Therapeutic Mitochondrial Delivery to Astrocytes for Ischemic Stroke

(Technical Paper)

Personal Risk Analysis of Direct-to-Consumer Genetic Testing

(STS Paper)

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Emma Taylor-Fishwick
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Technical Project Team Members
Caitleen Copeland

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

Introduction

The global direct-to-consumer genetic testing (DTC-GT) market is valued at approximately 824.1 million dollars, and is expected to increase to 6,364.5 million by 2028 (Markets, 2019). DTC-GT refers to genetic tests that are sold to the public but have no relation to the healthcare system (Badalato, Kalokairinou, & Borry, 2017). The DTC-GT services provide information such as disease risk percentage, ancestry tests, drug response, food intolerances, and preconception screening (Oliveri, Howard, Renzi, Hansson, & Pravettoni, 2016). The services, however, do pose personal risk and hazard to the consumers. The risks and hazards include privacy, incompatible results, and mis-interpretation of what the results signify (*Society News*, 2007; Kutz, 2010). Many of the risks and hazards are due to DTC-GT services not being associated with the healthcare system and thus not having to follow the Health Insurance Portability and Accountability Act (HIPPAA) (*Society News*, 2007). Additionally, the DTC-GT services do not always provide clear information on the limitations of the testing and also what the percent risks signify (Kutz, 2010). Therefore, this STS research topic will focus on assessing the personal risks that people take when becoming a consumer for DTC-GT services.

The DTC-GT services provides information on a percent risk of Parkinson's, Alzheimer's, and Cancers ("Boddy, 2017; Williamson & Duncan, 2002). New advancements in technology allow the DTC-GT services to test for new diseases, and soon these tests could include ischemic stroke, which is the occlusion of an artery in the brain by a clot (Roger et al., 2012). The current treatments for ischemic stroke involve breaking up the clot in order to restore blood flow (Ciccone et al., 2013). However, during an ischemic stroke, the mitochondria in the astrocytes, glial cells in the brain, become dysfunctional (Liu, Lu, Manaenko, Tang, & Hu, 2018). These dysfunctional mitochondria are passed from astrocytes to neurons and led to the

continuation of the ischemic cascade and neuronal death (Hayakawa et al., 2016). This technical project will focus on determining the type of mitochondria, from different tissues, that increases the ATP production and are uptaken by the astrocyte cells at the highest rate.

Technical Topic

Stroke is the leading cause of physical and intellectual disability and has a high rate of mortality in the United States (Yang, Mukda, & Chen, 2018). Approximately 795,000 people per year, in the United States, have a new or recurrent stroke, and of this population 87% are classified as ischemic stroke caused by a blood clot lodged in an artery supplying blood to the brain (Roger et al., 2012).

The current standard of treatment for ischemic stroke primarily focuses on restoring blood flow to the brain by removing the blockage (Ciccone et al., 2013). However, with the current standard of treatment, more than half of the patients either die or do not fully recover (Bhatia et al., 2010). The lack of full recovery is because the current treatments do not address the mitochondria that become dysfunctional after an ischemic stroke.

In healthy brain tissue, astrocytes, glial cells of the central nervous system, transfer mitochondria particles to the neurons via the calcium-dependent mechanisms of CD38 and the cyclin adenosine diphosphate (ADP) ribose signaling (Hayakawa et al., 2016; Robinson et al., 2017). The transfer of mitochondria particles is to allow for cell-to-cell communication between astrocytes and neurons. As a result, ATP production and viability of neurons is additionally increased (Hayakawa et al., 2016). However, after ischemic stroke, the astrocyte mitochondria become dysfunctional due to oxygen and glucose deprivation (Liu et al., 2018). These dysfunctional mitochondria have abnormalities in mitochondrial membrane potential, produce increased amounts of reactive oxygen species (ROS), and have decreased adenosine triphosphate

(ATP) production (Liu et al., 2018). When the dysfunctional mitochondria are circulated between astrocytes and neurons, it continues the ischemic cascade even after blood flow has been restored.

The transplantation of exogenous mitochondria from healthy tissue can help to reverse the effects of an ischemia-reperfusion injury, such as stroke, according to previous studies. In one clinical study of pediatric patients with myocardial ischemia-reperfusion injury following coronary artery occlusion and revascularization, transplantation of healthy mitochondria improved myocardial function within 24 to 48 hours after treatment (Emani & McCully, 2018). Another previous study has demonstrated that exogenous mitochondria injected systemically to a mouse with middle cerebral artery occlusion (MCAO), a model of ischemic stroke, can be taken up by neurons, astrocytes, and microglia (Liu et al., 2018). This treatment resulted in the upregulation of cell survival related signals in the MCAO mice. The study postulated that astrocytes have the ability to transfer healthy mitochondria (if they possess them) to rescue damaged neurons after stroke due to their cell-to-cell communication.

However, none of the previous studies tested mitochondria from different sources. In all previous studies, mitochondria have been harvested from skeletal muscle sources. Yet, mitochondria can also come from different tissues such as adipose and cardiac muscle. Peridroplet mitochondria from adipose tissue have enhanced bioenergetic and low fatty acid oxidation capacity. Their fusion-fission properties also differentiate them from cytoplasmic mitochondria (Benador et al., 2018). Myocardium mitochondria have a larger reliance on aerobic ATP when compared to skeletal muscle mitochondria (Miller, Rosenfeldt, Zhang, Linnane, & Nagley, 2003). Additionally, in a study completed by Song-Young Park, it was concluded that

cardiac and skeletal muscle mitochondria have similar oxidative phosphorylation capacities but vary in terms of respiratory control rate and nonphosphorylating respiration (Park et al., 2014).

Therefore, our team, under the guidance of Richard Price and Catherine Gorick, will develop a method to obtain optimal uptake of, and ATP production by, exogenous mitochondria in human astrocytes to provide a framework for a new therapeutic method to treat ischemic stroke. This technical project will utilize experimental resources of Price lab, such as mice, cell culture materials, and reagents, and information gathered via literature review. In order to accomplish this goal, we will first extract mitochondria from the various tissue sources mentioned above. Then, we will measure the ATP production of each type of mitochondria alone and after being taken up by cultured astrocytes. Additionally, we will measure the amount of mitochondria taken up by cultured astrocytes by methods of fluorescence. The experimentation specified will be conducted throughout the 2019-2020 school year.

STS Topic

Genealogy, the study of a person's lineage, has over 12 million people participating and has become the second most popular hobby in the United States (Bowen & Khoury, 2018). This popularity is due to the development of direct-to-consumer genetic testing (DTC-GT), which refers to genetic tests that are sold or advertised to the public but not associated with the healthcare system (Badalato et al., 2017). These genetic tests have decreased in cost significantly since first introduction. The reduction in cost is shown through 23andMe, a DTC-GT for ancestry, who's service cost has reduced from 999 dollars in 2007 to 99 dollars today (Roberts & Ostergren, 2013).This reduction in cost is due to the decreasing cost of genome sequencing (three billion dollars in 2000 to 10,000 in 2009) and due to advancements in SNPs (Sboner, Mu, Greenbaum, Auerbach, & Gerstein, 2011). SNPs are single nucleotide substitutions that occur in

greater than one percent of the population (Caulfield & McGuire, 2012; Sboner et al., 2011). SNPs are used by the majority of DTC-GT because they allow for a quick analysis of genomes for specific variations that are associated with certain ancestries (Zettler, Sherkow, & Greely, 2014).

The SNPs are used by DTC-GT services that are advertised for paternity testing, fetal gender determination, assessment of drug response, preconception screening, food intolerances, and disease risks (CCMG Ethics and Public Policy Committee et al., 2012; Oliveri et al., 2016). DTC-GTs for disease risks have become more prominent with 23andMe, creating a Personal Genome Service in addition to their ancestry analysis (Annas & Elias, 2014). Numerous other DTC-GT services have also arisen for disease risk analysis including 24Genetics, Atlas Biomed, Easy DNA, and Mapmygenome (Markets, 2019). These DTC-GT give the percentage of risk for diseases such as cancer, Parkinson's, Alzheimer's, and celiac disease (Boddy, 2017; Williamson & Duncan, 2002). However, with these disease risks, comes a lot of uncertainties that are felt by many of the consumers. In a study done with 3,640 customers of DTC-GT services, 50% had concerns regarding learning about their disease risk percentage, knowing how to feel about these risk percentage, and also about the privacy of their DNA data (Bloss et al., 2010).

The uncertainties and risks felt by consumers has led to discussion regarding DTC-GT services. One point of discussion involves the privacy of a consumer's DNA data. DTC-GT services are not subject to regulation by the Health Insurance Portability and Accountability Act (HIPPAA) because they are not connected to the health care system (*Society News*, 2007). Therefore, there is limited oversite on what the DTC-GT services do with a person's DNA. The lack of oversite was shown with the 23andMe and GlaxoSmithKline (GSK) partnership where 23andMe shared the genetic database with GSK (Philippidis, 2018). Another point of discussion

has been the accuracy of the DTC-GT services. In a study conducted by the United States Government Accountability Office, it was found that when a person's DNA was tested by four DTC-GT companies, the results for the disease risks varied (Kutz, 2010). In addition, the follow-up consultation provided by the DTC-GT services, regarding what a person's disease risk percentage means, varied depending on the company (Kutz, 2010). Some companies only provide general information and no expert advice (Kutz, 2010). This can lead to ambiguity and the consumer not knowing how to interpret, and sometimes misinterpreting, their results.

Risk analysis, as stated by Ulrich Beck in his book *A Critical Introduction to the Risk Society,* will be used to analyze the personal risks a consumer takes when participating in DTC-GT services (Mythen, 2004). Risk analysis will be used because it incorporates the concepts of 'man-made disasters,' or 'new risks,' which are both hazards and risks developed through modern society (Sørensen, 2018). These 'new risks' are defined as being difficult for insurance companies to price because the consequences cannot be calculated and involve "global, frequently irreparable damage" (Sørensen, 2018). There are critics about risk analysis including Beck's view of society not covering daily risks and the diagnostic procedure being shallow and generalized (Cottle, 1998; Sørensen, 2018). However, Beck's framework is currently the most cohesive in understanding the new era of risk (Sørensen, 2018). Therefore, in Spring 2020, risk analysis will be used to analyze the risks and hazards of DTC-GT services.

Research Question and Methods

The research question for the STS paper is as follows: what personal elements of risk are associated in DTC-GT testing? In order to analyze and answer this research question two methods will be used: documentary research method and discourse analysis. Documentary research methods will be used by reading papers discussing the benefits and risks of DTC-GT

testing. The papers will include discussion on the validity of the tests, the privacy regulations, and also the genome literacy (the ability to understand the genomic results). Papers that will be analyzed include "Direct-to-Consumer Genetic Testing: Perceptions, Problems, and Policy Responses," "Third party interpretation of raw genetic data: An ethical exploration," and "The real cost of sequencing: Higher than you think!" (Badalato et al., 2017; Caulfield & McGuire, 2012; Sboner et al., 2011).

Discourse analysis will also be conducted by analyzing YouTube videos of users discussing their experience using these DTC-GT tests, such as 23andMe. Five videos will be reviewed, and the terms that they use will be noted. Special attention will be given to terms such as risks, benefits, anxiety, peace of mind, and preparation. YouTube videos that will be analyzed including an "Unexpected Discovery: Kristin's 23andMe Story", "23andMe Story: Two of a Kind, Erika and Kristin", and "Empowering Herself: Sarah's 23andMe Story"(*23andMe Story*, 2016; *An Unexpected Discovery*, 2018; *Empowering Herself*, 2018).

Conclusion

The STS research will involve making a list of all of the risks/hazards associated with DTC-GT services and assessing the implications of these risks/hazards. This list will help to evaluate the risks/hazards that a consumer takes when performing DTC-GT services and also will allow for the consumers to become more educated about the DTC-GT services offered and their limitations.

For the technical topic, the mitochondria that increases ATP production and is uptaken by the astrocyte cells at the greatest rate will be determined. The determined mitochondria can then be used by future researchers as a method to create a new treatment for ischemic stroke. This

new treatment has the potential to decrease the number of people who have disabilities after ischemic stroke.

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