				8.98E-06
1	L	3R_26870374_SNP	FBgn0053920	*CG33920*	SYNONYMOUS_CODING	8.98E-06
1	L	2R_1687832_SNP				9.93E-06
1	L	3L_10261057_SNP	FBgn0011569	*can*	UPSTREAM	7.30E-06
1	L	3L_15504358_SNP	FBgn0000565	*Eip71CD*	INTRON	5.72E-06
1	L	3R_26870989_SNP	FBgn0010015	*CanA1*	INTRON	7.23E-06
1	L	2R_4356187_SNP	FBgn0033296	*Mal-A7*	DOWNSTREAM	6.10E-06
1	L	3R_26871052_DEL	FBgn0010015	*CanA1*	INTRON	8.51E-06
1	L	3R_26872447_SNP	FBgn0010015	*CanA1*	UTR_3_PRIME	8.21E-06
1	L	2R_5212477_SNP	FBgn0033403	*CG13739*	INTRON	9.71E-06
1	L	3R_26871306_SNP	FBgn0010015	*CanA1*	INTRON	9.19E-06
1	L	3R_26871309_SNP	FBgn0010015	*CanA1*	INTRON	9.19E-06
1	L	X_1798301_SNP	FBgn0023511	*Edem1*	DOWNSTREAM	4.08E-06
5	L	3L_8454729_SNP	FBgn0010825	*Gug*	INTRON	1.92E-08
5	L	2L_14425847_SNP	FBgn0028871	*Cpr35B*	UPSTREAM	6.99E-08
5	L	3L_9535396_SNP	FBgn0036010	*Ir67a*	NON_SYNONYMOUS_CODING	6.66E-07
5	L	3L_21253627_SNP	FBgn0004865	*Eip78C*	INTRON	1.49E-06
5	L	3L_2998427_SNP				3.15E-07
5	L	2L_7379041_SNP	FBgn0002938	*ninaC*	SYNONYMOUS_CODING	2.35E-06
5	L	X_20969348_SNP	FBgn0064123	*stg1*	INTRON	5.49E-06
5	L	X_20687828_SNP	FBgn0085387	*shakB*	INTRON	1.13E-06
5	L	3L_22706140_SNP	FBgn0037181	*CG11370*	NON_SYNONYMOUS_CODING	1.79E-05
5	L	3R_2811760_SNP	FBgn0260642	*Antp*	INTRON	1.04E-05
5	L	3R_1967895_SNP				4.12E-06
5	L	2L_8701419_SNP	FBgn0004914	*Hnf4*	INTRON	1.08E-05
5	L	3L_4394183_SNP	FBgn0035539	*slow*	INTRON	1.56E-06
5	L	2R_12509045_SNP	FBgn0034145	*CG5065*	NON_SYNONYMOUS_CODING	5.91E-06
5	L	2L_3670446_SNP	FBgn0261054	*Sfp24Bc*	INTRON	9.04E-06
5	L	3R_10659555_SNP	FBgn0264754	*btsz*	INTRON	5.58E-06
5	L	3R_25192358_SNP				4.72E-06
5	L	2L_7459166_SNP	FBgn0045495	*Gr28b*	INTRON	3.98E-05
5	L	2R_9550693_SNP	FBgn0000633	*fas*	INTRON	1.19E-05
5	L	3L_2999056_SNP				2.90E-06
5	L	2R_15935444_SNP				1.23E-05
5	L	3L_7455522_SNP				4.06E-06
5	L	X_6402103_SNP	FBgn0259242	*CG42340*	INTRON	2.01E-05
5	L	X_6402098_DEL	FBgn0259242	*CG42340*	INTRON	1.89E-05
5	L	2L_17470364_SNP	FBgn0000183	*BicD*	INTRON	1.07E-05
5	L	X_6104649_SNP				3.79E-05
5	L	3L_5418361_SNP				5.45E-06
5	L	X_8299253_SNP	FBgn0030035	*CG11190*	SYNONYMOUS_CODING	3.85E-05
5	L	3R_17142885_SNP	FBgn0001234	*Hsromega*	EXON	1.09E-05
5	L	2L_3372585_SNP	FBgn0085423	*CG34394*	INTRON	0.000103
5	L	3R_2944832_SNP	FBgn0261238	*Alh*	INTRON	1.45E-05
5	L	3L_4688618_SNP	FBgn0035567	*CG7514*	SYNONYMOUS_CODING	2.40E-05

5	L	3L_12717454_SNP				1.80E-05
5	L	3R_2739484_SNP	FBgn0260642	*Antp*	INTRON	1.34E-05
5	L	X_20938898_SNP	FBgn0031150	*bves*	INTRON	8.59E-06
5	L	3R_2740508_SNP	FBgn0260642	*Antp*	INTRON	1.38E-05
5	L	3L_2328425_SNP	FBgn0035331	*DmsR-1*	INTRON	1.22E-05
5	L	3R_17142517_SNP	FBgn0001234	*Hsromega*	EXON	5.27E-06
5	L	2L_9453764_SNP	FBgn0002973	*numb*	INTRON	2.93E-05
5	L	3R_2550742_SNP	FBgn0051481	*pb*	INTRON	4.05E-05
5	L	3R_2739833_SNP	FBgn0260642	*Antp*	INTRON	1.75E-05
5	L	3L_7444737_SNP	FBgn0259935	*CG42458*	INTRON	4.03E-06
5	L	2L_12957252_SNP				6.33E-06
5	L	3R_17142781_SNP	FBgn0001234	*Hsromega*	EXON	3.92E-06
5	L	3R_17142739_SNP	FBgn0001234	*Hsromega*	EXON	2.62E-06
5	L	3R_17142576_SNP	FBgn0001234	*Hsromega*	EXON	4.97E-06
5	L	3R_17142534_SNP	FBgn0001234	*Hsromega*	EXON	5.63E-06
5	L	3L_7442707_SNP	FBgn0259935	*CG42458*	INTRON	8.08E-06
5	L	3L_2999052_SNP				6.33E-06
5	L	3L_7442722_SNP	FBgn0259935	*CG42458*	INTRON	9.20E-06
5	L	3L_7459855_SNP	FBgn0035786	*Tsp66A*	INTRON	9.63E-06
5	L	X_20571210_SNP				3.92E-06

2. Endurance GWA results

Age	Trt	Chrom/Pos	FlyBase ID	Gene Symbol	Site Class	SNP *P*-value
1	C	3R_26470169_SNP	FBgn0051013	*CG31013*	INTRON	3.45E-07
1	C	X_20571194_SNP				7.76E-08
1	C	3R_24068525_SNP				6.25E-07
1	C	3R_26469967_SNP	FBgn0051013	*CG31013*	INTRON	3.01E-07
1	C	X_20571199_SNP				1.75E-07
1	C	3R_26468899_SNP	FBgn0051013	*CG31013*	DOWNSTREAM	7.64E-07
1	C	X_20571220_SNP				3.18E-07
1	C	X_20571225_SNP				3.18E-07
1	C	X_20571195_SNP				3.19E-07
1	C	2R_906244_SNP	FBgn0040849	*Ir41a*	INTRON	2.14E-06
1	C	3R_26470279_SNP	FBgn0051013	*CG31013*	SYNONYMOUS_CODING	1.49E-06
1	C	3L_5742867_SNP	FBgn0085447	*sif*	DOWNSTREAM	3.22E-06
1	C	3R_14045925_SNP	FBgn0038583	*CG7183*	SYNONYMOUS_CODING	2.89E-06
1	C	3L_13145353_SNP				3.10E-06
1	C	3L_13145307_SNP				3.80E-06
1	C	3R_12933323_SNP	FBgn0014141	*cher*	SYNONYMOUS_CODING	4.25E-06
1	C	X_20571192_SNP				3.21E-06
1	C	X_20571210_SNP				1.54E-06
1	C	X_10463425_SNP	FBgn0085443	*spri*	INTRON	8.12E-06
1	C	3L_2658433_SNP	FBgn0264606	*CG43955*	INTRON	5.42E-06
1	C	X_20571391_DEL				1.55E-06
1	C	X_10881200_SNP	FBgn0259241	*CG42339*	INTRON	9.09E-06
1	C	3L_5742903_SNP	FBgn0085447	*sif*	DOWNSTREAM	6.93E-06
1	C	3R_24114971_SNP				1.76E-05
1	C	2L_17056278_SNP				1.16E-05
1	C	3L_4922980_SNP				1.05E-05
1	C	X_20571201_MNP				2.19E-06
1	C	2L_12430105_SNP	FBgn0032431	*CG5435*	SYNONYMOUS_CODING	1.13E-05
1	C	X_20571211_INS				4.07E-06
5	C	2L_21909142_SNP	FBgn0032967	*CG1428*	DOWNSTREAM	2.23E-10
5	C	2L_21920643_SNP				5.56E-08
5	C	2L_21920650_SNP				5.56E-08
5	C	2L_21878705_SNP				2.48E-07
5	C	3L_8377863_SNP	FBgn0001253	*ImpE1*	INTRON	7.23E-08
5	C	2L_21941377_SNP				1.17E-06
5	C	2L_21743112_SNP	FBgn0086779	*step*	INTRON	7.44E-07
5	C	2L_21923221_SNP				1.87E-06
5	C	3L_8379798_SNP	FBgn0001253	*ImpE1*	NON_SYNONYMOUS_CODING	5.92E-07
5	C	X_16737118_SNP	FBgn0024941	*RSG7*	INTRON	2.04E-06
5	C	3L_8379799_SNP	FBgn0001253	*ImpE1*	SYNONYMOUS_CODING	6.37E-07
5	C	3L_8380005_SNP	FBgn0001253	*ImpE1*	NON_SYNONYMOUS_CODING	5.01E-07
5	C	2L_21909299_SNP	FBgn0032967	*CG1428*	DOWNSTREAM	1.53E-06
5	C	2L_21909304_SNP	FBgn0032967	*CG1428*	DOWNSTREAM	1.53E-06
5	C	2L_18945759_SNP	FBgn0262095	*CG42848*	UPSTREAM	6.68E-08
5	C	2L_21929985_SNP				1.38E-06
5	C	2L_18938333_SNP	FBgn0032723	*ssp3*	INTRON	7.74E-08
5	C	2L_13088247_SNP				1.02E-05

5	C	2L_13088409_SNP				1.02E-05
5	C	2R_2918687_SNP	FBgn0033128	*Tsp42Eg*	INTRON	1.20E-05
5	C	3L_16004067_SNP	FBgn0263601	*mib1*	INTRON	5.63E-07
5	C	2L_18945751_SNP	FBgn0262095	*CG42848*	UPSTREAM	4.82E-08
5	C	3L_8377428_SNP	FBgn0001253	*ImpE1*	INTRON	1.19E-06
5	C	2L_21848891_SNP				2.92E-06
5	C	2L_22002879_INS				4.47E-06
5	C	3L_18157137_SNP				1.16E-05
5	C	2L_18939130_SNP	FBgn0032723	*ssp3*	INTRON	1.52E-07
5	C	2L_21741296_SNP	FBgn0086779	*step*	DOWNSTREAM	3.20E-06
5	C	2L_21910025_SNP				4.88E-06
5	C	2L_21910054_SNP				4.88E-06
5	C	2L_18945924_SNP	FBgn0262095	*CG42848*	UPSTREAM	8.61E-08
5	C	2L_18946801_SNP	FBgn0262095	*CG42848*	SYNONYMOUS_CODING	8.61E-08
5	C	2L_18926292_SNP	FBgn0032723	*ssp3*	INTRON	4.18E-08
5	C	2L_18926452_SNP	FBgn0032723	*ssp3*	INTRON	5.40E-08
5	C	X_20409940_SNP	FBgn0259162	*RunxB*	INTRON	2.93E-06
5	C	3L_12824822_SNP	FBgn0036316	*CG10960*	INTRON	4.91E-07
5	C	3L_12824847_SNP	FBgn0036316	*CG10960*	INTRON	4.91E-07
5	C	3L_12830032_SNP	FBgn0036316	*CG10960*	INTRON	4.91E-07
5	C	2L_18923668_SNP	FBgn0032723	*ssp3*	INTRON	4.88E-08
5	C	3L_12830016_DEL	FBgn0036316	*CG10960*	INTRON	5.62E-07
5	C	3L_12823015_SNP	FBgn0036316	*CG10960*	INTRON	1.85E-07
5	C	2L_21847033_SNP				4.64E-06
5	C	3L_12725274_DEL				9.45E-08
5	C	2L_9077091_SNP	FBgn0032094	*CG12439*	UPSTREAM	7.64E-05
5	C	3L_12830005_SNP	FBgn0036316	*CG10960*	INTRON	9.02E-07
5	C	3L_12830009_SNP	FBgn0036316	*CG10960*	INTRON	9.02E-07
5	C	3L_12830011_DEL	FBgn0036316	*CG10960*	INTRON	9.02E-07
5	C	3L_12830014_SNP	FBgn0036316	*CG10960*	INTRON	9.02E-07
5	C	3R_19512464_SNP	FBgn0039102	*SPE*	NON_SYNONYMOUS_CODING	1.38E-06
5	C	X_12148384_INS	FBgn0259240	*Ten-a*	INTRON	1.33E-05
5	C	3L_8378366_SNP	FBgn0001253	*ImpE1*	INTRON	2.82E-06
5	C	2L_18947480_SNP	FBgn0262095	*CG42848*	DOWNSTREAM	1.62E-07
5	C	2L_21728683_SNP	FBgn0051619	*CG31619*	UTR_3_PRIME	5.64E-06
5	C	2L_21824028_SNP				9.98E-06
5	C	3L_21255282_SNP	FBgn0004865	*Eip78C*	INTRON	2.99E-06
5	C	2L_8312824_SNP	FBgn0032018	*CG7806*	SYNONYMOUS_CODING	2.03E-05
5	C	2L_19829160_SNP	FBgn0263873	*sick*	INTRON	3.67E-06
5	C	2L_8131794_SNP	FBgn0031988	*CG8668*	SYNONYMOUS_CODING	1.48E-07
5	C	3L_11249553_SNP				4.42E-06
5	C	3L_12821515_SNP	FBgn0036316	*CG10960*	INTRON	6.27E-07
5	C	2L_21594134_SNP				1.25E-05
5	C	3L_21253931_SNP	FBgn0004865	*Eip78C*	INTRON	4.82E-06
5	C	2L_21754583_SNP	FBgn0086779	*step*	INTRON	8.07E-07
5	C	3L_18156021_SNP	FBgn0036781	*CG13699*	UPSTREAM	1.83E-07
5	C	3L_16004055_SNP	FBgn0263601	*mib1*	INTRON	1.06E-06
5	C	3L_12724376_SNP				2.27E-06
5	C	3L_8379561_SNP	FBgn0001253	*ImpE1*	NON_SYNONYMOUS_CODING	8.36E-06
5	C	3L_2238442_SNP	FBgn0040507	*ACXD*	SYNONYMOUS_CODING	2.06E-07
5	C	2L_21823667_SNP				1.04E-05

5	C	3L_8374983_SNP	FBgn0001253	*ImpE1*	INTRON	2.65E-06
5	C	X_16737136_SNP	FBgn0024941	*RSG7*	INTRON	7.11E-06
5	C	3L_13119247_SNP				1.72E-05
5	C	2L_11701943_SNP				4.57E-06
5	C	3L_4097622_SNP	FBgn0035497	*CG14995*	NON_SYNONYMOUS_CODING	3.05E-06
5	C	3L_18155052_SNP	FBgn0036781	*CG13699*	INTRON	9.40E-07
5	C	X_20561193_SNP				7.39E-06
5	C	3L_12476856_SNP	FBgn0036287	*CG10663*	INTRON	5.22E-07
5	C	3L_18155170_SNP	FBgn0036781	*CG13699*	INTRON	9.33E-07
5	C	3L_10530403_SNP	FBgn0052062	*A2bp1*	INTRON	4.18E-06
5	C	2L_1877731_SNP	FBgn0051663	*CG31663*	INTRON	9.20E-05
5	C	X_20571391_DEL				1.56E-06
5	C	2R_16954770_DEL	FBgn0022700	*Cht4*	UPSTREAM	3.96E-06
5	C	2L_21725893_SNP	FBgn0051619	*CG31619*	INTRON	1.06E-05
5	C	2L_13088042_SNP				6.12E-07
5	C	2R_16955711_SNP	FBgn0034582	*Cht9*	SYNONYMOUS_CODING	4.26E-05
5	C	2L_6484832_SNP	FBgn0031820	*DLP*	NON_SYNONYMOUS_CODING	1.30E-06
5	C	3L_12125053_SNP	FBgn0046296	*CG11534*	UPSTREAM	8.78E-06
5	C	3L_3029602_SNP				1.48E-05
5	C	3L_16004079_INS	FBgn0263601	*mib1*	INTRON	2.62E-06
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5	C	3L_13296591_SNP	FBgn0264512	*CR43911*	DOWNSTREAM	4.44E-06
5	C	3L_13296592_SNP	FBgn0264512	*CR43911*	DOWNSTREAM	4.44E-06
5	C	3L_10530258_SNP	FBgn0052062	*A2bp1*	INTRON	8.38E-06
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5	C	3L_13251896_SNP	FBgn0023095	*caps*	INTRON	9.22E-06
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5	C	3L_11751885_SNP	FBgn0036202	*CG6024*	INTRON	2.13E-06
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5	C	2L_21999043_SNP				4.00E-06
5	C	3L_12656799_SNP				6.99E-06
5	C	3L_12724375_SNP				2.21E-06
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5	C	3L_9192943_SNP				8.66E-06
5	C	2R_396553_SNP	FBgn0264909	*CR44100*	DOWNSTREAM	5.01E-06
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5	C	2L_1061920_SNP	FBgn0003310	*S*	NON_SYNONYMOUS_CODING	7.26E-06
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5	C	3L_16004047_SNP	FBgn0263601	*mib1*	INTRON	2.30E-06
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5	C	2L_19976452_SNP	FBgn0264443	*CG43861*	INTRON	5.28E-07
5	C	3L_12822371_SNP	FBgn0036316	*CG10960*	INTRON	2.47E-06

5	C	3L_12964146_SNP	FBgn0036327	*CG10748*	DOWNSTREAM	1.71E-06
5	C	3L_12927379_SNP				6.46E-06
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5	C	3L_12927393_SNP				5.78E-06
5	C	2L_8132028_SNP	FBgn0031988	*CG8668*	SYNONYMOUS_CODING	6.24E-07
5	C	X_20571195_SNP				4.98E-06
5	C	3L_13002834_SNP	FBgn0036332	*CG11261*	NON_SYNONYMOUS_CODING	3.59E-07
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5	C	X_20571338_SNP				5.32E-06
5	C	X_21848777_SNP	FBgn0031183	*CG14621*	SYNONYMOUS_CODING	1.60E-06
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5	C	2L_8130921_SNP	FBgn0031988	*CG8668*	INTRON	9.72E-07
5	C	X_20571220_SNP				7.75E-06
5	C	X_20571225_SNP				7.75E-06
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5	C	2R_15139913_INS	FBgn0053453	*CG33453*	DOWNSTREAM	1.75E-06
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5	C	3R_3461679_SNP	FBgn0083963	*CG34127*	INTRON	6.16E-06
5	C	2L_21875209_SNP				9.96E-06
5	C	3L_13231487_SNP	FBgn0023095	*caps*	INTRON	9.18E-06
5	C	2L_13103081_SNP				3.79E-06
5	C	3L_11751918_SNP	FBgn0036202	*CG6024*	INTRON	9.90E-06
5	C	3L_18150461_SNP	FBgn0036781	*CG13699*	INTRON	7.22E-06
5	C	2R_288444_SNP	FBgn0260798	*Gprk1*	INTRON	8.13E-06
5	C	3L_9655763_SNP	FBgn0016081	*fry*	INTRON	1.53E-06
5	C	2L_11590944_DEL	FBgn0046212	*CG15841*	DOWNSTREAM	3.67E-07
5	C	3L_12727290_SNP	FBgn0261933	*SmD1*	INTRON	7.04E-06
5	C	2L_19936584_SNP	FBgn0263873	*sick*	INTRON	9.33E-07
5	C	3R_10521342_SNP	FBgn0265140	*Meltrin*	INTRON	2.62E-06
5	C	2R_15139909_SNP	FBgn0053453	*CG33453*	DOWNSTREAM	2.13E-06
5	C	2R_15139910_SNP	FBgn0053453	*CG33453*	DOWNSTREAM	2.13E-06
5	C	3L_12725007_SNP				3.96E-07
5	C	2R_396941_SNP	FBgn0050260	*tRNA:CR30260*	UPSTREAM	8.87E-06
5	C	3R_9786134_SNP	FBgn0016672	*lpp*	UTR_3_PRIME	8.77E-06
5	C	2R_15139905_DEL	FBgn0053453	*CG33453*	DOWNSTREAM	2.47E-06
5	C	3R_3461672_SNP	FBgn0083963	*CG34127*	INTRON	7.51E-06
5	C	3L_12833427_SNP	FBgn0036316	*CG10960*	INTRON	2.49E-06
5	C	2L_18936862_SNP	FBgn0032723	*ssp3*	INTRON	7.97E-06
5	C	2L_1058162_DEL	FBgn0003310	*S*	INTRON	6.55E-06
5	C	3L_21968394_SNP	FBgn0037146	*CG7470*	INTRON	4.03E-06
5	C	3L_12815870_SNP	FBgn0260965	*CG42588*	NON_SYNONYMOUS_CODING	3.70E-06
5	C	2L_13103067_SNP				5.17E-06
5	C	3L_12822965_SNP	FBgn0036316	*CG10960*	INTRON	5.26E-06
5	C	3L_12813843_SNP	FBgn0260965	*CG42588*	NON_SYNONYMOUS_CODING	4.34E-06
5	C	3L_12802921_SNP	FBgn0262714	*Sap130*	INTRON	4.23E-06
5	C	3L_12808938_SNP	FBgn0260965	*CG42588*	INTRON	4.23E-06

5	C	3L_12814032_SNP	FBgn0260965	*CG42588*	NON_SYNONYMOUS_CODING	4.23E-06
5	C	2L_19933914_SNP	FBgn0263873	*sick*	INTRON	1.29E-06
5	C	3L_13528968_DEL	FBgn0264001	*bru-3*	DOWNSTREAM	7.81E-06
5	C	2L_20405095_SNP	FBgn0032857	*CG10947*	INTRON	6.24E-06
5	C	3L_4417369_SNP	FBgn0035542	*DOR*	INTRON	2.76E-06
5	C	3L_11751967_SNP	FBgn0036202	*CG6024*	INTRON	9.01E-06
5	C	3L_18156038_SNP	FBgn0036781	*CG13699*	UPSTREAM	8.96E-06
5	C	2L_1061262_SNP	FBgn0003310	*S*	SYNONYMOUS_CODING	3.88E-06
5	C	X_12395843_SNP	FBgn0030396	*CG2556*	INTRON	9.28E-06
5	C	2L_19909272_SNP	FBgn0263873	*sick*	SYNONYMOUS_CODING	6.39E-06
5	C	2R_2995480_DEL	FBgn0263934	*esn*	INTRON	2.11E-06
5	C	2R_15139937_SNP	FBgn0053453	*CG33453*	DOWNSTREAM	3.87E-06
5	C	3L_18156032_SNP	FBgn0036781	*CG13699*	UPSTREAM	5.16E-06
5	C	3L_21968129_SNP	FBgn0037146	*CG7470*	INTRON	6.32E-06
5	C	3L_5878794_SNP				4.44E-06
5	C	2R_6218177_SNP	FBgn0261698	*CG42732*	INTRON	1.97E-06
5	C	3L_12816844_SNP	FBgn0260965	*CG42588*	INTRON	8.49E-06
5	C	3L_13528965_SNP	FBgn0264001	*bru-3*	DOWNSTREAM	7.26E-06
5	C	3L_13528966_INS	FBgn0264001	*bru-3*	DOWNSTREAM	8.10E-06
5	C	2R_14581030_SNP	FBgn0262103	*Sik3*	INTRON	5.03E-06
5	C	3L_12727267_SNP	FBgn0261933	*SmD1*	INTRON	5.56E-06
5	C	3L_12724801_SNP				4.80E-06
5	C	3L_12724811_SNP				4.72E-06
5	C	2L_8131935_MNP	FBgn0031988	*CG8668*	INTRON	5.17E-06
5	C	3L_3005820_SNP	FBgn0035385	*FR*	INTRON	7.56E-06
5	C	3L_12727288_SNP	FBgn0261933	*SmD1*	INTRON	6.15E-06
5	C	3L_4393248_SNP	FBgn0035539	*slow*	INTRON	2.33E-06
5	C	3L_10170407_SNP	FBgn0052057	*dpr10*	INTRON	1.67E-06
5	C	2R_15139977_SNP	FBgn0053453	*CG33453*	DOWNSTREAM	6.99E-06
5	C	3L_15642077_DEL	FBgn0262529	*CG43083*	INTRON	7.03E-06
5	C	2L_8130627_SNP	FBgn0031988	*CG8668*	INTRON	8.14E-06
5	C	X_19574743_SNP	FBgn0043903	*dome*	INTRON	8.80E-06
5	C	2L_8136459_SNP	FBgn0031990	*CG8552*	SYNONYMOUS_CODING	3.93E-06
5	C	3L_12725838_SNP	FBgn0261933	*SmD1*	DOWNSTREAM	6.79E-06
5	C	3L_11447064_SNP				5.43E-06
5	C	2L_18921415_SNP	FBgn0032723	*ssp3*	INTRON	6.77E-06
5	C	3L_757851_SNP				7.18E-06
5	C	3L_12411005_SNP	FBgn0020655	*Gap69C*	UPSTREAM	4.45E-06
5	C	2L_18919585_SNP	FBgn0032723	*ssp3*	INTRON	7.62E-06
5	C	2R_15139956_SNP	FBgn0053453	*CG33453*	DOWNSTREAM	9.10E-06
5	C	3L_12725885_SNP	FBgn0261933	*SmD1*	DOWNSTREAM	8.77E-06
5	C	2L_18920846_SNP	FBgn0032723	*ssp3*	INTRON	8.08E-06
5	C	2R_15139942_DEL	FBgn0053453	*CG33453*	DOWNSTREAM	9.70E-06
5	C	3L_12727296_SNP	FBgn0261933	*SmD1*	INTRON	8.65E-06
5	C	3L_12727300_SNP	FBgn0261933	*SmD1*	INTRON	8.65E-06
5	C	3L_11447058_SNP				6.82E-06
5	C	2L_13103053_SNP				1.77E-06
5	C	3L_4415856_SNP	FBgn0035542	*DOR*	INTRON	3.30E-06
5	C	3L_12726310_INS	FBgn0261933	*SmD1*	DOWNSTREAM	8.85E-06
5	C	3L_12648800_SNP				8.29E-06
5	C	2L_20487695_SNP	FBgn0262455	*mir-1*	DOWNSTREAM	4.33E-06

5	C	3R_2112850_SNP	FBgn0037421	*CG15594*	UPSTREAM	5.49E-06
5	C	3L_9240152_DEL				6.05E-06
5	C	3L_12661193_DEL				4.82E-06
5	C	3L_18155991_SNP	FBgn0036781	*CG13699*	UPSTREAM	4.90E-06
5	C	3L_4418646_SNP	FBgn0035542	*DOR*	INTRON	5.60E-06
5	C	2L_20487693_SNP	FBgn0262455	*mir-1*	DOWNSTREAM	5.54E-06
5	C	2L_13103091_SNP				3.40E-06
5	C	3L_12337936_SNP	FBgn0036278	*GRHRII*	INTRON	8.77E-06
5	C	2L_13102922_SNP				5.17E-06
5	C	3L_12849677_SNP	FBgn0036319	*Ent3*	UTR_3_PRIME	8.44E-06
5	C	3L_9239853_SNP				8.56E-06
5	C	3L_10984806_DEL	FBgn0013469	*klu*	INTRON	4.58E-06
5	C	3L_12722016_SNP				8.97E-06
5	C	3L_18924335_INS	FBgn0052204	*CG32204*	INTRON	9.12E-06
5	C	2L_5499546_SNP				9.06E-06
5	C	3L_12829558_SNP	FBgn0036316	*CG10960*	INTRON	8.73E-06
1	L	3L_4921245_SNP				3.24E-07
1	L	X_20571220_SNP				6.73E-07
1	L	X_20571225_SNP				6.73E-07
1	L	X_4216138_SNP	FBgn0040907	*mRpL33*	SYNONYMOUS_CODING	2.43E-06
1	L	X_16200557_SNP	FBgn0027521	*CG3679*	UTR_3_PRIME	3.06E-06
1	L	3L_2680111_SNP	FBgn0264606	*CG43955*	INTRON	3.44E-06
1	L	3R_23368163_SNP	FBgn0046887	*Gr98b*	UPSTREAM	2.43E-06
1	L	X_20571199_SNP				9.27E-07
1	L	3L_2680106_SNP	FBgn0264606	*CG43955*	INTRON	4.19E-06
1	L	X_4216132_SNP	FBgn0040907	*mRpL33*	SYNONYMOUS_CODING	5.37E-06
1	L	X_15540200_SNP				4.33E-06
1	L	X_16200544_SNP	FBgn0027521	*CG3679*	UTR_3_PRIME	5.14E-06
1	L	3R_26469967_SNP	FBgn0051013	*CG31013*	INTRON	3.22E-06
1	L	X_20571194_SNP				2.88E-06
1	L	X_15463002_SNP	FBgn0030653	*CG7860*	UPSTREAM	6.71E-06
1	L	2R_6493992_SNP	FBgn0263102	*psq*	INTRON	5.57E-06
1	L	3R_24068525_SNP				1.19E-05
1	L	3L_2680382_INS	FBgn0264606	*CG43955*	INTRON	8.13E-06
1	L	X_3060180_SNP	FBgn0004647	*N*	SYNONYMOUS_CODING	1.01E-05
1	L	3R_26470169_SNP	FBgn0051013	*CG31013*	INTRON	7.17E-06
1	L	X_18993792_SNP				1.24E-05
1	L	2R_7157512_SNP	FBgn0033603	*Cpr47Ef*	SYNONYMOUS_CODING	1.25E-05
1	L	X_20852792_SNP				1.21E-05
1	L	X_15462747_SNP	FBgn0030653	*CG7860*	UPSTREAM	9.83E-06
1	L	X_20571391_DEL				8.43E-06
1	L	X_20571195_SNP				6.94E-06
1	L	X_20571210_SNP				8.01E-06
1	L	X_20571201_MNP				6.80E-06
5	L	2L_19266299_SNP				2.84E-07
5	L	3L_9931950_SNP	FBgn0085385	*CG34356*	INTRON	1.89E-06
5	L	X_20571210_SNP				4.25E-07
5	L	X_20571391_DEL				4.58E-07
5	L	3L_21253931_SNP	FBgn0004865	*Eip78C*	INTRON	1.62E-06
5	L	2L_19265015_SNP				5.40E-06

5	L	2L_4708893_SNP	FBgn0085380	*CG34351*	INTRON	5.27E-07
5	L	X_20571211_INS				4.30E-07
5	L	X_6984903_SNP	FBgn0264270	*Sxl*	INTRON	1.10E-06
5	L	X_20571199_SNP				1.18E-06
5	L	X_20571194_SNP				1.63E-06
5	L	X_20571185_SNP				1.46E-06
5	L	X_15289425_SNP	FBgn0263257	*cngl*	INTRON	1.13E-05
5	L	X_20571201_MNP				1.72E-06
5	L	3L_3816723_SNP	FBgn0004888	*Scsalpha*	SYNONYMOUS_CODING	3.94E-06
5	L	3L_2316526_SNP				3.19E-06
5	L	X_20571338_SNP				2.04E-06
5	L	3L_13221139_SNP	FBgn0023095	*caps*	UPSTREAM	1.95E-05
5	L	X_20571254_SNP				3.68E-06
5	L	2L_19265020_SNP				5.37E-06
5	L	X_20571192_SNP				2.61E-06
5	L	X_10003992_SNP	FBgn0083940	*CG34104*	INTRON	1.00E-05
5	L	X_6984953_SNP	FBgn0264270	*Sxl*	INTRON	2.25E-06
5	L	3L_2316514_SNP				5.81E-06
5	L	X_20571195_SNP				4.13E-06
5	L	3L_3532034_SNP	FBgn0005640	*Eip63E*	INTRON	1.75E-05
5	L	X_20571220_SNP				3.68E-06
5	L	X_20571225_SNP				3.68E-06
5	L	2R_19606345_SNP	FBgn0034898	*CG18128*	UTR_3_PRIME	1.45E-05
5	L	3L_10037652_SNP	FBgn0040823	*dpr6*	INTRON	1.29E-05
5	L	3L_9931838_SNP	FBgn0085385	*CG34356*	INTRON	6.43E-06
5	L	3L_11249553_SNP				6.52E-06
5	L	X_20571241_SNP				5.93E-06
5	L	X_6983588_SNP	FBgn0264270	*Sxl*	INTRON	2.93E-06
5	L	3L_3816708_SNP	FBgn0004888	*Scsalpha*	SYNONYMOUS_CODING	7.15E-06
5	L	2R_16960337_SNP	FBgn0034583	*CG10527*	UPSTREAM	6.95E-06
5	L	2R_16960330_SNP	FBgn0034583	*CG10527*	UPSTREAM	7.73E-06
5	L	X_20571206_INS				5.97E-06
5	L	X_6997709_SNP	FBgn0029936	*CG4617*	SYNONYMOUS_CODING	8.30E-06
5	L	X_6975823_SNP	FBgn0264270	*Sxl*	INTRON	7.89E-06
5	L	X_7005884_DEL				9.80E-06
5	L	3L_2316496_DEL				7.64E-06
5	L	3L_3812744_SNP	FBgn0035464	*CG12006*	NON_SYNONYMOUS_CODING	8.75E-06
5	L	3L_2316502_SNP				9.31E-06

Appendix B. Candidate gene information

Candidate genes associated with each phenotype (1) climbing speed and (2) endurance. C = untreated food, L = Lisinopril-treated food. FBgn = FlyBase gene.

1. Climbing speed candidate gene information

Age (Weeks)	Treatment	FlyBase ID	Gene Symbol	Biological Processes	Molecular Function
1	C	FBgn0037327	*PEK*	ATP-binding, protein kinase activity	related to early onset diabetes
1	C	FBgn0036376	*Liprin-Beta*	protein binding, axon target recognition, neuromuscular synaptic growth	protein binding
1	C	FBgn0011715	*Snr1**	wing morphgenesis, muscle organ development	RHABDOID TUMOR PREDISPOSITION SYNDROME 1; RTPS1
1	C	FBgn0262018	*CadN2*	calcium ion binding, axon extension involved in axon guidance	macular dystrophy, cancer
1	C	FBgn0037328	*RpL35A*	structural constituent of ribosome, regulation of growth and hormone	ribosomal protein
1	C	FBgn0037330	*mRpL44*	ribonuclease III activity, structural constituent of ribosome, mitochondrial translation	oxidative phosphorylation
1	C	FBgn0015360	*oxt*	acetylglucosaminyltransferase activity, sugar/sulphate metabolic processes	transferase enzymes which act upon xylose
1	C	FBgn0015542	*sima**	cellular response to insulin stimulus, regulation of transcription, DNA-templated	hypoxia related, receptor
1	C	FBgn0035876	*PEX2*	ubiquitination, spermatid development	PEROXISOME BIOGENESIS
1	C	FBgn0003175	*px**	imaginal disc-derived wing vein morphogenesis	
1	C	FBgn0027655	*htt*	dendric/synaptic transport	related to huntingtins
1	C	FBgn0085447	*sif*	nucleotide exchange factor, actin organization	cancer/nucleotide exchange factor
1	C	FBgn0033296	*Mal-A7*	carbohydrate metabolic process	sugar breakdown
1	C	FBgn0004795	*retn*	DNA binding/ transcription factor, muscle organ development, neuro development, glucose metabolic processes	DNA interactions
1	C	FBgn0051116	*CIC-a*	chloride channel activity	chloride voltage gated channel activity
1	C	FBgn0051632	*sens-2*	metal ion binding	zinc binding, growth factor
1	C	FBgn0000565	*Eip71CD**	determination of adult lifespan, response to oxidative stress	oxidative stress
1	C	FBgn0264857	*iab-8*	no info	
1	C	FBgn0264495	*gpp*	gene regulation	gene regulation
1	C	FBgn0040823	*dpr6*	sensory perception of chemical stimulus	cell adhesion
1	C	FBgn0004870	*bab1*	DNA/protein binding, imaginal disc-derived leg morphogenesis	DNA binding

1	C	FBgn0034606	*ASPP*	protein tyrosine kinase activator activity	protein regulation, cancer supression
1	C	FBgn0010015	*CanA1*	hydrolase activity, regulation of sleep/immune response	phosphatase activity
1	C	FBgn0010114	*hig*	synaptic target recognition	susceptbility to diseases
1	C	FBgn0263929	*jvl*	dorsal appendage formation, chaeta morphogenesis	
1	C	FBgn0052274	*Drsl1*	defense response to fungus	
1	C	FBgn0023511	*Edem1**	calcium ion binding, determination of adult lifespan, ER unfolded protein response	ER response
1	L	FBgn0037327	*PEK*	ATP-binding, protein kinase activity	related to early onset diabetes
1	L	FBgn0037330	*mRpL44*	ribonuclease III activity, structural constituent of ribosome, mitochondrial translation	oxidative phosphorylation
1	L	FBgn0011715	*Snr1**	wing morphgenesis, muscle organ development	RHABDOID TUMOR PREDISPOSITION SYNDROME 1; RTPS1
1	L	FBgn0037328	*RpL35A*	structural constituent of ribosome, regulation of growth and hormone	ribosomal protein
1	L	FBgn0027655	*htt*	dendric/synaptic transport	related to huntingtins
1	L	FBgn0023511	*Edem1**	calcium ion binding, determination of adult lifespan, ER unfolded protein response	ER response
1	L	FBgn0037332	*Hcs*	biotin-[propionyl-CoA-carboxylase (ATP-hydrolyzing)] ligase activity	holocarboxylase synthetase activity
1	L	FBgn0038295	*Gyc88E*	CO, NO, O2, heme, protein binding, respond to reactive oxygen species, cGMP synthesis	guanylate cyclase activity
1	L	FBgn0034606	*ASPP*	protein tyrosine kinase activator activity	protein regulation, cancer supression
1	L	FBgn0036376	*Liprin-Beta*	protein binding, axon target recognition, neuromuscular synaptic growth	protein binding
1	L	FBgn0264495	*gpp*	gene regulation,histone methylation	gene regulation
1	L	FBgn0005658	*Ets65A*	DNA binding	cancer, transcription factor
1	L	FBgn0000565	*Eip71CD**	determination of adult lifespan, response to oxidative stress	oxidative stress
1	L	FBgn0037899	*RpL24-Like*	structural constituent of ribosome	ribosomal protein
1	L	FBgn0264857	*iab-8*	no info	
1	L	FBgn0013469	*klu*	metal ion/nuc acid binding, neurogenesis, tricarboxylic acid cycle, positive regulation of compound eye retinal cell programmed cell death	metal ion binding

1	L	FBgn0035936	*Tsp66E*	no info	related to prostate cancer, protein coding
1	L	FBgn0032586	*Tpr2*	unfolded protein binding	protein coding
1	L	FBgn0037382	*Hpr1*	mRNA export, signal transduction	protein coding
1	L	FBgn0022702	*Cht2*	chitin development	protein coding
1	L	FBgn0037301	*Mms19*	DNA repair	cell assembly
1	L	FBgn0261258	*rgn*	carbohydrate binding, tissue regeneration	cell regeneration
1	L	FBgn0034058	*Pex11*	peroxisome fission/organization	peroxisome biogenesis
1	L	FBgn0003175	*px**	imaginal disc-derived wing vein morphogenesis	
1	L	FBgn0013576	*mtd**	imaginal disc-derived wing morphogenesis, immune response	oxidation resistance
1	L	FBgn0036518	*RhoGAP71E**	imaginal disc-derived leg morphogenesis, GTPase activator activity	GTPase activity
1	L	FBgn0010434	*cora**	adult somatic muscle development, heart process, maintenance of imaginal disc-derived wing hair orientation	protein coding
1	L	FBgn0004870	*bab1**	imaginal disc-derived leg morphogenesis, DNA bidning	DNA binding
1	L	FBgn0051561	*Osi16*	no info	
1	L	FBgn0010282	*TfIIFalpha*	DNA binding and transcriptional regulation	transcription factor
1	L	FBgn0010114	*hig*	synaptic target recognition	susceptbility to diseases
1	L	FBgn0004795	*retn*	DNA binding/ transcription factor, muscle organ development, neuro development, glucose metabolic processes	DNA interactions
1	L	FBgn0010015	*CanA1*	hydrolase activity, regulation of sleep/immune response	phosphatase activity
1	L	FBgn0259173	*corn*	microtubule/protein binding	protein binding
1	L	FBgn0262018	*CadN2*	Calcium ion binding, cell adhesion/development	protein coding for cell adhesion
1	L	FBgn0010909	*msn*	ATP binding, regulation of glucose metabolic process, kinase activity	protein coding for kinase
1	L	FBgn0011569	*can*	spermatid development,	related to hypoxia, TATA box binding
1	L	FBgn0033296	*Mal-A7*	carbohydrate metabolic process	sugar breakdown
5	C	FBgn0003896	*tup*	chaeta morphogenesis, heart development, muscle cell fate determination	transcription factor, protein coding
5	C	FBgn0052423	*shep*	adult locomotory behavior, RNA binding	RNA binding
5	C	FBgn0020655	*Gap69C*	GTP binding	GTPase protein
5	C	FBgn0004865	*Eip78C*	regulation of glucose metabolic process, hormone receptor activity	protein receptor
5	C	FBgn0064123	*stg1*	channel regulator activity	Calcium voltage gated channel subunit

5	C	FBgn0003310	*S*	stem cell fate commitment (eye photoreceptor)	
5	C	FBgn0026592	*Fie*	no info	
5	C	FBgn0035542	*DOR*	steroid hormone receptor binding	protein coding, cancer related
5	C	FBgn0260660	*mp*	carbohydrate binding, cardiac muscle atrophy, mitochondrion organization, skeletal muscle atrophy	protein coding for collagenous structures
5	C	FBgn0261514	*nimA*	sensory perception of pain	receptor coding
5	C	FBgn0015381	*dsf*	zinc ion binding, hormone receptor	receptor coding
5	C	FBgn0000395	*cv-2*	BMP binding, imaginal disc-derived wing morphogenesis, imaginal disc-derived wing vein specification	BMP binding
5	C	FBgn0019890	*Smg5*	NOT ? gene silencing by miRNA	mRNA decay factor
5	C	FBgn0004623	*Gbeta76C*	activation of phospholipase C activity, G-protein coupled receptor, transduction	G-protein, signal transduction
5	C	FBgn0003090	*pk*	zinc ion binding, imaginal disc-derived leg joint morphogenesis	Epilepsy, myopathy related
5	C	FBgn0023495	*Lip3*	lipase activity	Lipase activity
5	C	FBgn0031150	*bves*	no info	Protein coding
5	C	FBgn0033935	*Sin1*	dendrite morphogenesis	protein kinase activity
5	C	FBgn0031309	*Tfb4*	nucleotide-excision repair	transcription factor activity
5	C	FBgn0024277	*trio*	Rho guanyl-nucleotide exchange factor activity,imaginal disc-derived leg morphogenesis, mushroom body development	exchange factor activity
5	C	FBgn0029091	*CS-2*	chitin synthase activity	synthase activity
5	C	FBgn0003016	*osp*	no info	protein coding
5	C	FBgn0262737	*mub*	regulation of alternative mRNA splicing, via spliceosome, sleep	protein coding
5	C	FBgn0032536	*Ance-3*	peptidyl-dipeptidase activity	Renal/blood pressure functions
5	C	FBgn0036896	*wnd*	ATP binding, protein kinase activity	protein kinase activity
5	C	FBgn0002973	*numb*	embryonic heart tube development, pericardial nephrocyte differentiation, muscle cell fate specification	protein coding
5	C	FBgn0013469	*klu*	metal ion/nuc acid binding, neurogenesis, tricarboxylic acid cycle, positive regulation of compound eye retinal cell programmed cell death	metal ion binding
5	C	FBgn0030897	*Frq1*	calcium ion binding, neuromuscular junction development	neuronal protein
5	C	FBgn0033652	*ths*	growth factor activity, glial cells, heart development	
5	C	FBgn0263930	*dally*	imaginal disc-derived leg morphogenesis, imaginal	development morphogenesis

				disc-derived wing morphogenesis, imaginal disc-derived wing vein morphogenesis, regulation of imaginal disc growth	
5	C	FBgn0262139	*trh*	limb development	obesity related
5	C	FBgn0085387	*shakB*	jump response, reponse to light	
5	C	FBgn0003138	*Ptp61F*	negative regulation of insulin receptor signaling pathway.	protein phosphatase activity related to diabetes
5	C	FBgn0051721	*Trim9*	zinc binding, neurogenesis	related to mental retardation
5	C	FBgn0260642	*Antp*	heart development, muscle cell fate specification	related to skeletal abnormalities
5	C	FBgn0263144	*bin3*	snRNA binding, negative regulation of translation	protein binding
5	C	FBgn0020617	*Rx*	regulation of glucose metabolic process	related to muscular degeneration
5	C	FBgn0263934	*esn*	zinc ion binding	Epilepsy, myopathy related
5	C	FBgn0004456	*mew*	cell adhesion molecule binding, imaginal disc-derived wing morphogenesis, muscle attachment	cell adhesion
5	C	FBgn0016797	*fz2*	Wnt-protein binding, imaginal disc-derived wing margin morphogenesis	Wnt signaling pathway
5	C	FBgn0085408	*Shroom*	actin filament binding	related to mental retardation
5	C	FBgn0004514	*Oct-TyrR*	G-protein receptor activity	obesity related
5	C	FBgn0000464	*Lar*	motor neuron axon guidance, protein tyrosine phosphatase activity	protein phosphatase activity related to cancer
5	C	FBgn0000183	*BicD*	regulation of endocytosis, protein binding	RNA transport
5	C	FBgn0035539	*slow*	calcium ion binding, regulation of imaginal disc-derived wing size, muscle attachment	membrane receptor protein coding?
5	C	FBgn0015039	*Cyp9b2*	heme binding, iron binding	related to oxidative stress
5	C	FBgn0032264	*Lip4*	triglyceride lipase activity	protein conding for lipase
5	C	FBgn0000547	*ed*	dorsal appendage formation, imaginal disc-derived wing morphogenesis, muscle organ morphogenesis	related to muscular degeneration
5	C	FBgn0036298	*nst*	magnesium ion binding, trachea development	related to immunodeficiency
5	C	FBgn0035385	*FR*	adult locomotory behavior	
5	C	FBgn0041622	*Or69a*	olfactory receptor activity	
5	C	FBgn0036010	*Ir67a*	ligand-gated ion channel activity	ion receptor
5	C	FBgn0053516	*dpr3*	store-operated calcium entry	
5	C	FBgn0032723	*ssp3*	no info	ER protein

5	C	FBgn0263995	*cpo*	chemical synaptic transmission, olfactory	RNA binding
5	C	FBgn0034602	*Lapsyn*	nervous system development	disease susceptibility
5	C	FBgn0036333	*MICAL-like*	actin/zinc binding	cell binding protein
5	C	FBgn0000633	*fas*	cardioblast cell fate determination	cell adhesion and cancer
5	C	FBgn0026597	*Axn*	heart development, imaginal disc pattern formation, imaginal disc-derived wing morphogenesis, Wnt signal	hepato cancer
5	C	FBgn0000257	*car*	determination of adult lifespan	renal dysfunction
5	C	FBgn0033438	*Mmp2*	adult fat body development, imaginal disc fusion, thorax closure	related to heart disease
5	C	FBgn0015542	*sima*	cellular response to insulin stimulus	related to hypoxia
5	C	FBgn0035608	*blanks*	RNA interference	RNA binding
5	C	FBgn0265296	*Decam2*	homophilic cell adhesion via plasma membrane adhesion molecules	cell adhesion
5	C	FBgn0003068	*per*	age-dependent response to oxidative stress, determination of adult lifespan, locomotor rhythm	circadian rhythm
5	C	FBgn0261054	*Sfp24Bc*	multicellular organism reproduction	peptidase inhibitor
5	C	FBgn0036278	*GRHRII*	corazonin receptor activity, acetaldehyde metabolic process	protein binding
5	C	FBgn0025697	*santa-maria*	carotenoid metabolic process	scavenger receptor
5	C	FBgn0003435	*sm*	determination of adult lifespan, RNA bidnig	nuclear binding
5	C	FBgn0044020	*Roc2*	cullin family protein binding, ubiquitin protein ligase activity	protein ubiquitination
5	C	FBgn0038165	*Task6*	potassium channel activity	potassium channel activity
5	L	FBgn0010825	*Gug*	imaginal disc-derived leg morphogenesis,larval somatic muscle development	related to anormalities of the heart
5	L	FBgn0028871	*Cpr35B*	structural constituent of chitin-based cuticle	protein binding
5	L	FBgn0036010	*Ir67a*	ligand-gated ion channel activity	ion receptor
5	L	FBgn0004865	*Eip78C*	regulation of glucose metabolic process, hormone receptor activity	protein receptor
5	L	FBgn0002938	*ninaC*	ATP binding, motor activity, phototransduction	movement
5	L	FBgn0064123	*stg1*	channel regulator activity	Calcium voltage gated channel subunit
5	L	FBgn0085387	*shakB*	jump response, reponse to light	
5	L	FBgn0260642	*Antp*	heart development, muscle cell fate specification	related to skeletal abnormalities
5	L	FBgn0004914	*Hnf4*	lipid biosynthetic process	related to diabetes
5	L	FBgn0035539	*slow*	calcium ion binding,	membrane

				regulation of imaginal disc-derived wing size, muscle attachment	receptor protein coding?
5	L	FBgn0261054	*Sfp24Bc*	multicellular organism reproduction	peptidase inhibitor
5	L	FBgn0264754	*btsz*	actin filament organization,morphogenesis of embryonic epithelium, protein transport	protein encoding
5	L	FBgn0045495	*Gr28b*	immune response	
5	L	FBgn0000633	*fas*	cardioblast cell fate determination	cell adhesion and cancer
5	L	FBgn0000183	*BicD*	regulation of endocytosis, protein binding	RNA transport
5	L	FBgn0001234	*Hsromega*	positive regulation of cellular protein metabolic process	
5	L	FBgn0261238	*Alh*	zinc ion binding, instar larval development, larval somatic muscle development	cancer related
5	L	FBgn0031150	*bves*	no info	Protein coding
5	L	FBgn0035331	*Dms-R1*	myosuppressin receptor activity, adult locomotory behavior	G-protein receptor
5	L	FBgn0002973	*numb*	embryonic heart tube development, pericardial nephrocyte differentiation, muscle cell fate specification	protein coding
5	L	FBgn0051481	*pb*	regulation of glucose metabolic process, specification of segmental identity, labial segment	related to hearing
5	L	FBgn0035786	*Tsp66A*	no info	

2. Endurance candidate gene information

Age (Weeks)	Treatment	FlyBase ID	Gene Symbol	Biological Processes	Molecular Function
1	C	FBgn0040849	*Ir41a*	ligand-gated ion channel activity	receptor related to epilepsy, mental retardation
1	C	FBgn0085447	*sif*	guanyl-nucleotide exchange factor activity, actin cytoskeleton organization, positive regulation of filopodium assembly	protein coding
1	C	FBgn0014141	*cher*	determination of adult lifespan, actin binding, motor neuron axon guidance	actin coding
1	C	FBgn0085443	*spri*	Ras GTPase binding, guanyl-nucleotide exchange factor activity, axon extension	Ras GTPase activity
1	L	FBgn0040907	*mRpL33*	structural constituent of ribosome, translation	protein for translation
1	L	FBgn0046887	*Gr98b*	taste receptor activity	no info
1	L	FBgn0263102	*psq*	DNA binding, imaginal disc-derived wing morphogenesis	DNA domain binding
1	L	FBgn0004647	*N*	calcium ion binding, determination of adult lifespan, metabolic, morphogenesis	related to many muscular functions
1	L	FBgn0033603	*Cpr47Ef*	structural constituent of chitin-based cuticle	no info
5	C	FBgn0001253	*ImpE1*	imaginal disc eversion	component of basement membrane
5	C	FBgn0086779	*step*	imaginal disc-derived wing vein morphogenesis,positive regulation of multicellular organism growth, regulation of actin organization	GEP activity
5	C	FBgn0024941	*RSG7*	intracellular signal transduction	signal transduction
5	C	FBgn0032723	*ssp3*	no info	ER protein
5	C	FBgn0033128	*Tsp42Eg*	no info	protein for retinal membrane
5	C	FBgn0263601	*mib1*	imaginal disc-derived leg/wing morphogenesis, lateral inhibition, Notch signaling pathway	ubiquitin protein
5	C	FBgn0259162	*RunxB*	ATP, DNA binding.	Transcription factor and protein for peroxisome biogenesis
5	C	FBgn0039102	*SPE*	defense response, immune response	no info
5	C	FBgn0259240	*Ten-a*	synaptic growth at neuromuscular junction	transmembrane protein, for nucleus
5	C	FBgn0004865	*Eip78C*	regulation of glucose metabolic process, hormone receptor activity	protein receptor
5	C	FBgn0263873	*sick*	actin filament organization, phototransduction	neuronal protein

5	C	FBgn0040507	*ACXD*	cyclic nucleotide biosynthetic process	adenylate cyclase activity
5	C	FBgn0052062	*A2bp1*	imaginal disc-derived wing vein specification, mRNA/nucleotide binding	RNA bindin protein
5	C	FBgn0022700	*Cht4*	chitinase activity	chitinase activity
5	C	FBgn0034582	*Cht9*	chitinase activity	chitinase activity
5	C	FBgn0031820	*DLP*	determination of adult lifespan, response to oxidative stress	transcriptional regulator
5	C	FBgn0003997	*W*	many functions related to cell death/growth	no info
5	C	FBgn0036260	*Rh7*	G-protein coupled photoreceptor activity	protein receptors
5	C	FBgn0264512	*CR43911*	no info	no info
5	C	FBgn0023095	*caps*	motor neuron axon guidance	protein receptors
5	C	FBgn0264909	*CR44100*	no info	no info
5	C	FBgn0003310	*S*	stem cell fate commitment (eye photoreceptor)	no info
5	C	FBgn0016081	*fry*	chaeta, antennal, dendrite morphogenesis	microtubule binding, transcriptional coactivator
5	C	FBgn0260798	*Gprk1*	imaginal disc-derived wing vein specification,G-protein coupled receptor signaling pathway	G protein coupled receptor
5	C	FBgn0261933	*SmD1*	poly(A) RNA binding	RNA protein binding
5	C	FBgn0265140	*Meltrin*	protein oligomerization, zinc binding, metallopeptidase activity	metallopeptidase activity
5	C	FBgn0050260	*tRNA:CR30260*	ACA codon-amino acid adaptor activity	no info
5	C	FBgn0016672	*Ipp*	inositol-1,4-bisphosphate 1-phosphatase activity	phosphatase activity
5	L	FBgn0004865	*Eip78C*	regulation of glucose metabolic process, hormone receptor activity	protein receptor
5	L	FBgn0264270	*Sxl*	growth factor activity, mRNA binding, imaginal disc growth	RNA binding protein
5	L	FBgn0263257	*cngl*	intracellular cyclic nucleotide activated cation channel activity, cation transport	no info
5	L	FBgn0004888	*Scsalpha*	cofactor binding, tricarboxylic acid cycle	protein ligase activity
5	L	FBgn0023095	*caps*	motor neuron axon guidance	protein receptors
5	L	FBgn0005640	*Eip63E*	developmental growth, metamorphosis	cylin kinase activity
5	L	FBgn0040823	*dpr6*	sensory perception of chemical stimulus	cell adhesion

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