RESVERATROL INFLUENCE ON LIFESPAN AMONG *CAENORHABDITIS ELEGANS* GENETIC VARIANTS & IMPLICATIONS FOR PERSONALIZED MEDICINE

by

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A THESIS

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Patrick Phillips

Precision medicine represents a modern transition toward utilizing patient genetics to create specific, individualized treatment options for a variety of diseases. To demonstrate the significance of personalized patient care, we can examine the variation in responses to drugs among genetic variants of the same species. *Caenorhabditis elegans (C. elegans)* is a model organism for human research, beneficial for collecting mass data from genetically identical individuals due to their hermaphroditic reproduction and relatively short lifespan. Experimentation with the nematode species *C. elegans* and drug applications may suggest variability in effects when examining different strains or slight genetic variants. Differences in drug or compound effectiveness among strains of the same species would indicate the importance of individualized care to maximize positive outcomes. In this experiment, we analyzed the lifespans of four strains of *C. elegans* when exposed to the antioxidant compound, resveratrol, compared to a control with no compound. Resveratrol is known to extend the nematode lifespan in wild-type or "non-mutant" lines. We also examined mutant lines of these four strains, each with the *daf-16* transcription factor gene knocked-out

through CRISPR technology. These mutant lines underwent the same procedures as their corresponding wildtype strains to compare lifespan changes between control and resveratrol exposure. The magnitude of difference in drug effectiveness among individuals of the same species indicates a need to investigate genetic variation and personalize treatment plans that are more efficient and effective.

As a passionate Human Physiology major with a long-term goal of working in medicine, I am always captivated by the transitioning platform for medical care. The advancements in science pave way for greater disease prevention and treatment with the potential of bettering the lives of countless individuals. This Clark Honors College thesis through the University of Oregon is a compilation of current personalized medicine research with the goal of analyzing the innumerable possibilities and potential future directions of Westernized medicinal care. Furthermore, the body of my thesis will include personal research conducted through an Ecology and Evolution laboratory to examine lifespan differences among strains of the roundworm *Caenorhabditis elegans* exposed to an antioxidant compound. The goal is to better understand the interactions between genetic makeup and drug responsiveness. This will provide correlative evidence and implications surrounding the importance of personalized treatments in humans, and provides the basis for examining the necessary considerations and conditions required to transition toward individualized care, including components beyond genetic analysis and targeting disease as well as the risks and ethical concerns surrounding the future of personalized medicine.

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Introduction

Personalized medicine encompasses a variety of diagnosis and treatment techniques, all of which target specific types of diseases predicated upon differences among individual patients. Important consideration factors include health status, age, medical history, genetic profile and individual environment. Some aspects of modern medicine are slowly making the transition toward cell and gene therapies for many disease-treatment models, specifically in genetic or rare diseases. This individualized care has received a great deal of attention in the medical community in recent years. The interest in prevention, diagnosis and treatment modalities targeted to specific diseases based upon individual patient variability has sparked a discourse about and innovation in the science community. Most recently, cell and gene therapies for cancer and gene therapy for severe combined immunodeficiencies have proved highly valuable and effective therapies in various experimental and some clinical settings (Bauer, 2017). Genetic analysis is key in these cases. When combined with a holistic understanding of the patient as an individual entity, the likelihood for health increase and disease elimination grows substantially.

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The modern medical system involves physicians diagnosing disease to then formulate plans to improve quality of life. However, many modern treatments are primarily based on what will help the average individual rather than extensively analyzing the individual patient in need. Every person has a unique variation of the human genome, and this genetic variation can play a large role in individual health, especially when combined with distinct behavior and unique influences from the environment. Individual genotype or genetic makeup dictates unique phenotype, the

observable characteristics. One idiosyncratic demonstration of biological variation among people occurs in individual drug responsiveness. Because DNA encodes for proteins and drugs interact directly with proteins, the genetic makeup of an individual can directly impact how a drug interacts with the body. Many of the current prescriptions and treatment options are standardized for what works for the average in prior medical trials. For example, it has been shown that ADHD medicine only works for one out of ten preschoolers, many of the most widely used cancer drugs are effective for about 25% of patients, and typical depression drugs have a success rate of only 60% (Goldstein, 2005). With the availability of genetic analysis technology and a greater understanding of the components that affect human health, modern medicine is slowly transitioning to personalized care to create both prevention and treatment plans. As technology advances in this manner, medical research must focus on the factors that influence drug effectiveness among individuals diagnosed with the same disorder. Due to the impossibility of analyzing a large subset of humans with identical genomes, environmental factors and lifestyle choices, there is a call to utilize model organisms such as *Caenorhabditis elegans* to understand the implications of such factors on medical diagnosis and treatment.

One principle of personalized medicine is the treatment of patients based on identification of genetic mutations or abnormalities that cause disease, providing treatment options based on an individual's genetic makeup. This personalized, precision treatment is based on a single patient system rather than utilizing general disease diagnoses, leading to a higher probability of therapeutic success and likely increased cost efficiency in the healthcare sector. Genome sequencing, analyzing the genetic

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makeup of an individual, can function to diagnose disease while also playing a role in drug prescription and dosage allotment. Drug effectiveness can depend on genetic background, as an individual's DNA encodes for proteins that have receptors for specific drugs that aid in their transport or function as enzymes for important bodily reactions. The precision medicine movement could allow detection of known protein markers (called biomarkers) in individuals, giving physicians insight to cater drug type and dosage based on what will be most efficiently utilized by the individual's body (Abou-El-Enein, 2017). Because drugs and treatments interact directly with human proteins, understanding individual make-up in pivotal in efficient medical care. Analyzing protein-protein interactions and understanding the genome's role in disease pathways will lead to a greater ability to directly treat individuals based on their unique characteristics. The importance of medical research using model organisms is immense in this area, as many molecular pathways are conserved among organisms.

These transitions have brought significant commercialization challenges to the surface, however, driven by high developmental costs and difficulties surrounding circulation of genetic information and the diagnostic results based off them. The increasing development of technology, especially in the westernized world, has potential to change the modern medical techniques to cater care for an individual and his or her individual ailment. Troubles arise when the rapidly changing technological advances surpass the experimental and clinical trials. Many of the difficulties surrounding the adoption of a personalized medical approach stem from the lagging clinical trials and policy development around the quickly rising technological possibilities. It is important to recognize and address these obstacles while evaluating

the future of modern medicine and the possibilities genomic analysis can provide. Individualized, personalized, precision medicine has the potential to become the most efficient and effective medicine model science can offer and with the considerations of the pitfalls and difficulties in the transition, it is imperative that physicians cater therapeutic techniques to each individual patient holistically by utilizing the genomic technology readily available.

Background Information

The human genome consists of approximately 3 billion nucleotide base pairs bound in a molecular strand called deoxyribonucleic acid (DNA), coiled into 23 pairs of chromosomes residing in the nucleus of every cell. Each of these chromosomes contains hundreds to thousands of genes with specific instructions to make RNA which then can translate and produce proteins. Humans have an estimated 30,000 genes that encode proteins and each of these genes, on average, can make three proteins (Human Genome Project). In 1988, the Human Genome Project was created by a committee of the U.S. National Academy of Sciences, working with the National Institutes of Health and the Department of Energy to create physical and genetic maps of the human genome. They successfully mapped the first complete human genome in 2003 and was made readily available to the public (Human Genome Project). Since the completion of this genetic mapping, scientists have been developing new technologies to interoperate and understand the human sequences in the context of medical advances and improving human health (Human Genome Project).

Important steps in genome-based research have been ongoing the last ten years in attempts to understand how parts of the genetic code work together in various pathologies. As genome sequences techniques are becoming faster, less expensive and more readily accessible, the development of diagnostic tools based, in part, on genetic sequences will lead to a powerful form of preventive medicine and disease treatment techniques (Human Genome Project). Determining protein biomarkers is an imperative part of the process to streamline efficient and effective care. Regions in the human genome correspond to specific proteins and biochemical pathways that could help

physicians locate the root cause of disease or dysfunction (Schork, 2015). They accurately and reproducibly represent a quantifiable biological characteristic, giving an objective measure of disease or overall health status (Skevaki, 2015). By measuring the protein biomarker availability and responses, genetic characteristics can be inferred and specifically targeted for effective therapy. Physicians can use this tool to better understand disease processes, allowing for early detection, treatment and disorder management, especially in the context of noncommunicable diseases (NCDs). These chronic disease, such as cardiovascular disease, cancer, chronic respiratory disease and diabetes are prevalent worldwide, with every 3 deaths each year attributed to an NCD (Skevaki, 2015).

A Personalized Approach

Researchers have discovered hundreds of genes with variations or mutations contributing to a variety of human illnesses. It has also been determined that treatments of many diseases have varied results, due in part to genetic variability in patients. This demonstrates the importance of targeting the molecular causes of some diseases, using diagnostic tests based on genetics or other molecular mechanisms for more effective and efficient therapies catered to the individual (Hamburg, 2010). Many of the current leaders in medical interventions including the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) have begun the transition toward a personalized medicine approach. The overall goal is to focus on the best mechanism of therapy development to optimize correct drug administration and the right time with the right dose (Hamburg, 2010).

Determining the most clinically significant genetic markers has proven difficult and the development of policy surrounding such a novel approach to patient care leads to a myriad of challenges. The treatment itself, determined by a personalized and genetic analysis approach, will vary depending on the circumstances and results may inform a need for diet or lifestyle changes, specific drug treatments or possibly medical surveillance (Human Genome Project).

The term precision medicine has also been used in the context of focusing clinical applications on the individual rather than the average human. Among the top ten highest-grossing drugs in the United States, the likelihood that the therapy actual helps an individual is between 1 in 25 and 1 in 4, thus the most popular drugs have less than 25% efficiency (Schork, 2015). Some drugs, such as statins to routinely lower cholesterol, benefit as few as 1 in 50. Others are even harmful to certain ethnic groups because of the bias towards white Western participants in experimental studies (Schork, 2015). Because the data collected in many of the therapeutic drug trials are controlled on factors like genetics, lifestyles and diets, the results may indicate the need to study and validate the effectiveness of therapy in those that responded positively to determine the biological mechanisms (Stenson, 2014). An article in a 2015 issue of *Nature* entitled, “Personalized medicine: Time for one-person trials”, includes data showing the ten highest-grossing drugs in the United States and the number of people they help with each prescription demonstrate this dramatic difference in drug effectiveness. It shows that the top drugs in the nation prescribed by medical professionals fail to improve conditions of between 3 and 24 people for every person they do benefit. This table is labeled “imprecision medicine”, showing the ineffectiveness of the most popular drugs

and emphasizing the need to transition towards a precision medicine practice, providing personalized prevention and treatment for patients.

Inherited diseases account for up to 70% of all admissions to children's hospitals and 10% of admissions to adult hospitals. Many of the most common inherited disorders like cystic fibrosis and sickle cell anemia lack treatments beyond supportive care. Several modes of gene therapy allow for the manipulation of genetic mutations to correct the known genetic causes of various inherited diseases (Mingozzi, 2011). Two of the most common gene therapy treatments utilize either the transfer of stem cells—with the potential to differentiate into selected tissues—or utilization of viral machinery to manipulate the human genetic code. Some diseases showing promising gene therapy outcomes have included cystic fibrosis, hemophilia, heart failure and Parkinson's disease (Mingozzi, 2011). Cardiovascular disease (CVD) treatment is one area that would benefit greatly from the transition to personalized medical care, as CVDs remain the leading cause of death in the United States (Lee, 2012).

Many studies are identifying genetic variations underlying risks of both rare and common diseases, providing information for newly discovered genes, proteins and pathways that may be powerful drug targets. Today, however, there is insufficient evidence or identification of potential therapeutic targets for many of these diseases. Academic researchers, including many NIH-supported centers, must screen thousands of chemicals to find potential drug candidates for commercial development (Hamburg, 2010). With the more personalized approach, development in genetic informatics must match up with the pharmaceutical and biomedical technologies as well. To do so, a collaborative approach would be imperative**.** Utilizing the model organism

Caenorhabditis elegans, it is possible to isolate genetic differences to determine their role in drug effectiveness, keeping all environmental factors consistent and analyzing many organisms in a short timeframe. To effectively transition to personalized care, the interplay of genetics and therapeutic treatment needs to be better understood.

Experimental Findings: *Caenorhabditis elegans*

Experimental Background Information

Caenorhabditis elegans is a model organism for studies in biological development, evolution and human diseases. It was the first multicellular organism to have its genome completely sequenced in 1998, thus there are more tools for genetic manipulation for this organism than nearly all others. Additionally, it is easy to culture and maintain in the lab with a rapid life cycle of 3 days from egg to egg-laying adult and an average lifespan of 18 to 20 days. Its microscopic size—approximately 1 millimeter long—makes cultivation and environmental manipulations easy to manage (Balla, 2013). The animals can also be frozen for years and thawed when needed, important when reproducing experimental results and maintaining identical *C. elegans* lines for future use. Most important for this study, *C. elegans* is a self-fertilizing hermaphrodite, meaning a single animal can populate a plate and a single strain can be replicated with the same genetic makeup (Corsi, 2015). Within a single strain, all organisms are genetically identical, while across strains there are slight genetic differences, despite being members of the same species.

There are numerous similarities in both cellular and molecular processes among *C. elegans* and other animals, including metabolism, organelle structure, gene regulation and protein biology. At least 38% of the *C. elegans* protein-coding genes have orthologs—pathways of similar genetic origin evolved by speciation retaining the same function—found in the human genome. Approximately 40% of genes with known association to human diseases have clear orthologs in the *C. elegans* genome (Balla,

2013). Consequently, research and results surrounding *C. elegans* is often translated to possible human outcomes, especially in the context of the newly developed precision medicine initiative. Because the genetic makeup across the human species can *C. elegans* is similar, the experimentation with the nematode can provide general trends that should be, to some degree, mirrored in human subjects. Furthermore, it is impossible to do large scale experimentation on genetically identical individuals utilizing mice, monkeys or humans. The utilization of *C. elegans* allows for reproducibility that other model organisms cannot provide.

The genetic sequences are known for all observed strains and "knock-out" lines (with a genetic deletion of the *daf-16* region) have been created with CRISPR-cas9 editing technology, affecting an insulin-like pathway similar to that in humans. This deletion, or mutation, has been shown to decrease lifespan in the *C. elegans*. Similar pathways called insulin-like growth factors exist in other organisms, including mammals and humans, which carry out similar functions. In mammals, for example, the FOXO-transcription factor activates genes that mediate oxidative stress, heat shock, innate immunity and metabolism (Hesp, 2015). With an expected difference in lifespan, the drug effectiveness on the mutant strains may have a differing magnitude of change when compared to the non-mutant wild-type trials among each strain. To relate the mechanisms examined in *C. elegans* to human applications, studies have been conducted to determine orthologs—genes evolved from common ancestral trait—between the species. In the case of the *C. elegans* DAF-16 pathway, humans have four homologous FOXO transcription factors that influence age-related disease such as cancer, osteoporosis and diabetes mellitus (Hesp, 2015). A greater understanding of the

DAF-16 transcription factor mediated stress responses in *C. elegans* increased insight into age-related disease. Recent research on components of the insulin-like signaling pathway—affecting metabolism and longevity—and DAF-16 in *C. elegans* has facilitated the discovery of protein homologs in humans, which regulate aging and many age-related diseases (Hesp, 2015). Observation of both the DAF-16 pathway and drug exposure on lifespan has not yet been conducted under this context, thus my experimentation will further explore the metabolic interactions of the pathway in *C. elegans* for human application as well.

The examined *C. elegans* strains were exposed to an antioxidant compound called resveratrol, shown to elongate lifespan among various wild-type nematode strains and other model organisms. Resveratrol, with the chemical name *tran*-3,4',5-tridydorxystilbene, is a naturally compound found in the skin of various plants such as grapes and peanuts (Fischer, 2016). Many beneficial effects have been recently studied, including anti-inflammatory and antioxidant characteristics and modulation of different intracellular signaling pathways. Antioxidant activity and radical scavenging may be responsible for the life-prolonging effects of resveratrol, diminishing the amount of reactive oxygen species involved in aging (Fischer, 2016). It may also increase AMP-activated protein kinase (AMPK) phosphorylation, regulating the SIRT1 pathway. Small molecule activators of this pathway have been developed as therapies for the treatment of type 2 diabetes, as AMPK helps cells uptake glucose independently of insulin (Farghali, 2013).

Aging is often described as a risk factor for disease, but can be defined as a disease itself with a 100% mortality rate (Bulterijs, 2015). All individuals that are alive

and age eventually die. The medical definition of a disease is a “harmful abnormality of bodily structure and function” and aging most definitely falls into such a category. The causes of aging are becoming increasingly clear, most of which can be reduced to a cellular and molecular level with recognizable signs and symptoms like other commonly defined diseases and disorders (Bulterijs, 2015). In this experiment, nematode age is the dependent factor, with aging treated as a progressive disease and the administered compound as a disease prevention treatment. Analyzing the *daf-16* knock-out lines allow observation of compound effectiveness in organisms with accelerated disease symptoms, as the mutants lack an important transcription factor in an insulin-like pathway influencing longevity. With the environmental factors held constant, the effectiveness of the compound for aging treatment will be dependent solely on genetic variation across the *C. elegans* strains.

Data analysis after experimentation will provide conclusions that may have implications to the recent precision medicine initiative. By determining the magnitude of lifespan variance between *C. elegans* with slight genetic difference given resveratrol, we can observe the importance of genetic consideration when administering drugs to humans in a clinical setting. The results from this experiment could emphasize the importance of genetic analysis in medical treatment.

Purpose & Hypothesis

The specific purpose of this experiment is to determine the variance in lifespan among four strains of *Caenorhabditis elegans* and mutant lines of each strain when exposed to the same compound, the anti-oxidant resveratrol. By determining the effects of drugs across slightly genetically different strains of the same species along with a

mutant variant of each strain, we can explore the relationship between drug effectiveness and genetic makeup. It is hypothesized that controlled resveratrol application to four *C. elegans* strains and their respective *daf-16* mutants will result in varied changes in lifespan due to the slight genetic variability among strains and differential drug effectiveness.

Methods

The 4 *Caenorhabditis elegans* strains selected for this experiment included N2, JU775, CB4856 and MY16. Each strain is a unique *C. elegans* wild isolate originating from various geographic locations. Previously generated *daf-16* mutants for these strains were also thawed and maintained in an identical manner, including PX591 ($N2^{\Delta}$), PX595 ($JU775^{\Delta}$), PX597 ($CB4856^{\Delta}$) and PX606 ($MY16^{\Delta}$). Each mutant was genetically manipulated through CRISPR-cas9 technology and identical sequences were deleted from each strain to ensure homogeny.

The worm strains utilized in this experiment were thawed from frozen stocks in a single lab and all standard operating procedures were followed in their growth and maintenance. This includes plate and drug preparations and allowing thawed worms to grow three generations before experimental use. All worm cultures were maintained at 20°C with 80% humidity. Before placed on scanner plates, they were cultivated on nematode growth media (NGM). A streptomycin-resistant bacteria strain (OP50-1) was the food source for each plate throughout experimentation.

Populations of the same age were obtained by three-hour egg lays with first-day adults, which were then removed while the eggs developed into the next generation adults. Upon the first day of adulthood, these worms were moved to NGM plates

containing the compound FUdR, inhibiting subsequent reproduction without changing the lifespan on the adult worms. Between 25 and 40 worms were transferred to FUdR plates with and without resveratrol application, allowing comparison between drug and control conditions. The resveratrol plates were prepared by dissolving the solid compound in dimethylsulfoxide (DMSO) to create a stock solution that could be added to sterile water before application. Specifically, 10.0μL of stock solution (40mM resveratrol in DMSO) was added to 166.7μL for each plate. The resveratrol concentration on each scanner plate with 7mL NGM was 100μM. This solution was distributed across the entire plate surface and allowed to dry in a sterile hood until all liquid was absorbed into the agar. Control plates also contained the same concentration and volume of DMSO.

Lifespan data for the four hermaphroditic *C. elegans* strains and their respective *daf-16* mutant lines was generated through an automated lifespan machine (ALM). The ALM is a set of modified high resolution scanners that collect images of worms on plates, typically collected every hour. The collected images are analyzed automatically by software that determines if a worm is dead or alive based on movement, as shown in Figure 1.

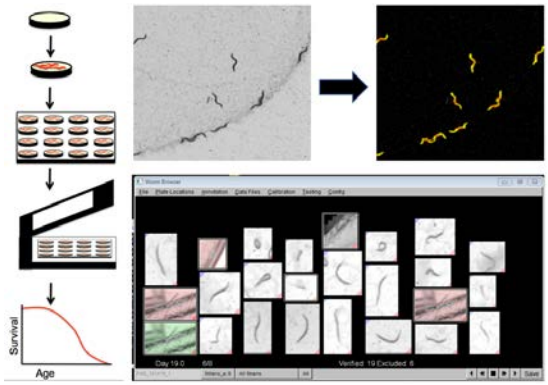

Figure 1: Automated lifespan machine imaging software

The high-resolution image taken by the scanner is then analyzed by a software that detects subtle movements across the collected images. When worms have completely stopped moving, the ALM records time of death, which is then checked for each worm by hand after data collection is complete. A survival plot is generated, showing the percent of worms surviving over time.

In this experiment, each ALM scanner held sixteen specialized plates with and without compound and approximately 40 worms on each plate. In the experimental duration, four scanners were utilized at the same time to ensure controlled environmental conditions. The plates were randomized on the scanner bay to avoid error due to unforeseen heat distribution or air flow in the experimental area. The room, however, was climate controlled due to the sensitivity of nematodes to temperature changes.

The lifespan analysis of each worm was collected through the ALM programming and the time of death of each worm was confirmed by hand, evaluating the time at which each worm stopped all movement. Survival plots were generated automatically along with data for each plate. Exactly 1114 worms were examined in the lifespan data analysis, including all four strains and their respective mutants.

Figures

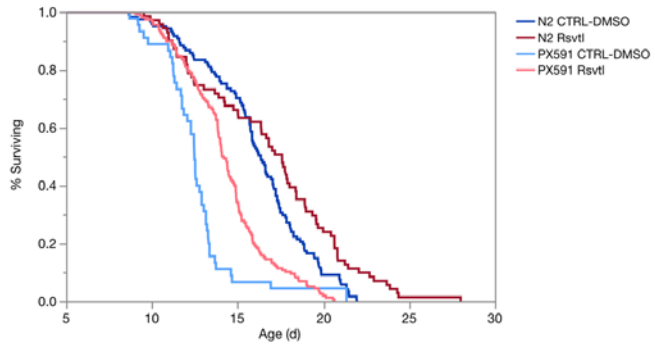

Figure 2: Survival Plot for N2 and N2$^{\Delta}$

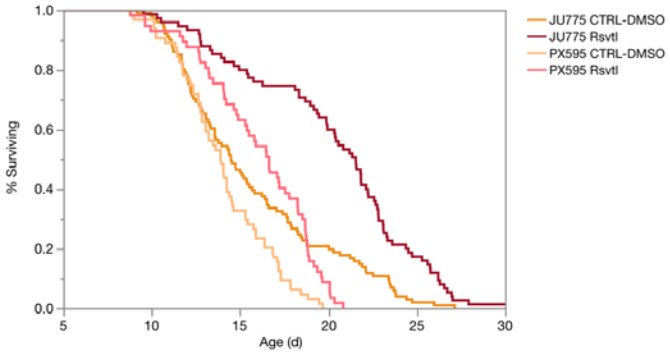

Figure 3: Survival Plot for JU775 and JU775$^{\Delta}$

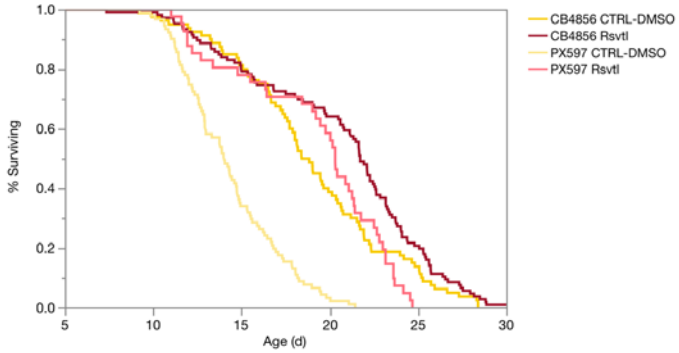

Figure 4: Survival Plot for CB4856 and CB4856$^{\Delta}$

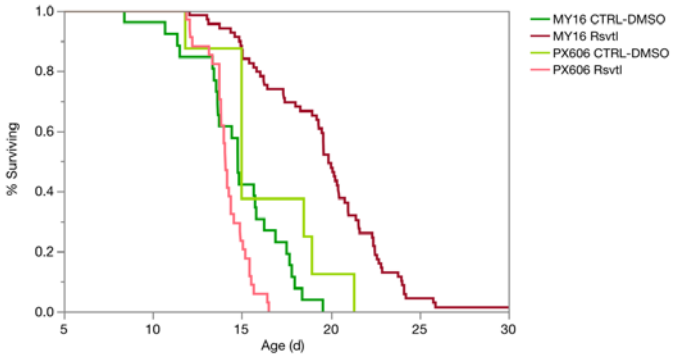

Figure 5: Survival Plot for MY16 and MY16$^{\Delta}$

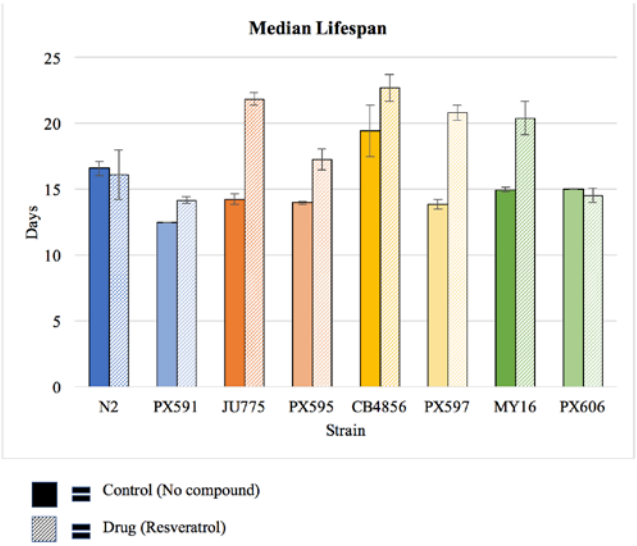

Figure 6: Median Lifespan

This figure depicts the average median lifespans of all *C. elegans* strains and their respective mutant strains both with and without resveratrol.

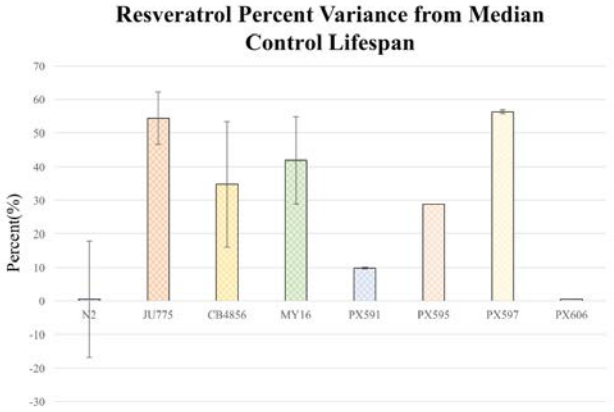

Figure 7: Resveratrol effectiveness among *C. elegans* strains.

The percent variance from the control average median lifespan with exposure to resveratrol indicates the effectiveness of the compound in extending lifespan.

Tables

Strain + Condition	Number of worms analyzed (n)	Average Lifespan (days)	Median Lifespan (days)
N2 CTRL	121	16.1331	16.343
N2 Rsvtl	71	16.8875	17.582
PX591 CTRL	45	12.6498	12.478
PX591 Rsvtl	158	14.3051	14.135
Combined	**395**	**15.1406**	**14.885**

Table 1: N2 and $N2^{\Delta}$ ALM Data

Data collected for N2, showing both the average lifespan and the median lifespan of the strain with and without drug. Also includes number of worms analyzed (n) for each condition.

Strain + Condition	Number of worms analyzed (n)	Average Lifespan (days)	Median Lifespan (days)
JU775 CTRL	101	15.7272	14.478
JU775 Rsvtl	75	20.3196	21.541
PX595 CTRL	64	14.032	13.905
PX595 Rsvtl	57	16.0621	16.635
Combined	**297**	**16.5859**	**15.749**

Table 2: JU775 and $JU775^{\Delta}$ ALM Data

Data collected for JU775, showing both the average lifespan and the median lifespan of the strain with and without drug. Also includes number of worms analyzed (n) for each condition.

Strain + Condition	Number of worms analyzed (n)	Average Lifespan (days)	Median Lifespan (days)
CB4856 CTRL	80	19.0231	18.619
CB4856 Rsvtl	106	20.5835	21.718
PX597 CTRL	91	14.3319	14.02
PX597 Rsvtl	41	19.1754	20.301
Combined	**318**	**18.2204**	**18.098**

Table 3: CB4856 and CB4856$^{\Delta}$ ALM Data

Data collected for CB4856, showing both the average lifespan and the median lifespan of the strain with and without drug. Also includes number of worms analyzed (n) for each condition.

Strain + Condition	Number of worms analyzed (n)	Average Lifespan (days)	Median Lifespan (days)
MY16 CTRL	26	14.8578	14.78
MY16 Rsvtl	69	19.604	19.874
PX606 CTRL	8	16.3168	14.989
PX606 Rsvtl	34	14.1919	14.093
Combined	**137**	**17.1681**	**15.833**

Table 4: MY16 and MY16$^{\Delta}$ ALM Data

Data collected for MY16, showing both the average lifespan and the median lifespan of the strain with and without drug. Also includes number of worms analyzed (n) for each condition.

Strain	Average Percent Variance (%)	Standard Error
N2	0.534	17.36
JU775	54.5	7.819
CB4856	34.7	18.66
MY16	41.9	12.99
PX591	9.90	0.3131
PX595	28.7	
PX597	56.4	0.54387
PX606	0.486	

Table 5: Average percent lifespan variance of all strains with exposure to resveratrol

Results

Resveratrol was shown to increase lifespan to some degree across all strains. The degree to which resveratrol affected *C. elegans* lifespan varied across the strains for both wild-type (WT) and mutant (*daf-16*). For example, of the WT strains, it was found that JU775 had the greatest percent difference from control median lifespan with resveratrol exposure, a 54.5% increase. In contrast, the WT strain N2 showed only a 0.534% increase in lifespan with resveratrol application. The other WT strains CB4856 and MY16 showed an increase in lifespan of 34.7% and 41.9% respectively.

The *daf-16* mutants showed inconsistancy in resveratrol response across the strains as well, although to different degrees than their respective WT strains. The strain PX597 ($CB4856^{\Delta}$) showed the most dramatic response to the compound with a 56.4% increase in median lifespan as compared to control conditions. Alternatively, the lowest

percent variance from control was shown in PX 606 ($MY16^{\Delta}$) with a 0.486% increase in median lifespan on average. The other two mutant strains, PX591 ($N2^{\Delta}$) and PX595 ($JU775^{A}$) showed an increase of 9.90% and 28.7% respectively.

Looking at the magnitude of drug effectiveness between WT and respective *daf-16* mutants, some strains shows increased drug responsiveness with mutation and others showed decreased drug responsiveness. The WT strain N2 showed little responsiveness with a 0.534% increase in median lifespan, while $N2^{\Delta}$ exposed to resveratrol showed an increased lifespan of 9.90% as compared to control. Similarly, the CB4856 WT strain showed a diminished resveratrol response as compared to its respective mutant, $CB4856^{\Delta}$ with a 34.7% increase in median lifespan versus the 56.4% increase in average median lifespan from control conditions. The WT strain JU775 showed a 54.5% increase in lifespan while its *daf-16* knock-out line had only a 28.7% increase in lifespan with resveratrol exposure. Finally, the WT strain MY16 experienced a 41.9% increase in lifespan as compared to its control while the mutant line $MY16^{\Delta}$ was only minimally affected by resveratrol exposure, showing a 0.486% increase in lifespan.

There are, however, some limitations to this study. While over one-thousand *C. elegans* organisms were utilized in this experimentation, there needs to be further trials to ensure reproducibility and validity of the collected data. Some obstacles encountered during lifespan data collection included plate contamination and “fogging”, in which condensation on the scanner caused obscured image collection in the ALM. Worms on plates with fogging or excessive contamination could not be detected by the ALM software and had to be excluded from the collected data.

Experimental Conclusions

The antioxidant compound resveratrol was found to increase median lifespan of four *C. elegans* strains and their *daf-16* knock-out mutants, however to varying degrees. The wildtype N2 and mutant MY16$^{\Delta}$ had little responsiveness to the resveratrol compound while wildtype JU775 and mutant CB4856$^{\Delta}$ both showed an increase in median lifespan by over 50%. Interestingly, much of the resveratrol research surrounding *C. elegans* and lifespan changes are conducted with the WT N2 strain, which showed the least significant response to the compound as compared to the other WT strains in this experiment. This may indicate the need to diversify strain usage for drug effectiveness experimentation in *C. elegans* and potentially spans to other model organisms and their various strains as well.

The variance between the wildtype strains and their *daf-16* knockouts with resveratrol also differed in magnitude and overall trend. There was no consistency in the wildtype or the mutant being more sensitive to resveratrol's lifespan elongation effects, thus it is difficult to determine if resveratrol is acting directly on the Daf-16 pathway or by another unknown mechanism. In most mutant lines (excluding MY16$^{\Delta}$ with an n=8), reveratrol application seemed to at least partially rescue the lifespan diminishing effect of knocking out the *daf-16* gene. Future research is needed to determine these specific intercellular interactions.

In conclusion, if genetic background was not influencing compound effectiveness, one would expect the percent variance in median lifespan across all *C. elegans* strains to be nearly identical, as the magnitude of change would remain consistent. With all environmental factors relatively constant throughout the

experimentation and the utilization of a control group, the only variance across strain and condition throughout this experiment is in the slight genetic differences that exist among *C. elegans* strains, with all organism of the same strain being genetically identical. The results indicate that the slight genetic variation across organisms of the same species plays an important role in compound effectiveness. Furthermore, the varied trends observed between the four strains and their *daf-16* mutants indicate that identical mutations across slightly genetically varied organism can result in further variance in drug response. Such a variance in drug effectiveness demonstrates the important role genetics play in drug responsiveness of multicellular organisms, potentially extending to a greater call to individualized, personalized medical care in humans. If slight genetic variations across *C. elegans* result in significant differences in treatment effectiveness, there is a high likelihood that the ubiquitous variation among human genome sequences also plays a pivotal part in medical treatment efficiency and overall success.

The Big Picture

Aspects of patient environment and socioeconomic status play a pivotal role in overall well-being and health. Personalized medicine procedures need to include a greater level of physician-patient communication in these areas to ensure the most well-rounded and complete medical care. While the transition toward genetic screening, biomarkers and individualized gene therapy has the support of proper technological advancements, there are a multitude of factors that influence the potential success of personalized medicine in the Western society.

Economic Considerations

There is ongoing discourse surrounding personalized medicine analyses and diagnoses techniques and their ability to provide an economic value, addressing the omnipresent difficulties with innovative new technologies that are accessible and affordable. A study conducted by the American College of Medical Genetics and Genomics conducted a cost-utility analysis of personalized medicine, utilizing quality-adjusted life years (QALYs) as the measure of success. It examined the impact a personalized and genetic centric approach would have on the top eight causes of mortality in the United States (excluding accidents and suicides): heart disease, malignant neoplasms, chronic lower respiratory disease, cerebrovascular disease, Alzheimer disease, diabetes mellitus, nephritis and influenza/pneumonia (Phillips, 2014). They also examined the high-expenditure conditions, including heart conditions and cancer. Much of the cost to QALY ratio (72%) indicated personalized care provided

better health, but with a higher cost. It was found that very few studies indicated an overall cost savings (Phillips, 2014).

Information on clinical utility, economic value, affordability and public health implications are essential to assess these new techniques. There is also a call to balance innovation and affordability. Responsibility for such a balance is shared among patients, providers, industry, payers and professional organizations. These issues are becoming more prevalent with the more readily available whole-genome sequencing. There is great importance, however, in considering diagnostic value and the need for innovation despite economic values and projections**.**

Unfortunately, researchers face pressures from the public to develop cell therapies that could reach clinics in a relatively short time period and with very little funding, making personalized treatment difficult to conceptualize. The transition towards personalized medicine may be best facilitated by utilization of patient advocacy groups, especially in the early developmental process. They may help by addressing concerns around commercialization, especially surrounding the concerns that the technologies may not be accessible at a reasonable cost (Bauer, 2017).

Data Storage and Use

The Human Gene Mutation Database (HGMD) was created in attempt to collect and classify all gene lesions responsible for human inherited disease. While this database does not contain somatic or mitochondrial mutations, other platforms such at MitoMap and COSMIC cover the genetic gaps (Stenson, 2014). These genome databases can be used for extensive meta-analyses on different gene mutations that cause human inherited disease, demonstrating mutation evolution among populations

along with practical use in clinical settings. Large banks of known mutations allow the physicians and technicians to compare known disease causing sequences with those of the patient's genome, proving practical for many diagnoses. The information may also be utilized by human molecular geneticist, genome scientists, molecular biologists, and those specializing in biopharmaceuticals and bioinformatics (Stenson, 2014). Evidence provided by such technology supports pathological authenticity and detection of detrimental gene lesions along with the general importance of a knowledgebase for bioinformatics that emphasize personalized genomic testing (Stenson, 2014). Harnessing the power and potential of big data could prove essential in the rapidly advancing field of bioinformatics. Developers in the field have increasingly been transforming cell and gene therapies for the market in efforts to potentially benefit from the analysis of large subsets of data (Bauer, 2017)

Ethical Considerations

Personalized medicine requires a collective approach and those charged with the convergence of bioinformatics and patient data face ethical considerations and many possible dilemmas. Ethical challenges are particularly pressing with genomic analysis due to vast amounts of sensitive data generated and collected. Furthermore, genetic analysis has a certain probabilistic character and the frequent uncertain significance of purely considering genetic information leaves potential implications for the patients and their relatives (Vos, 2017). Bridging the current translational gap between ethical proceedings and genomic research practice is essential before modern medicine can transition toward personalized care and the development of successful, large genomic databases (Vos, 2017). Broad consent has become the accepted consent model for

genomics research, with participants giving permission for a broad range of approved but unspecified future research use of their stored samples (Vos, 2017). It is difficult to anticipate the types of risks and benefits when the goal is increasing knowledge and technological advances. There are difficulties in determining the level of regulation for genetic tests that both protect patients and encourage innovation (Hamburg, 2010).

To ensure reliability in genetic analysis, research outside clinical medicine must first be completed and reproduced. Returning individual genetic results that are analytically and clinically valid would be an obligation of those collecting the information and analyzing the results. Along with this obligation lies the principal of beneficence, respecting the autonomy and reciprocity toward clinical patients and research participants as well. There are difficulties surrounding the disclosure of all genetic information, however, as too much information may cause adverse psychological, social and financial consequences for patients (Vos, 2017). The variable interpretation of genetic results makes full disclosure of genetic results cumbersome and potentially detrimental. However, unsolicited findings in genetic research may be beneficial to later research and mass genetic analysis, aiding the overall advancement of knowledge. Moral conflicts may arise with researcher decision making, finding a balance between producing generalizable knowledge and warning participants of potential harm (Vos, 2017). Addressing the growing concerns surrounding the ethical development of personalized medicine and genetic data storage and distribution, there needs to be integration and communication between physicians, researchers and the public to avoid unethical treatment of patients (Bauer, 2017). The transition toward

mainstream personalized, precision medicine is in the ongoing relationship between patients and physicians (Schork, 2015).

Patient History, Environmental Conditions and Race

Medical care includes more than disease diagnosis and treatment. Disease prevention and increased patient well-being are also important responsibilities of medical physicians and researchers. For example, increasing research has been conducted on the elongation of human lifespan and, more importantly, health-span. This recently coined term represents the amount of time in an organism's life with optimum health. In these years, the individual is generally healthy and free from serious, chronic illness.

Mental health would be a huge component of successful individualized care, as it varies immensely person to person and may impact physical health components as well. Those with a greater exposure to stress, for example, have increased rates of heart disease, weight gain, diabetes and stroke (Mitchell, 2014). Additionally, the stress response is not merely in one's head. It has been shown that improperly managed, chronic stress damages cells and leads to a decreased health-span and often decreased overall lifespan as well (Bulterijs, 2015).

Environmental factors and social hierarchies also have a significant impact on overall health, which physicians frequently disregard when diagnosing and creating treatment options. The social determinants of health (SDOH) are the socioeconomic conditions distributed among populations that influence individual health status. These include social structures and economic systems that directly affect physical environment, health services and various societal factors (CDC). This is exemplified in

various aspects of Westernized society; for instance, African American boys who grow up in highly disadvantaged environments have shorter telomeres than boys who grow up in highly advantaged environments (Mitchell, 2014). Telomeres are repetitive nucleotide sequences at each end of a chromosome, protecting from DNA deterioration associated with aging and age-related diseases (Shammas, 2012). Social environment and telomere length (TL) is moderated by genetic variation in the serotonin and dopamine pathways. In other words, the environment is directly affecting their DNA. This shows a direct gene-social environment interaction using TL as a biomarker for stress exposure. Research has suggested many possible behavioral mediators with a negative correlation between stress and TL, including stress, mental illness like depression, and obesity. Behavior and environment, consequently, are social predictors of health that physicians may need to account for in the shift toward personalized care.

While physicians in the current medical practice do not know how an individual patient will respond to a drug treatment, they often rely on information from previous studies and experiences to make surface level inferences. According to the American Heart Association, heart failure is more common among African Americans than Caucasians and the disease symptoms occur at an earlier age, progressing more rapidly among blacks (Root, 2003). While this data represents accurate data, physicians relying on these statistics when creating preventative care and treatment options to black patients may mistreat individuals who do not fall within the statistical range. With few exceptions, people classified as “black” have a worse overall health profile than those classified as “white” (Root, 2003). Furthermore, while doctors do not know how individuals will respond to different drugs, given a correlation between drug response

and race, they will invariably begin treatment based on what has worked on the majority of those falling in that predetermined category.

Treating the average and utilizing a one-size-fits-all model of medical care is impossible considering the impact of external factors on a cellular and molecular level, as individual behavior, exposure, and predetermined biases affect physical health immensely. A shift toward personalized care must encompass more than genetic analysis and manipulation. Treating a patient as an individual with specific needs requires a greater breadth of knowledge around all the factors that directly impact human health and how those interplay for each human in a unique manner.

Future Directions

Determining a path to personalized medicine is immensely challenging given the many factors that determine individual health and well-being along the endless societal considerations that are required for such an approach. The success of personalized medicine will depend on accurate and efficient diagnostic tests to identify who will benefit from potential targeted therapies (Hamburg, 2010). For example, a test for the human epidermal growth factor receptor type 2 (HER2) is currently used in breast tumor diagnostics, indicating overexpression and predicting a better response for the medication trastuzumab (Hamburg, 2010). Such an indication allows for more effective therapy without a trial-and-error approach that a one-type-fits-all treatment approach would permit. In order to apply such a technique to other diseases and disorders, there must be a collective greater understanding of biomarkers. Randomized control trials give evidence for the effectiveness of medical interventions, but such trials include patients with predefined characteristics and such a lack of diversity that the results only show the effectiveness of a drug in a precise subgroup of people. Medical research needs to incorporate a greater diversity of subjects to connect the intricate protein-protein interactions and mechanisms back to individual genetic background.

With a greater breadth of knowledge on disease mechanisms and genetic linkage, genomic editing technology may be an upcoming component in the personalized medicine transition. The potential of the CRISPR (clustered regulatory interspaced short palindromic repeats) mechanism for genome editing could change the way medical professionals treat genetically linked diseases dramatically. Such technology allows for precise gene editing by injection of specified bacterial plasmids,

allowing relatively accurate addition or deletion of specific DNA base pairs. As the mechanisms become better understood, this method will become more efficient and robust with different targeting ranges and specificities (Chen, 2017).

Finally, a greater understanding of the environmental factors that affect health are necessary for the progression toward individualized and highly efficient medical care. The effects of mental health, diet, exercise and even socioeconomic level can influence individuals at a cellular and genetic level, influencing overall health and well-being. More medical research must be conducted surrounding the various external impacts

Conclusions

Technological advances and an increasing availability of bioinformatics techniques in personalized care have inspired the medical community to expand the bounds of modern medicine. With an increasing number of deaths each year due to disease with genetic links, the United States may have the opportunity to be in the forefront of individualized therapies due to the access to the most innovative technology used by some of the most innovative scientists and medical researchers. The quality of life of many individuals affected by genetic disorders could be altered drastically, and those with underlying genetic mutations that may later cause harm will have a better chance of combatting any illness encoded in their genome.

The obstacles to reach personalized medical care cannot be ignored, however, and the continued discourse around the subject is prudent to developing the most efficient individualized care model, both in terms of effective treatment and economic

productivity. Thousands of years ago, Hippocrates stated “it is far more important to know what person the disease has than what disease the person has”, and the sentiment holds true in medicine today (Lee, 2012). This holds true in modern medical care, but current care models have shifted away from the treatment of the individual and toward the treatment of the average patient, focusing on the list of symptoms rather than their root causes. With some economic sacrifices and ethical considerations, personalized medicine and genome sequencing technology can open a world of medical care options and will result in overall societal health gains that even extend beyond the local bounds. Human beings are genetically diverse and, consequently, face unique health concerns. Physicians and medical researchers need to aim their focus on individualized, holistic, all-encompassing care to provide the most effective treatment options for all.

Bibliography

Abou-El-Enein, M., Duda, G. N., Gruskin, E. A., & Grainger, D. W. (2017). Strategies for Derisking Translational Processes for Biomedical Technologies. *Trends in Biotechnology*, *35*(2), 100–108. https://doi.org/10.1016/j.tibtech.2016.07.007

Agyeman, A. A., & Ofori-Asenso, R. (2015). Perspective: Does personalized medicine hold the future for medicine? *Journal of Pharmacy & Bioallied Sciences*, *7*(3), 239–44. https://doi.org/10.4103/0975-7406.160040

Bass, T. M., Weinkove, D., Houthoofd, K., Gems, D., & Partridge, L. (2007). Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. *Mechanisms of Ageing and Development*, *128*(10), 546–552. https://doi.org/10.1016/j.mad.2007.07.007

Bauer, G., Abou-El-Enein, M., Kent, A., Poole, B., & Forte, M. (2017). The path to successful commercialization of cell and gene therapies: empowering patient advocates. *Cytotherapy*, *19*(2), 293–298. https://doi.org/10.1016/j.jcyt.2016.10.017

Bulterijs, S., Hull, R. S., Björk, V. C. E., & Roy, A. G. (2015). It is time to classify biological aging as a disease. *Frontiers in Genetics*, *6*, 205. https://doi.org/10.3389/fgene.2015.00205

Chen, W., Rezaizadehnajafi, L., & Wink, M. (2013). Influence of resveratrol on oxidative stress resistance and life span in Caenorhabditis elegans. *The Journal of Pharmacy and Pharmacology*, *65*(5), 682–8. https://doi.org/10.1111/jphp.12023

Corsi, A., Wightman, B., & Chalfie, M. (n.d.). A Transparent window into biology: A primer on Caenorhabditis elegans. Retrieved May 9, 2016, from http://www.wormbook.org/chapters/www_celegansintro/celegansintro.pdf

Farghali, H., Canová, N. K., Lekić, N., & Farghali, H. (2013). Resveratrol and Related Compounds as Antioxidants With an Allosteric Mechanism of Action in Epigenetic Drug Targets. *Physiol. Res*, *62*, 1–13. Retrieved from www.biomed.cas.cz/physiolres

Fischer, N., Büchter, C., Koch, K., Albert, S., Csuk, R., & Wätjen, W. (2017). The resveratrol derivatives trans-3,5-dimethoxy-4-fluoro-4′-hydroxystilbene and trans-2,4′,5-trihydroxystilbene decrease oxidative stress and prolong lifespan in *Caenorhabditis elegans*. *Journal of Pharmacy and Pharmacology*, *69*(1), 73–81. https://doi.org/10.1111/jphp.12657

FitzPatrick, D. (2004, October 1). Mutants: On Genetic Variety and the Human Body. *American Journal of Human Genetics*. Elsevier. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1182069/

Ginsburg, G. S., & Willard, H. F. (2009). Genomic and personalized medicine: foundations and applications. *Translational Research*, *154*(6), 277–287. https://doi.org/10.1016/j.trsl.2009.09.005

Goldstein, D. B. (2005). The genetics of human drug response. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *360*(1460), 1571–2. https://doi.org/10.1098/rstb.2005.1686

Hamburg, M. A., & Collins, F. S. (2010). The Path to Personalized Medicine. *New England Journal of Medicine*, *363*(4), 301–304. https://doi.org/10.1056/NEJMp1006304

Hesp, K., Smant, G., & Kammenga, J. E. (2015). Caenorhabditis elegans DAF-16/FOXO transcription factor and its mammalian homologs associate with age-related disease. *Experimental Gerontology*, *72*, 1–7. https://doi.org/10.1016/j.exger.2015.09.006

Hsieh, T., & Wu, J. M. (2010). Resveratrol: Biological and pharmaceutical properties as anticancer molecule. *BioFactors (Oxford, England)*, *36*(5), 360–9. https://doi.org/10.1002/biof.105

Human Genome Project Completion: Frequently Asked Questions - National Human Genome Research Institute (NHGRI). (n.d.). Retrieved March 24, 2017, from https://www.genome.gov/11006943/human-genome-project-completion-frequently-asked-questions/

Kimura, K. D. (1997). daf-2, an Insulin Receptor-Like Gene That Regulates Longevity and Diapause in Caenorhabditis elegans. *Science*, *277*(5328), 942–946. https://doi.org/10.1126/science.277.5328.942

Kobayashi, Y., Furukawa-Hibi, Y., Chen, C., Horio, Y., Isobe, K., Ikeda, K., & Motoyama, N. (2005). SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. *International Journal of Molecular Medicine*, *16*(2), 237–43. https://doi.org/10.3892/IJMM.16.2.237

Lee, M.-S., Flammer, A. J., Lerman, L. O., & Lerman, A. (2012). Personalized medicine in cardiovascular diseases. *Korean Circulation Journal*, *42*(9), 583–91. https://doi.org/10.4070/kcj.2012.42.9.583

Lee, R. Y. N., Hench, J., & Ruvkun, G. (2001). Regulation of C. elegans DAF-16 and its human ortholog FKHRL1 by the daf-2 insulin-like signaling pathway. *Current Biology*, *11*(24), 1950–1957. https://doi.org/10.1016/S0960-9822(01)00595-4

Lin, K. (1997). daf-16: An HNF-3/forkhead Family Member That Can Function to Double the Life-Span of Caenorhabditis elegans. *Science*, *278*(5341), 1319–1322. https://doi.org/10.1126/science.278.5341.1319

Mingozzi, F., & High, K. A. (2011). Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. *Nature Reviews Genetics*, *12*(5), 341–355. https://doi.org/10.1038/nrg2988

Mitchell, C., Hobcraft, J., McLanahan, S. S., Siegel, S. R., Berg, A., Brooks-Gunn, J., … Notterman, D. (2014). Social disadvantage, genetic sensitivity, and children's telomere length. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(16), 5944–9. https://doi.org/10.1073/pnas.1404293111

Phillips, K. A., Ann Sakowski, J., Trosman, J., Douglas, M. P., Liang, S.-Y., & Neumann, P. (2014). The economic value of personalized medicine tests: what we know and what we need to know. *Genetics in Medicine : Official Journal of the American College of Medical Genetics*, *16*(3), 251–7. https://doi.org/10.1038/gim.2013.122

Rascón, B., Hubbard, B. P., Sinclair, D. A., & Amdam, G. V. (2012). The lifespan extension effects of resveratrol are conserved in the honey bee and may be driven by a mechanism related to caloric restriction. *Aging*, *4*(7), 499–508. https://doi.org/10.18632/aging.100474

Reynolds, R. M., & Phillips, P. C. (2013). Natural variation for lifespan and stress response in the nematode Caenorhabditis remanei. *PloS One*, *8*(4), e58212. https://doi.org/10.1371/journal.pone.0058212

Root, M. (2003). The Use of Race in Medicine as a Proxy for Genetic Differences. *Philosophy of Science*, *70*(5), 1173–1183. https://doi.org/10.1086/377398

Schork, N. J. (2015). Personalized medicine: Time for one-person trials. *Nature*, *520*(7549), 609–611. https://doi.org/10.1038/520609a

Skevaki, C., Van den Berg, J., Jones, N., Garssen, J., Vuillermin, P., Levin, M., … Thornton, C. A. (2016). Immune biomarkers in the spectrum of childhood noncommunicable diseases. *Journal of Allergy and Clinical Immunology*, *137*(5), 1302–1316. https://doi.org/10.1016/j.jaci.2016.03.012

Stenson, P. D., Mort, M., Ball, E. V., Shaw, K., Phillips, A. D., & Cooper, D. N. (2014). The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Human Genetics*, *133*(1), 1–9. https://doi.org/10.1007/s00439-013-1358-4

Vos, S., van Delden, J. J. M., van Diest, P. J., & Bredenoord, A. L. (2017). Moral Duties of Genomics Researchers: Why Personalized Medicine Requires a Collective Approach. *Trends in Genetics*, *33*(2), 118–128. https://doi.org/10.1016/j.tig.2016.11.006

Wang, X. (2016). Gene mutation-based and specific therapies in precision medicine. *Journal of Cellular and Molecular Medicine*, *20*(4), 577–80. https://doi.org/10.1111/jcmm.12722

Ye, K., Ji, C.-B., Lu, X.-W., Ni, Y.-H., Gao, C.-L., Chen, X.-H., … Guo, X.-R. (2010). Resveratrol Attenuates Radiation Damage in Caenorhabditis elegans by Preventing Oxidative Stress. *Regular Paper J. Radiat. Res*, *51*, 473–479. https://doi.org/10.1269/jrr.10009