Examining the Influence and Role of Pharmacogenetics among Children with Autism Spectrum Disorder

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EXAMINING THE INFLUENCE AND ROLE OF PHARMACOGENETICS AMONG CHILDREN WITH AUTISM SPECTRUM DISORDER

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EXAMINING THE INFLUENCE AND ROLE OF PHARMACOGENETICS AMONG CHILDREN WITH AUTISM SPECTRUM DISORDER

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I am blessed to have two mothers to dedicate my work for,

Amal Bullock your strong soul still reminding me of God’s generosity that enlightens my path in life. Your last words keep motivating me to move forward and have faith in God no matter how hard this life may hit me.

Huda Melky your love, care and support fill my spirit with hope and brighten my way to the pinnacle of success. Thanks for being a mother, a friend, a family and more than anything I have hoped for.

My loving gratitude to my sister Noor Shaker who never gets tired of supporting me and directing me with her enriched experience.

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Pharmacogenetics is the study of genomic-guided individualized drug prescription that plays an important role in preventing the severe adverse effects of drugs, decreasing the time and cost of therapeutic choices, and directing healthcare professionals to choose medications that are effective and safe. It is noteworthy that this approach becomes highly beneficial in patients suffering from chronic diseases or disorders, since these conditions may require multiple and long term pharmacological therapies, as in children with autism spectrum disorder (ASD). However, public acceptance is a major challenge when implementation of pharmacogenetics merges into clinical practice. The purpose of this study is a) to investigate, among small cohort group of children with ASD, several genetic variants of enzymes that influence the metabolism of commonly prescribed drugs to treat ASD and b) to inspect the knowledge of, attitude towards and future expectations with regards to pharmacogenetics among parents of children with ASD. A group of 15 school-aged participants with ASD were recruited for the study. Approximately 5 ml of venous blood was drawn for each participant to analyze the genotype of enzymes implicated in drug metabolism via pharmacogenetics testing. Thereafter, the parents of these children attended a training session to help them gain a better understanding of the pharmacogenetics results depicted in the drug panel results. A pre-training and post-training survey was conducted to assess the knowledge of, attitude towards and future expectations of pharmacogenetics among the children’s parents.
Chapter One: Introduction

The number of diagnoses of autism spectrum disorder (ASD) is escalating. The CDC reports that one in every 68 children is diagnosed with ASD in the United States. This fact is indicative of the increased demand for pharmacological treatment options (Bowker, D'Angelo, Hicks, & Wells, 2011). The concomitant use of multiple therapeutic modalities to treat ASD creates uncertainty about the efficacy of each method separately. Several pharmacological therapies are used for different behaviors associated with ASD; however, their efficacy is not ensured and there may be severe adverse effects in some patients (Bowker et al., 2011; Zane, Davis, & Rosswurm, 2008). This emphasizes the need to seek further testing that will help physicians make more targeted and effective pharmacological choices for the patients of children with ASD in order to avoid adverse effects of the prescribed medications (Bowker et al., 2011; Zane et al., 2008).

Pharmacogenetics is the study of the genes that affect the response of medications in the body. Most of the genes that are commonly tested play an important role in regulating the family of enzymes responsible for the metabolism of drugs. Pharmacogenetics testing is highly promising in the field of medicine in order to prevent the occurrence of adverse effects of medications and giving insight into appropriate, personally-tailored dosage guidelines to assure drug safety and efficacy (Wolf, Smith, & Smith, 2000), (Ensom et al., 2001), (McCarthy, 2001). However, public acceptance is a major challenge when the implementation of pharmacogenetics merges into clinical practice. Therefore, educating the general population about the importance of pharmacogenetics and measuring beliefs and expectations is highly significant in successfully applying this approach in clinical settings.
Autism Spectrum Disorder (ASD)

Autism Spectrum Disorder (ASD) is a neurodevelopmental disability characterized by restricted interests, repetitive behaviors and deficits in communication and social reciprocity skills (White, Keonig, & Scahill, 2007). Due to the prevalence of children identified with ASD (1 in 68 children), there is a need for interventions targeting the specific characteristics children and adolescents with ASD display (Center for Disease Control and Prevention, 2014).

Characteristics of Autism Spectrum Disorder

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), there are five factors that make up the diagnostic criteria for ASD. The first feature includes deficits, which persist over time, in social communication and social interaction. These deficits can include, but are not limited to, abnormal social approach, failure to grasp the pragmatics of normal conversation, failure to start or participate in social interactions, abnormal eye contact and body language, failure to develop and maintain social relationships, difficulties initiating or maintaining friendships, and a lack of interest in peers. The second factor includes restricted and repetitive behaviors and interests. Examples of this type of behavior include, but are not limited to, repetitive speech, insistence on adhering to routines, highly restricted perseverative interests with an abnormal intensity, and unusual sensory interests. The third factor says that the symptoms must be present in the early developmental period. The fourth factor says that the symptoms cause clinically significant impairment in certain areas of functioning. The final factor says that the symptoms are not better explained by another disability.
Pharmacological treatment of ASD

Medications are one of the multiple interventions used to treat ASD. The concomitant use of multiple therapies to treat ASD is common due to the comorbidity nature of the disease; thus detecting the efficacy of specific treatments is further complicated (Bowker et al., 2011; Zane et al., 2008). However, medications that are used to treat comorbidities associated with ASD, rather than the main symptoms of the disorder, have been reported to be one of the most frequently discontinued treatment methods (Bowker et al., 2011; McPheeters et al., 2011). Therefore, the increasing demands on pursuing pharmacological choices, the lack of knowledge regarding the efficacy of medications and the alarming adverse effects of some of the drugs, coupled with the high cost of these modalities, prompts the need for further investigation in the field (Bowker et al., 2011; Zane et al., 2008).

Current research regarding the most commonly prescribed medications used in the treatment of ASD addressed the following classes of drugs: antipsychotics, antidepressants and anti-ADHD agents (McPheeters et al., 2011). Clinical use of these medications improves challenging behaviors (irritability, aggression and self-injurious behavior), repetitive behavior and hyperactivity respectively. Although antipsychotics, particularly aripiprazole and risperidone, have been approved to be efficacious, adverse effects were observed when using these agents, as 30% of patients using aripiprazole were susceptible to weight gain. The adverse effects of atypical antipsychotics manifested by weight gain, sedation and extrapyramidal symptoms (McPheeters et al., 2011; Stachnik & Gabay, 2010). Two other common SSRI (a class of antidepressants) medications - citalopram and fluoxetine – are used to treat individuals with ASD having specific
problem behaviors associated with the condition. For example, fluoxetine improves repetitive behavior and citalopram ameliorates challenging behavior.

Another class of medications that was used by 30-85% of children with ASD is anti-ADHD, these neurostimulant medications were prescribed to provide a short-term relief for symptoms of hyperactivity and impulsivity (Dalsgaard, Nielsen, & Simonsen, 2013; McPheeters et al., 2011). Regardless of the beneficial effects of medications used to treat (ASD), close monitoring is required as they may cause serious adverse effects because of the long term use of these medications (McPheeters et al., 2011).

Having expectations of the possibility to develop potential adverse effects of the drugs, can protect the patients from being predisposed to adverse effects that can be avoided. That critical goal in addition to saving money and giving the time for other therapeutic choices to be prescribed, can be accomplished by requesting a pharmacogenetics test (Bowker et al., 2011; Wolf et al., 2000; Zane et al., 2008).

**Pharmacogenetics (PGx)**

*What is Pharmacogenetics?*

Pharmacogenetics (PGx) is the study of genes that affect the response of the medications in the body. In some literature pharmacogenetics is used interchangeably with pharmacogenomics; however, other literature defined pharmacogenomics as the study of the interaction effects in between genes (Zhang, Bruce, Hayden, & Rieder, 2014). Wolf et al., 2000 divided the study of PGx into two aspects:

- The study of genes associated with specific diseases that can be targeted by medications
• The study of genetic variability that can alter drug response

Medications demonstrate their actions on the body by working at three levels: the enzymes metabolizing the drug, the signaling pathway, and the receptor on the targeted tissues (Ensom et al., 2001). Most of the identified genetic variation is a result of single nucleotide polymorphism SNPs that change the coding for a group of enzymes implicated in metabolizing medications (Ensom et al., 2001). The outcome of these SNPs may range from delayed response, increased toxicity, extended effect, adverse reaction or drug-drug interactions (Wolf et al., 2000). Thus, DNA analyzed by the pharmacogenetics testing would provide an individualized information about the efficacy and safety of the prescribed medications (McCarthy, 2001).

**Beneficial Role of Pharmacogenetics**

One of the studies indicated that 1 in 15 hospital admissions is related to side effects of drugs (Wolf et. al., 2000); whereas among children, adverse effects count for 2-4% of hospital admissions and 3% of out-patient visits (Zhang et al., 2014). Therefore, being knowledgeable of the personalized adverse effects prospectively, by detecting the genetic coding of enzymes responsible for the metabolism of the prescribed drugs, would reduce the cost, time, and harmful events (Bowker et al., 2011; Zane et al., 2008).

Many patients are deprived from pharmacological choices because the standard dose of this medication may accumulate in their body leading to drug related toxicity. The situation becomes even more critical when the pharmacological choices are lifesaving as in oncology patients (Drew, 2016). Pharmacogenetics testing enables physicians to bypass all these obstacles by establishing a constant “personal pharmacogenetics profile”. This profile can provide recommendations of the type,
dosing, and alternative of the drugs that may be prescribed during the patient’s life time; consequently, healthcare providers can make their decisions based on the genetic information of the patient (Wolf et al., 2000).

**Knowledge and Attitude towards Pharmacogenetics**

The future of pharmacogenetics is highly promising; nonetheless, public acceptance is still the cornerstone of pharmacogenetics’ prosperity (Innocenti, Iyer, & Ratain, 2000; Zhang et al., 2014). Research has shown that people’s enthusiasm about pharmacogenetics varies between the general population and those who are suffering chronic diseases (Zhang et al., 2014). Adding to that, the baseline understanding of pharmacogenetics and health condition had a significant effect on the individual’s level of comfort to perform a pharmacogenetics testing (Innocenti et al., 2000). It is noteworthy to mention that even those that showed interest in PGx testing, after testing, they are often not compliant with the test results (Zhang et al., 2014). In other words, they would take the medication regardless of the fact that it can cause serious side effects or even if the drug is contraindicated in respect to their genetic variability. Therefore, educating public about pharmacogenetics plays an important role in a successful pharmacogenetics implementation in the clinical settings (Zhang et al., 2014).

**Cytochrome P450 (CYP450) family of enzymes**

Most of the genes investigated in PGx drug panels encode enzymes of the cytochrome P450 family of enzymes. These enzymes are synthesized in the liver and they are responsible for metabolizing numerous prescribed drugs. Different alleles are analyzed for each gene. In this study, alleles investigated were chosen due to a) their association with the alteration of enzymatic activity, b) their association with increasing
side effects of specific drugs and c) their role in drug-drug interaction. Different alleles are associated with decreased CYP450 enzymatic activity, causing a decrease in the metabolism of certain drugs. The rate at which a drug is metabolized determines its effects on the targeted tissue. For example, decreasing drug metabolism in respect to inhibited enzyme activity can lead to increased drug concentration and consequently increase its toxicity. Alternately, inducing enzyme activity leads to higher rate of drug degradation and therapeutic levels will not be reached as a result of decreased drug bioavailability.

Multiple drugs can play an important role in altering the effects of CYP450 enzymes by inducing or inhibiting these enzymes. Below are common drugs/supplementation that affect CYP450 activities:

**Inducers:**

Phenytoin, phenobarbital, rifampin, carbamazepine, ginseng, oral contraceptive and griseofulvin.

**Inhibitors:**

Ritonavir, amiodarone, cimetidine, macrolides, cranberry juice, omeprazole, quinidine and SSRIs.

**Genes targeted in the PGx drug panel**

The following section will describe 12 different genes that are targeted in the PGx drug panel. These genes were selected because of their importance in the metabolism of commonly prescribed drugs. For each drug the following information will be presented: clinical relevance, drug-drug interaction, and targeted alleles of interest.

**CYP1A2**
On the genetic mapping CYP1A2 was found on chromosome 15q24.1 sharing the same 5' flanking region with CYP1A1. This enzyme is highly susceptible to the stimulatory effects of many drugs such as carbamazepine, phenytoin and rifampin. Similarly, a wide spectrum of drugs inhibit CYP1A2 like isoniazid, zileuton and rofecoxib. Those effects do not control the clearance of all drugs that have first pass metabolism in the liver, only drugs that is catabolized by the same enzyme would show a variation in their bioavailability. For instance, zileuton has an expressive pharmacokinetics effects on antipyrene and propranolol, while its effect on other agents; that are not metabolized by CYP1A2, were unnoticeable. Two alleles were tested; CYP1A2*1F and CYP1A2*1K, studies showed that patients with CYP1A2*1K tend to have a significant decrease in the enzyme activity. On the opposite side, environmental factors such as tobacco smoking induce enzyme activation so increasing drug dosage would be required. The importance of this enzyme came from its ability to activate many endogenous chemical substances in the body and convert pro-carcinogens compounds into carcinogens through its oxidative characteristics (S. F. Zhou, Wang, Yang, & Liu, 2010).

CYP2B6

This gene takes its significance from being a highly polymorphic gene implicated in the transcription, splicing and translation of mRNA which create a variation in protein expression. Adding to that, the excretion of enzymes coded by this gene is not only limited to the liver but it was also found in extrahepatic systems as the respiratory and gastrointestinal tissues. One of the characteristics these tissues share, is their ability to provide a protection barrier against different environmental toxins, which highlight the
impotence of this gene in detoxifying these chemicals. One of the most studied drugs metabolized by CYP2B6 is efavirenz which is used in combination with other agents to treat HIV positive patients. Efavirenz belongs to NNRTIs; a group of drugs that inhibit viral DNA synthesis from RNA template. Close monitoring of drug concentration should be considered as it has a low therapeutic index. Efavirenz toxicity can cause neuropsychiatric symptoms that is found to be associated with Q172H variant on CYP2B6*6 allele. It was found that the presence of this allele was associated with a down regulation in protein translation which leads to 50-75% decrease in enzyme activity that cause decrease in dug elimination and increase drug toxicity (Hedrich, Hassan, & Wang, 2016; Y. Li et al., 2013; Zanger & Klein, 2013).

*CYP2C19*

This highly variable gene; more than 80 SNPs has been related to CYP19, is responsible for the metabolism of wide range of drugs such as:

- Proton pump inhibitors (omeprazole): used to treat ulcer disease induced by helicobacter pylori and GERD.
- Anti-depressants (citalopram, clomipramine): to treat depression.
  - (Amitriptyline): treat depression and migraine headaches.
- Aromatase Inhibitors (AI): used in substitution to anti estrogenic agents in patients who lack CYP2D6 gene activity, AI has been prescribed to postmenopausal women to suppress estrogen conversion in the peripheral tissues.
Different alleles were studied in comparison to the wild type \((CYP2C19 \, ^{*1/*1})\) that codes for a normal metabolizer. It was found that the homozygosity of \(CYP2C19 \, ^{*17}\) allele was associated with lower drug concentrations because of increased enzyme activity. The increase in enzymatic metabolic functions was mediated by activation of transcription factors that enhance gene transcription of CYP2C19 gene. This phenotype variant plays its effect on the metabolism of anti-depressants and proton pump inhibitor agents (Hicks et al., 2013; Sim et al., 2006).

\textit{CYP2D6}

The CYP2D6 is the most detected gene in PGx testing as it is an important indicator in the metabolism of psychiatric medications (Wolf et al., 2000). Although this enzyme present in small quantities in the CYP450 family, its role is highly relevant as it accounts for metabolizing more than 25\% of the most commonly prescribed drugs. In addition, studying this gene polymorphism is very important as it interferes highly with the pharmacokinetics of nearly 50\% of the most commonly prescribed drugs. This enzyme metabolizes chemotherapeutic agents like tamoxifen; anti estrogenic agent used to treat breast cancer, by converting it to its active metabolites. Increased active amounts of this enzyme are found to be related to lower drug concentrations and higher adverse effects of the affected drug. For instance, more active levels of CYP2D6 leads to lower concentrations of tamoxifen and increase vomiting severity as an adverse effect of this agent. MDMA, which is a psychoactive drug that increases the stimulant neurotransmitter levels in the synaptic cleft, is metabolized by CYP2D6. Interestingly, it is a potent inhibitor of CYP2D6 and dramatically decreases the enzyme levels, thus continuous use of these substances leads to overdose toxicity as the enzyme that degrade these
metabolites is blocked. Decrease enzyme activity was associated with *10 and *14 allele. Concomitant use of drugs that inhibit CYP2D6 may lead to inactivation of the gene and predispose the patient to be a poor metabolizer even though, the patient genotype detected as normal metabolizer (Hicks et al., 2013; Ingelman-Sundberg, 2005; Mas et al., 2012; Wolf et al., 2000; S.-F. Zhou, 2009).

**CYP3A**

CYP3A is a very important enzyme in drug metabolism as it is considered as the most active enzyme responsible for clearance of the majority of prescribed drugs like midazolam, erythromycin, cyclosporine and diltiazem. High quantities of this enzyme are commonly detected in both the liver and intestines. Genetic mutations of this enzyme are implicated in the alteration of enzymatic activity. Research aimed to detect potential causes of genetic mutations of this gene, showed that the variation in exons and introns likely alters enzymatic activity and quantity.

Several drugs may either enhance or inhibit CYP3A activity, thus drug-drug interactions should be cautiously checked among individuals with altered CYP3A activity, as they may be at higher risk of serious and even life threatening side effects. For example, antifungal agents, nitromedazole, verapamil and diltiazem are all CYP3A inhibitors. Administration of these agents concomitantly with other agents metabolized by the same enzyme’ like erythromycin, would lead to decrease erythromycin clearance with an increase in its toxic effects. Erythromycin toxicity may cause QT prolongation that may develop to torsade de point and sudden cardiac death. Similarly, the consumption of grapefruit; which is CYP3A inhibitor; simultaneously with Calcium
Channel Blockers (CCB) can lead to extensive vasodilation and reflex tachycardia as a side effect of calcium channel blockers’ adverse effects.

Interestingly, this phenomenon of drug-drug interaction can aid in some of the treatment methodologies to eradicate certain viruses. Antiretroviral medications, which are used to slow the rate of Human Immunodeficiency Virus HIV replication, are required to be in high concentrations in order to reach the therapeutic benefits. To achieve this goal, ritonavir is administered with other protease inhibitors to increase their level in the plasma. Ritonavir is a CYP3A inhibitor which decreases CYP3A enzymatic function in both the liver and the intestines. Other protease inhibitors are metabolized by the same enzyme (CYP3A); therefore, their plasma concentration would be increased appropriately in response to the attenuation of CYP3A enzyme activity. Another mechanism by which ritonavir increases other drug concentrations in the body is by blocking the P-glycoprotein on the intestinal wall. P-glycoprotein, also known as Multi Drug Resistance Protein-1 (MDR1), has an ATP dependent efflux pump on the cell membrane that prevents the accumulation of drugs intracellularly. Consequently this action inhibits the absorption of several drugs and facilitates their excretion in the intestinal lumen (Bofitto, 2004; Lamba, Lin, Schuetz, & Thummel, 2012; Wilkinson 2005; Yasuhara, 2006).

ANKK1/DRD2

These two genes act as a receptor in the dopaminergic pathway. Dopamine is the main neurotransmitter that controls movement regulation, cognitive status and prolactin secretion. It acts via three different physiological pathways: nigrostriatal, mesocortical and tuberoinfundibular. Damage of dopaminergic neuron in the basal ganglia contributes
to Parkinson disease. In contrast, the increase in dopamine is implicated in schizophrenia and Huntington disease. Antipsychotics medications antagonize the D2 receptor causing drug-induced Parkinsonism by blocking D2 receptors in the nigrostriatal pathway. The irreversible effects of inducing tardive dyskinesia is highly associated with the A2-A2 genotype on Taq1A in DRD2 gene. Other studies have shown that patients who have \textit{DRD2}*A1 allele were more susceptible to experience hyperprolactinemia as a side effect of using antipsychotics (Bakker, van Harten, & van Os, 2008; Huang et al., 2009; Young et al., 2004).

\textbf{COMT}

This gene codes for the COMT enzyme that is found in the liver and brain. It plays an important role in the degradation of catecholamines neurotransmitters. Decreased activity of this enzyme causes accumulation of dopamine; increased dopamine concentration is one of the drug mechanisms used to treat Parkinson disease. Polymorphisms of the COMT gene result from the substitution of valine amino acid with methionine (Val158Met). The outcome of this variation is a methionine variant that has a lower action on catalyzing neurotransmitters in the dopaminergic pathway. The increased dopamine concentration, as a result of the methionine variant, is likely responsible for the wide variation in physiological responses to neurostimulants, such as amphetamine, used to treat ADHD. Research showed that people with valine variant (wild type Val/Val) of the COMT gene tend to have better drug efficacy to neurostimulant agents as they have lower pre-treatment concentration of dopamine in their synapses, compared to those with the methionine variant. Similarly, modulating the dosage of morphine is required depending on the genotype. Higher doses of morphine were administered to patients
holding homozygous valine genotype, compare to those with homozygous methionine genotype, in order to provide the same level of pain relief (Bellgrove et al., 2005; Meyer-Lindentberg et al., 2006; Rakvag et al., 2005).

**OPMR1**

Analgesics antagonize opioid receptors in the body, such as mu beta-endorphin opioid receptor (OPMR1), and alterations in the OPMR1 gene influence a wide range of variation in response to opioids among patients. Although different loci in the OPMR1 gene still need to be further investigated, strong associations were found between altered pain threshold and the single nucleotide polymorphism G variant at position 118 (A118G). This missense mutation substitutes the amino acid asparagine with aspartic acid, resulting in decreased glycosylation on the N-terminal site. This missense mutation is associated with altered pain sensitivity, so dosage modulation of opioids is required accordingly. Patients who are homozygous to guanine variant (G/G) genotype require higher doses of morphine compared to those with the normal adenine (A/A) genotype. However, there are still controversial speculations regarding the heterozygous (A/G) genotype. Research indicates that the fluctuation in opioid response is dependent on the clinical setting such as labor, cancer, postoperative or chronic disease. Additionally, other investigations suggest that ethnicity may be a potent contributor to this diversity (Hwang et al., 2014; Rhodin et al., 2013; Shabalina et al., 2009). To summarize, pharmacogenetics testing implications in aesthetic drugs still at the early beginnings and further inspections of mutations in other genes; like ABCB1, CYPs, or UGT’s, that may be involved in response variation yet to be studied (Hwang et al., 2014; Rhodin et al., 2013; Shabalina et al., 2009).
**SLCO1B1**

Solute Carrier Organic Anion Transporter family member 1B1 (SLCO1B1) is the gene encoding the protein responsible for the transport of different chemicals, including drugs, to the liver in order to be metabolized. This gene was found to be implicated in several clinical implications such as bladder cancer, hematologic malignancy, and myopathy (J. Li, Wang, X.-R., Zhai, X.-W., Wang, H.-S., Qian, X.-W., Miao, H., & Zhu, X.-H., 2015). The following are examples of its clinical relevance:

- The allele GG/AG at SLCO1B1 rs2306283 gene has been associated with bladder cancer, decrease arsenic detoxification thought to be the culprit of this process. Arsenic is a carcinogen that is involved in toxic reduction reaction and it predispose to squamous cell carcinoma in the bladder, it is commonly associated with occupational exposure (Bui, Fujimoto, Kubo, Inatomi, & Matsumoto, 2014; Gribble et al., 2013).

- SLCO1B1 on chromosome 12 codes for a membrane transporter called OATP1B1 responsible for the efflux of one methotrexate; a chemotherapeutic agent used in the treatment of acute lymphoblastic leukemia ALL, which is the most common hematologic malignancy in children. Increase in methotrexate toxicity was found to be related to two different genotype in the SLCO1B1 gene; rs4149081AA and rs11045879CC (J. Li, Wang, X.-R., Zhai, X.-W., Wang, H.-S., Qian, X.-W., Miao, H., & Zhu, X.-H., 2015).

- One of the commonly prescribed drugs that are highly affected by SLCO1B1 variant is statins. Statins are lipid lowering agents that decrease LDL, increase HDL and decrease the risk of cardiovascular complications. The most common observed adverse effect of using statins is myopathy, which may range from moderate to severe in some cases. Studies showed that there is a strong association between the statin-induces
myopathy and variant 521T>C (rs4149056) on the *5 allele which decrease the function of SLCO1B1. Furthermore, TT genotype was found to prevent the development of myopathy, while C allele predispose to myalgia (Group, 2008; Linde, Peng, Desai, & Feldman, 2010; Stewart, 2013).

**MTHFR**

Methylenetetrahydrofolate reductase is the rate-limiting enzyme that irreversibly converts 5, 10 methylenetetrahydrofolates to 5 methylenetetrahydrofolates (5 MTHF). It is encoded by the MTHFR gene. In order for folate to be in the active form (5 MTHF), it first needs to be reduced by the MTHFR enzyme. Folate plays an important role in the synthesis of nitrogenous bases in DNA and RNA. It serves as a coenzyme for 1-carbon transfers in the cysteine cycle that regulate homocysteine levels in the blood. A mutation in MTHFR gene, found on chromosome 1 (p36.3), may lead to impaired DNA repair, DNA hypomethylation, folate deficiency and even an increase in homocysteine levels (Del Greco et al., 2011). Below is a description of the different allelic variants detected in MTHFR gene that are clinically relevant:

- **MTHFR gene mutation and kidney pathology:** It was proposed that the main predictor of 5 MTHF activity as a metabolite that facilitate homocysteine conversion to cysteine is a SNPs on MTHFR gene; C677T polymorphism. This mutation is implicated in reduced enzyme MTHFR activity causing hyperhomocysteinemia, increased homocysteine levels predispose to increase risk of thrombosis and cardiovascular diseases. Moreover, C677T polymorphism in combination with another mutation; A1298C (rs1801131, Glu429Ala) in Exon 7, showed to cause hypertensive nephrosclerosis in patients with renal failure; additionally, both mutations can lead to
hyperhomocysteinemia. Interestingly, these complications were noticeable in individuals with the C677T mutation alone, but not with individuals who express just the A1298C polymorphism (Fung et al., 2012).

- **MTHFR and cancer risk:** The association between MTHFR A1298C polymorphism and malignancy has been found to be related to the cancer site and ethnicity. A1298C was associated with increased risk for lymphoma and cervical cancer; controversially, it was linked to decrease colorectal cancer risk. Both increase and decrease malignancy risk was detected in Asian population in specific (Zhu et al., 2016).

- **MTHFR and BNP (B-type natriuretic peptide):** It was proposed that the variant on a specific gene may show interaction with variants located on other genes; therefore, MTHFR gene was studied among a group of genes MTHFR-CLCN6-NPPA-NPPB gene cluster. Although the four genes were associated with BNP levels; BNP plasma levels are used to rule out heart failure, robust association was found with MTHFR C688T polymorphism. This relation between the variant in the gene cluster and the BNP was also related to hypertension; however, systolic blood pressure was highly related to a SNP on MTHFR gene as well. The influence of C677T polymorphism is not exclusive to the pathology of the disease solely, but it was related to the treatment choices; for example, the response to ACEI; anti-hypertensive medication, is affected by the C677T polymorphism (Del Greco et al., 2011; Fung et al., 2012).

- **MTHFR gene and ASD:** MTHFR deficiency is associated with increased oxidative and decreased reduced metabolites in the transsulfuration pathway of homocysteine, that creates a metabolic imbalance and increase the risk of oxidative stress which was noticed in autistic children. Additionally, MTHFR gene heterozygote mutation
has been strongly associated with hyperhomocysteinemia which predispose to cardiovascular diseases as it increases the oxidative stress and decreases nitric oxide that has vasodilatory effects; consequently, MTHFR gene variant underlies an early onset and increase susceptibility to a hypercoagulable state (James et al., 2006; Kim, Lee, Kim, Kim, & Yoo, 2013; Pramukarso, Faradz, Sari, & Hadisaputro, 2015). Folate and B12 vitamin supplementation are administered in patients who have MTHFR deficiency to reduce the risk of thrombosis (Kim et al., 2013). These vitamins are also used in the treatment of autism as it improves the behavioral symptoms; however research showed that prenatal vitamin supplementation played an important role in preventing autism occurrence in the offspring(Schmidt et al., 2011).
Chapter Three: Methodology

Participants

Participants were recruited from clients participating in the KAP (Kelly Autism Program) at Western Kentucky University (WKU). The KAP is housed in the Suzanne Vitale Clinical Education Complex (CEC) on the WKU campus, and the program provides a variety of services to those diagnosed with ASD, as well as to their families.

A research team member was responsible for identifying potential participants and contacting their caregivers/legal guardians to share information about the study. All participants and their caregivers were involved to give utmost consideration to the unique and specific needs of the participants, who represent a particularly vulnerable population. All of the investigators have extensive experience with conducting research with vulnerable populations, in general, and with children with ASD, in particular. This interdisciplinary research team brings unique strengths and skills that involve consenting children and their parents in primary data collection activities and are aware of the necessary steps that must be taken to safeguard them not only from undue risks. All potential participants were given an informational brief (Appendix A) and a copy of the caregiver informed consent (Appendix B). In each case, participants and their caregivers were aware of risks and requirements for participating. It was communicated that participation was completely voluntary and would not affect their KAP programming in any way. The testing that was performed in this study was performed with the best interests of the child in mind. All participants for this study met the following inclusion criteria:
1) There was documentation that the student has been identified by a third party professional as having an Autism Spectrum Disorder,

2) The student was between the ages of 7-17,

3) The student had a current or past sleep disturbance, as measured by caregiver report,

4) The student was currently taking a daily medication.

As the study sample for this project was composed of minors with a disability, there were multiple steps to the consent process. All caregivers had to complete a written informed consent (Appendix B). Additionally, four separate assent documents were created targeting the unique communication methods of participants with ASD (Appendices D-G).

A research team member was available for in-person meetings or phone conferences to answer all questions the caregivers might have had regarding the study. Once parents signed the consent document, they were asked to identify their child's most common communication method (Appendix C).

This information was used to obtain child consent. Caregivers were asked to identify one of four categories of communication:

(a) The child can sign his/her name to give consent;

(b) The child can check a box to give consent;

(c) The child can use sign language to give consent, or

(d) The child can use a communication device to give consent.

Once informed caregiver consent was obtained, one of the investigators or their designee acquired participant assent.
One of the investigators trained in working with students with ASD read one of the four assent documents associated with the child’s most common method of communication. The investigator was instructed to read the script exactly as it was written and then prompt a response from the participant. Once informed caregiver and participant assent had been obtained, the PI then collected information about whether the participant met inclusion criteria. If the participant met inclusion criteria, he/she was placed in a pool to be randomly selected for inclusion in the study. If the participant did not meet inclusion criteria, a letter was sent to the informal caregiver explaining that the student was not eligible for participation in the study. A total of fifteen (15) participants with ASD were selected to participate in the study.

**Blood Sampling**

The blood draw procedures coincide with the guidelines set forth by the U.S. Department of Health and Human Services Office for Human Protections Research. Blood samples were obtained using Universal Precautions at rest, and approximately 5 ml of venous blood was drawn from the cubital or cephalic vein of each participant’s arm. Each participant had only one blood draw. All blood draws will be done by a skilled professional trained in phlebotomy from the Exercise Physiology laboratory in the School of Kinesiology, Recreation & Sport (KRS).

The phlebotomist doing the blood draw also had a brief training by the investigators regarding blood draws from children with ASD. The training was centered on the guidelines and suggestions in the “Phlebotomy Toolkit for Providers Treating Children with Autism”, and it was ensured that the research group was adequately prepared with proper accommodations and supports. Additionally, one of the
investigators (specifically trained to work with students with ASD) was present during the entire duration of the blood draw for each participant. To minimize risks to study participants and to identify potential support prior to bringing children in front for a blood draw, a checklist was provided to the parent to ensure that it is a good fit for the child and that the parent believes the child was prepared to have his/her blood drawn (Appendix C).

Blood draws were done in a classroom at the CEC that is designated for blood draws during the duration of this study. The participants were recruited from programs at SVCEC, so they were in a familiar, comfortable environment. The duration of each blood draw depended on the behavior and willingness of each participant but it was anticipated that they will take anywhere from two minutes to fifteen minutes. If at any time, the participant expressed anger or discomfort with the blood draw, the blood draw was immediately stopped.

_Blood draw procedures were as follows:_

1. Wrap a tourniquet around the patient's upper arm to stop blood flow;
2. Sterilize the puncture site with alcohol;
3. Insert the needle into the vein with the bevel up;
4. Attach the appropriate test tube to the needle. Allow the blood to fill the test tube;
5. Remove the tourniquet to restore blood flow;
6. Place a gauze pad over the site while withdrawing the needle; and
7. Apply firm pressure to the site until bleeding has stopped

_Blood Storage and Transport_
Individual vials of participants’ blood labeled and coded appropriately for anonymity. It was stored in the KRS Exercise Physiology laboratory at a temperature of -80°C until it is shipped to MyGenetx in Franklin, Tennessee for genetic analysis. Shipping was done in small batches (3–5 vials per batch) via FedEx. All procedures for maintaining integrity of the blood, as well as appropriate measures for confidentiality, were followed for shipping.

**Genetic Analysis of Blood**

All genetic analyses of participants’ blood was conducted at MyGenetx in Franklin, Tennessee. MyGenetx is a molecular and general chemistry laboratory involved in research concerning pharmacogenetics, chronic disease management, oncology, and endocrinology, as well as other current health issues.

*Genetic analysis procedures were as follows:*

1. Next generation sequencing (NGS) is performed in three steps that begin with the generation of a template. Template preparation consists of building a library of nucleic acid and amplifying that library.

2. Sequencing libraries are constructed by fragmenting the DNA sample and ligating adapter sequences onto the ends of the DNA fragments.

3. Once constructed, libraries are clonally amplified in preparation for sequencing.

4. Following template preparation, nucleic acid sequencing occurs from the amplified libraries; the platform relies on sequencing by synthesis.

5. The library fragments act as a template, off of which a new DNA fragment is synthesized.
6. The sequencing occurs through a cycle of washing and flooding the fragments with the known nucleotides in a sequential order. As nucleotides incorporate into the growing DNA strand, they are digitally recorded as sequence.

7. Once sequencing is complete, raw sequence data must undergo several analysis steps.

8. A generalized data analysis pipeline for NGS data includes preprocessing the data to remove adapter sequences and low-quality reads, mapping of the data to a reference genome or de novo alignment of the sequence reads, and analysis of the compiled sequence.

9. Analysis of the sequence can include a wide variety of bioinformatics assessments, including genetic variant calling for detection of SNPs or idles (i.e., the insertion or deletion of bases), detection of novel genes or regulatory elements, and assessment of transcript expression levels.

10. Analysis can also include identification of both somatic and germline mutation events that may contribute to ASD and sleep disturbance.

11. Analysis of the data will include incorporation of proteomic, metabolomics, transcriptomic, immunomic, signaling pathways and disease data to identify unique molecular connections. These unique connections are placed under increasing stringency to identify genes with the highest probability of connection to ASD and sleep disturbance.

12. The overarching goal of the analysis is to identify genes that play a role in the pathogenesis and progression of ASD and to identify potential therapeutic targets.
13. The identification of targets can allow for the personalization of therapy for each patient based on the individual genomic map that is produced.

All information was de-identified before delivery to the MyGenetx laboratories. MyGenetx never had information that connects an individual participant with their blood draw. Electronic data were numerically coded for anonymity and stored in a password-protected document on a password-protected computer.

**Drug Panel**

The results of the pharmacogenetics testing were reported as a summary of the selected genes with their specific genotype, phenotype and the alleles that were tested. Most of the potential drugs, affected by the detected genes that may be used currently or over the participant’s life time, were listed. According to the personalized genotype, medications were classified as drugs to use with standard precautions, drugs to use with caution and drugs that need to consider alternatives.

Following these classifications, a detailed dosing guidance; as predicted by the participant’s genotype; was provided for each medication belongs to the use with caution category. A description of individual’s level of sensitivity to the drug and the recommended dosage adjustment to reach the desired response was proposed. Adding to that, all the adverse effects that may occur as a response to high drug doses or as a complication of environmental factors; such as smoking or smoking cessation; was mentioned. Close monitoring of specific body vital signs, organ functions or blood cell count measurements was advised when using drugs that may cause alteration in these parameters. Moreover, drug substitution was suggested for the category of agents classified as consider alternatives. At the end, a focused literature review including an
assay interpretation, clinical utility and clinical implementations of each gene was provided.

**Survey and Training Session**

*Survey*

A pre-training survey was designed to estimate the knowledge of, attitudes towards, and future expectations of pharmacogenetics among the guardian’s participants. These surveys were administered before the pharmacogenetics results training session (lead by a research team member). The level of knowledge was detected by asking questions about their understanding of the healthcare application of pharmacogenetics and about how familiar they are with pharmacogenetics. Other questions in the survey related to their attitude measured how interested they were in learning about pharmacogenetics or how they rate the impotence and beneficial role of pharmacogenetics. Additionally, their level of confidence regarding communication with their doctor about pharmacogenetics was assessed. Likewise, the parent’s expectations that pharmacogenetics testing results would enable their physicians to prescribe better choices of drugs were questioned. Participants were directed to answer the survey to the best of their understanding.

Post-training surveys, identical to the pre-training surveys, were administered to compare how participants responded to the training session.

*Training Session*

A training session was conducted to help the participants gain better understanding of the results depicted in the drug panel. A brief introduction describing the principles of how and why an individual may respond to medications was given.
The leader of the training session described how individuals respond to medications differently, despite the medication having the same mechanism of action. Participants were informed that this concept is the basis of an emerging field of study called pharmacogenetics, which is the study of gene-medication interaction. Additional information regarding the explanation of how genetic variations likely cause individualized responses to different medications was explained.

During the training session, a summary of the steps needed to for pharmacogenetics testing was described as following:

1. Conduct a blood draw
2. DNA is isolated in the lab
3. Unique marker in DNA are identified
4. These markers give clues about how medicines will work, because medicines work differently in different people

The training session explained how the genes selected to be analyzed are implicated in the metabolism of the most commonly prescribed medications that the children may be taking currently or may take during their life time. These genes control the efficacy of the drugs and classify medications in three categories: standard precautions, use with caution (for drugs that cause adverse effects) and use alternatives (either because of reduced response or increased sensitivity to the drug).
Chapter Four: Attitudes, Knowledge and Future Expectations towards Pharmacogenetics among Families of Children with Autism Spectrum Disorder

Abstract

Pharmacogenetics is the study of genomic-guided individualized drug prescription that plays an important role in preventing the severe adverse effects of drugs, decreasing the time and cost of therapeutic choices, and directing healthcare professionals to choose medications that are effective and safe. It is noteworthy that this approach becomes highly beneficial in patients suffering from chronic diseases or disorders, since these conditions may require multiple and long term pharmacological therapies, as in children with autism spectrum disorder (ASD). However, public acceptance is a major challenge when implementation of pharmacogenetics merges into clinical practice. The purpose of this study is to inspect the knowledge of, attitude towards and future expectations with regards to pharmacogenetics among parents of children with ASD. A group of 15 school-aged participants with ASD were recruited for the study. Approximately 5 ml of venous blood was drawn for each participant to analyze the genotype of enzymes implicated in drug metabolism via pharmacogenetics testing. Thereafter, the parents of these children attended a training session to help them gain a better understanding of the pharmacogenetics results depicted in the drug panel results. A pre-training and post-training survey was conducted to assess the knowledge of, attitude towards and future expectations of pharmacogenetics among the children’s parents.
Introduction

*Autism Spectrum Disorder (ASD)*

Autism Spectrum Disorder (ASD) is a neurodevelopmental disability characterized by restricted interests, repetitive behaviors and deficits in communication and social reciprocity skills (White, Keonig, & Scahill, 2007). Due to the prevalence of children identified with ASD (1 in 68 children), there is a need for interventions targeting the specific characteristics children and adolescents with ASD display (Center for Disease Control and Prevention, 2014).

*Characteristics of Autism Spectrum Disorder*

According to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V), there are five factors that make up the diagnostic criteria for ASD. The first feature includes deficits, which persist over time, in social communication and social interaction. These deficits can include, but are not limited to, abnormal social approach, failure to grasp the pragmatics of normal conversation, failure to start or participate in social interactions, abnormal eye contact and body language, failure to develop and maintain social relationships, difficulties initiating or maintaining friendships, and a lack of interest in peers. The second factor includes restricted and repetitive behaviors and interests. Examples of this type of behavior include, but are not limited to, repetitive speech, insistence on adhering to routines, highly restricted perseverative interests with an abnormal intensity, and unusual sensory interests. The third factor says that the symptoms must be present in the early developmental period. The fourth factor says that the symptoms cause clinically significant impairment in certain areas of functioning. The final factor says that the symptoms are not better explained by another disability.
Pharmacological treatment of ASD

Medications are one of the multiple interventions used to treat autism. The concomitant use of multiple therapies was common due to the comorbidity nature of the disease; thus detecting the efficacy of the treatment was further complicated (Bowker et al., 2011; Zane et al., 2008). However, medications; that are used to treat comorbid rather than main symptoms of the disease, has been reported to be one of the most frequently discontinued treatment methods (Bowker et al., 2011; McPheeters et al., 2011). Therefore, the increasing demands on pursuing pharmacological choices, the unclarity of efficacy and the alarming adverse effects of some of the drugs with the high cost of these modalities, prompt the need for further investigation in the field (Bowker et al., 2011; Zane et al., 2008).

Research that reviewed the most commonly prescribed medications used in the treatment of autism addressed the following classes of drugs; antipsychotics, SSRIs (as part of anti-depressants) and anti-ADHD agents (McPheeters et al., 2011), clinical use of these agents improves challenging behaviors (irritability, aggression and self-injurious behavior), repetitive behavior and hyperactivity respectively. Although antipsychotics; particularly aripiprazole and risperidone, are proved to be efficacious, sever adverse effects were observed when using these agents as 30% of patients using aripiprazole were susceptible to weight gain. The adverse effects of atypical antipsychotics manifested by weight gain, sedation and extrapyramidal symptoms (McPheeters et al., 2011; Stachnik & Gabay, 2010).

Although both citalopram and fluoxetine are agents from the SSRIs category, their targeted benefits were found to be divergent. While fluoxetine improves repetitive
behavior, citalopram ameliorates challenging behavior. Other class of medications that was used by 30-85% of children with ASD is anti-ADHD, these neurostimulant medications were prescribed to provide a short-term relief for symptoms of hyperactivity and impulsivity (Dalsgaard et al., 2013; McPheeters et al., 2011). Regardless of the beneficial effects of medications used to treat autism, close monitoring is required as they may cause serious adverse effects because of the long term use of these medications (McPheeters et al., 2011).

Having a Prospective expectations of the possibility to develop potential adverse effects of the most commonly prescribed drugs for patients with ASD, can protect the patients from being predisposed to avoidable adverse effects of these drugs in addition to saving money and giving the time for other therapeutic choices to be prescribed. That critical goal can be accomplished by requesting a pharmacogenetics testing (Bowker et al., 2011; Wolf et al., 2000; Zane et al., 2008)

**Pharmacogenetics (PGx)**

What is pharmacogenetics?

Pharmacogenetics is the study of genes that affect the response of the medications in the body. In some literature pharmacogenetics is used interchangeably with pharmacogenomics; however, other literature defined pharmacogenomics as the study of the interaction effects in between genes (Zhang et al., 2014). A number of literature divided the study of PGx into two aspects:

- The study of the genes associated with specific diseases that can be targeted by medications
The study of genetic variability that can alter drug response (Wolf et al., 2000).

Medications demonstrate its action on the body by working at three levels: the enzymes metabolizing the drug, the signaling pathway and the receptor on the targeted tissues (Ensom et al., 2001). Most of the identified genetic variation is a result of single nucleotide polymorphism SNPs that change the coding for a group of enzymes implicated in metabolizing medications (Ensom et al., 2001). The outcome of these SNPs may range from delayed response, increased toxicity, extended effect, adverse reaction or drug-drug interactions (Wolf et al., 2000). Thus, DNA analyzed by the pharmacogenetics testing would provide an individualized information about the efficacy and safety of the prescribed medications (McCarthy, 2001).

**Beneficial Role of Pharmacogenetics**

One of the studies indicated that 1 in 15 hospital admissions is related to side effects of the drugs (Wolf et al., 2000); whereas among children, adverse effects count for 2%-4% of hospital admissions and 3% of out-patient visits (Zhang et al., 2014). Therefore, being knowledgeable of the personalized adverse effects prospectively; by detecting the genetic coding of enzymes responsible for the metabolism of the prescribed drugs, would reduce the cost, time and harmful events (Bowker et al., 2011; Zane et al., 2008).

Many patients are deprived from pharmacological choices because the standard dose of this medication may accumulate in their body leading to drug related toxicity. The situation becomes even more critical when the pharmacological choices are lifesaving as in oncology patients (Drew, 2016). Pharmacogenetics testing enable the
physicians to overpass all these obstacles by establishing a constant “personal pharmacogenetics profile”. This profile can provide recommendations of the type, dosing and alternative of the drugs that may be prescribed during the patient’s life time; consequently, healthcare providers can make their decisions based on the genetic information of the patient (Wolf et al., 2000).

Knowledge and Attitude towards Pharmacogenetics

The future of pharmacogenetics is highly promising; nonetheless, public acceptance is still the cornerstone of pharmacogenetics’ prosperity (Innocenti et al., 2000; Zhang et al., 2014). Research showed that people enthusiasm about pharmacogenetics vary between general population and those who are suffering chronic diseases (Zhang et al., 2014). Adding to that, the baseline understanding of PGx and the health condition had a significant effect on the individual’s level of comfort to perform a pharmacogenetics testing (Innocenti et al., 2000). It is noteworthy to mention that even though public showed interest to do a PGx testing, they do not seem to be compliant to the test results. In other words, they would take the medication regardless of the fact that it can cause serious side effects or even if the drug is contraindicated in respect to their genetic variability. Therefore, educating public about pharmacogenetics plays an important role in a successful pharmacogenetics implementation in the clinical settings (Zhang et al., 2014).

Methods

Nine parents of children with ASD; who were recruited to do the pharmacogenetics testing, attended a training session and performed a survey.

Survey
A pre-training survey was designed to estimate the knowledge of, attitudes towards, and future expectations of pharmacogenetics among the guardian’s participants. These surveys were administered before the pharmacogenetics results training session (lead by a research team member). The level of knowledge was detected by asking questions about their understanding of the healthcare application of pharmacogenetics and about how familiar they are with pharmacogenetics. Other questions in the survey related to their attitude measured how interested they were in learning about pharmacogenetics or how they rate the impotence and beneficial role of pharmacogenetics. Additionally, their level of confidence regarding communication with their doctor about pharmacogenetics was assessed. Likewise, the parent’s expectations that pharmacogenetics testing results would enable their physicians to prescribe better choices of drugs were questioned. Participants were directed to answer the survey to the best of their understanding.

Post-training surveys, identical to the pre-training surveys, were administered to compare how participants responded to the training session.

*Training Session*

A training session was conducted to help the participants gain better understanding of the results depicted in the drug panel. A brief introduction describing the principles of how and why an individual may respond to medications was given. The leader of the training session described how individuals respond to medications differently, despite the medication having the same mechanism of action. Participants were informed that this concept is the basis of an emerging field of study called pharmacogenetics, which is the study of gene-medication interaction. Additional
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During the training session, a summary of the steps needed to for pharmacogenetics testing was described as following:

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Results

We conducted the survey pre and post-training to observe the effect of children’s parents awareness on the implementation of pharmacogenetics. Nine parents of children with ASD, who were recruited for the study, attended the training session. Results comparing the variability in parents’ response before and after training regarding attitude, knowledge and future expectations about pharmacogenetics are demonstrated in Table 1. Results showed that participants’ understanding of healthcare application of pharmacogenetics was significantly increased after the training session ($p=0.008$).
Additionally, participants’ familiarity about pharmacogenetics as well as their rating of the importance of pharmacogenetics testing was significantly increased with a P value of (p=0.035). However, some of the attitudes and knowledge aspects trend to be increased in response to training, since: 1) their comfort to talk to their physicians about pharmacogenetics and their rating of beneficial role of pharmacogenetics reached a P value of 0.081, 2) hearing about pharmacogenetics showed a trend to be increased as it has a P value of 0.051.
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How would you rate the importance of pharmacogenetics testing?

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13. How interested are you in doing more pharmacogenetics testing?

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14. How comfortable are you talking to your doctor about pharmacogenetics?

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15. How comfortable are you talking to your child’s doctor about pharmacogenetics?

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19. I was hesitant to have my child participate in the research study because it involved genetic analysis.
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|                          | How much have you heard about pharmacogenetics?                          |
|                          | 1(11%)                                                                   |

|                          | How familiar are you with pharmacogenetics?                             |
|                          | 4(44%)                                                                   |

|                          | I have a good understanding of the healthcare application of             |
|                          | Pharmacogenetics?                                                        |
|                          | 1(11%)                                                                   |
Learning about how my body metabolizes medications could make my medications safer and more effective. I would benefit from learning about how specific genes in the body can affect the metabolism of medications. I would ask my doctor to use pharmacogenetics results to determine the best drug for me.

10. I would request a pharmacogenetics test to determine my best choice of medicine in the future.

16. I would ask my child’s doctor to use my child’s pharmacogenetics results to determine the best medication for them.

Discussion

Our study was designed to detect the significance of educating the public towards pharmacogenetics. Our participants were from families have a child with ASD who may chronically be in need to pharmacological interventions, hence the gene-drug interactions study is highly beneficial in this special population. The baseline survey before the training indicated a limited knowledge of pharmacogenetics among participants which was significantly improved after the training. This finding emphasizes the need to pursue awareness plans about pharmacogenetics.
Chapter Five: Pharmacogenetics Study among Children with Autism Spectrum Disorder

Abstract

Pharmacogenetics is the study of genetic-guided individualized drug prescription that plays an important role in: preventing severe adverse effects of the drugs, decreasing the time and cost of therapeutic choices and directing healthcare professionals to choose medications that are effective and safe. It is noteworthy that this approach becomes highly beneficial in patients suffering from chronic diseases or disorders, since these conditions may require multiple and long term pharmacological therapies; as in children with autism spectrum disorder ASD. The purpose of this study is two-fold: a) to investigate the link between genes previously associated with ASD and also implicated in pharmacological therapies commonly used in this population and b) to report the genotype frequency of genes known to influence drug metabolism among small cohort of children with ASD. Group of 15 school aged children with ASD were recruited for the study, venous blood was drawn to analyze the genotype of enzymes implicated in drug metabolism. Results showed that 66% of our cohort have a mutation to the CYP2C19; similarly, 72%, 66% and 30% have a mutation in the MTHFR, COMT and OPRM1 gene respectively. The mentioned mutations were associated with ASD and detected as genes that are involved in drug metabolism as well.

Key Words: Pharmacogenetics, ASD, Genes of ASD.

Key Message: As the number of children with ASD is escalating, the demand for pharmacological choices is increasing. Thus, it is highly significant to build a treatment plan for this special population that; interestingly, seems to share a group of genetic variants that are associated with developing the disorder and implicated in metabolizing a
group of most commonly prescribed drugs in patients with ASD. Pharmacogenetics is an evolving test that is highly promising in the future of medicine as it aids in creating an individualized pharmacological profile, though its use in the clinical settings is still limited. That profile aims to help healthcare professionals make better treatment choices especially in patients with chronic diseases and those who are in continuous need for medications as in children with ASD. That may even enlighten the idea of having gene-based treatment plan for gene associated diseases or disorders.

**Introduction**

Autism Spectrum Disorder (ASD) is a neurodevelopmental disability characterized by restricted interests and repetitive behaviors and deficits in communication and social reciprocity skills (White et al., 2007). Due to the prevalence of children identified with ASD (1 in 68 children), there is a need for interventions targeting the specific characteristics children and adolescents with ASD display (Center for Disease Control and Prevention, 2014).

**Characteristics of Autism Spectrum Disorder**

According to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V), there are five factors that make up the diagnostic criteria for ASD (Association, 2013). The first feature includes deficits, which persist over time, in social communication and social interaction. These deficits can include, but are not limited to, abnormal social approach, failure to grasp the pragmatics of normal conversation, failure to start or participate in social interactions, abnormal eye contact and body language, failure to develop and maintain social relationships, difficulties initiating or maintaining friendships, and a lack of interest in peers. The second factor includes restricted and
repetitive behaviors and interests. Examples of this type of behavior include, but are not limited to, repetitive speech, insistence on adhering to routines, highly restricted perseverative interests with an abnormal intensity, and unusual sensory interests. The third factor says that the symptoms must be present in the early developmental period. The fourth factor says that the symptoms cause clinically significant impairment in certain areas of functioning. The final factor says that the symptoms are not better explained by another disability. For the purposes of this research, the focus will be on the first and second factor relating to the characteristics children with ASD—social and communication deficits and adherence to perseverative interests (Association, 2013). A variety of different factors have been implicated in increasing the susceptibility to ASD incidence, such as the nutritional status (Castro et al., 2016; Schmidt et al., 2011), genetic variants and the maternal effects whether it is maternal inheritance or prenatal care including nutrition(Castro et al., 2016; Hettinger et al., 2012).

Medications are one of the multiple interventions used to treat ASD, concomitant use of multiple therapies was common due to the comorbidity nature of the disease; thus detecting the efficacy of the treatment was further complicated (Bowker et al., 2011; Zane et al., 2008). Due to the wide range of adverse effects of pharmacological therapies, medications are typically one of the most frequently discontinued treatment methods (Bowker et al., 2011; McPheeters et al., 2011). Therefore, the increasing demands of pursuing more effective pharmacological therapies to treat ASD, the unclarity of their efficacy, the alarming adverse effects of drugs commonly prescribed to children diagnosed with ASD, coupled with the high cost of these modalities, prompts the need for further
Pharmacogenetics is the study of genes, more specifically variations within certain genes, which affect the response of the medications in the body. Most of the identified genetic variations in enzymes, transporters, receptors, and other factors impacting drug metabolic pathways are a result of single nucleotide polymorphisms (SNPs), which can ultimately alter the body’s response to that medication (Ensom et al., 2001). The outcome of these SNPs may range from delayed response, increased toxicity, extended effect, adverse reaction or drug-drug interactions (Wolf et al., 2000). Thus, DNA analyzed by the pharmacogenetics testing provides individualized information about the efficacy and safety of the prescribed medications (McCarthy, 2001).

Wolf et. al., (2000) indicated that 1 in 15 hospital admissions is related to side effects of the drugs. Therefore, being knowledgeable of the personalized adverse effects prospectively; by detecting the genetic coding of enzymes responsible for the metabolism of the prescribed drugs, would reduce the cost, time and harmful events (Bowker et al., 2011; Zane et al., 2008).

CYP2C19 is highly variable gene tested in pharmacogenetics; more than 80 SNPs has been related to CYP19. Different alleles were studied in comparison to the wild type (CYP2C19 *1/*1) that codes for a normal metabolizer. It was found that the homozygosity of CYP2C19 *17 allele was associated with lower drug concentrations because of increased enzyme activity. The increase in enzymatic metabolic functions was mediated by activation of transcription factors that enhance gene transcription of
CYP2C19 gene. This phenotype variant plays its effect on the metabolism of anti-
depressants (Hicks et al., 2013; Sim et al., 2006).

CYP2D6 is the most detected gene in the pharmacogenetics testing as it facilitates
in dose control when using psychiatric medications (Wolf et al., 2000). Although this
enzyme present in small quantity in the CYP450 family, its role is highly relevant as it
accounts for metabolizing more than 25% of the most commonly prescribed drugs. In
addition, studying this gene polymorphism is very important as it interferes highly with
the pharmacokinetics of half of the commonly prescribed drugs. Concomitant use of
drugs that inhibit CYP2D6 may lead to inactivation of the gene and predispose the patient
to be a poor metabolizer even though, the patient genotype detected as normal
metabolizer (Hicks et al., 2013; Ingelman-Sundberg, 2005; Mas et al., 2012; Wolf et al.,

COMT gene codes for the COMT enzyme that is found in the liver and brain, it
plays an important role in the degradation of catecholamines neurotransmitters. Decrease
activity of this enzyme cause accumulation of dopamine; increase dopamine
concentration is one of the drug mechanisms used to treat Parkinson disease.
Polymorphism of the COMT gene is the result of the substitution of valine amino acid
with methionine (Val158Met); the outcome is a methionine variant that has lower action
on catalyzing neurotransmitters in the dopaminergic pathway. The increase in dopamine
concentration in methionine variant revealed the causes of response variation for
neurostimulants; such as amphetamine, which is used to treat ADHD. Research showed
that people with valine variant (wild type Val/Val) of the COMT gene tend to have better
drug efficacy to neurostimulant agents as they have lower pre-treatment concentration of
dopamine in their synapses compared to those with the methionine variant (Bellgrove et al., 2005; Meyer-Lindenberg et al., 2006; Rakvag et al., 2005).

Analgesics play their role in the body as an agonist at opioid receptors; mu beta-endorphin (OPRM1), wide range of variation in response to opioids among patients was reported. Strong association was found between altered pain threshold and the SNPs G variant at position 118 (A118G) on the OPRM1 gene. This missense mutation; substitutes the amino acid asparagine with aspartic, results in decrease glycosylation on the N-terminal site with altered pain sensitivity so dosage modulation of opioids is required accordingly. Patients who are homozygous to guanine variant (G/G) genotype required higher doses of morphine compared to those with the normal adenine (A/A) genotype. However, there are still controversial speculations regarding the heterozygous (A/G) genotype. Research showed that the fluctuation in opioid response is dependent on the clinical setting such as labor, cancer, postoperative or chronic disease. Further investigations highlighted the ethnicity as a potent contributor to this diversity.

Methylenetetrahydrofolate reductase (MTHFR) is the rate-limiting enzyme that irreversibly convert 5,10 methylenetetrahydrofolates to 5 methylenetetrahydrofolates (5 MTHF) and it is encoded by the MTHFR gene. In order for folate to be in the active form (5 MTHF), it should go through reduction reaction by the MTHFR enzyme. Folate plays an important role in the synthesis of nitrogenous bases in DNA and RNA, it is a coenzyme for 1-carbon transfer in the cysteine cycle that regulates homocysteine levels in the blood; that being said, a mutation in MTHFR gene; found on chromosome 1 (p36.3), may lead to impaired DNA repair, DNA hypomethylation, folate deficiency and even an increase in homocysteine level (Del Greco et al., 2011).
MTHFR deficiency is associated with increased oxidative and decreased reduced metabolites in the transculturation pathway of homocysteine, that creates a metabolic imbalance and increase the risk of oxidative stress which was noticed in autistic children (James et al., 2006). Additionally, MTHFR gene heterozygote mutation has been strongly associated with hyperhomocysteinemia which predispose to cardiovascular diseases as it increases the oxidative stress and decreases nitric oxide that has vasodilatory effects; consequently, MTHFR gene variant underlies an early onset and increase susceptibility to a hypercoagulable state (James et al., 2006; Kim et al., 2013; Pramukarso et al., 2015). Folate and B12 vitamin supplementation are administered in patients who have MTHFR deficiency to reduce the risk of thrombosis (Kim et al., 2013). Interestingly, these vitamins are also used in the treatment of autism as it improves the behavioral symptoms; additionally, research showed that prenatal vitamin supplementation played an important role in preventing autism occurrence in the offspring (Schmidt et al., 2011). The purpose of this study is two-fold: 1) to investigate the link between genes previously associated with ASD and also implicated in pharmacological therapies commonly used in this population and 2) to report the genotype frequency of genes known to influence drug metabolism among small cohort of children with ASD.

Methodology

Participants

Participants were recruited from clients participating in the KAP (Kelly Autism Program) at WKU (Western Kentucky University). Participant characteristics are displayed in Table 1. In each case, participants and their caregivers were aware of risks
and requirements for participating made clear that participation is completely voluntary and will not affect their KAP programming in any way. The testing that was performed in this study was performed with the best interests of the child in mind. All participants for this study met the following inclusion criteria:

1. There is documentation that the student has been identified by a third party professional as having an ASD.
2. The student is between the ages of 7-17
3. The student currently takes a daily medication

As the study sample for this project was composed of minors, there were multiple steps to the consent process. All caregivers had to complete a written informed consent indicating requirements for participation, risks involved, and benefits. Four separate assent documents were created targeting for the unique communication methods of participants with ASD.

All participants and their caregivers were involved to give utmost consideration to the unique and specific needs of the participants, who represent a particularly vulnerable population. This information would be used to obtain child consent. Caregivers would be asked to identify one of four categories of communication: the child can: sign his/her name to give consent; check a box to give consent; use sign language to give consent, or the child can use a communication device to give consent.

Once informed caregiver consent was obtained, one of the investigators or their designee would acquire participant assent. A total of fifteen (15) participants with ASD were selected to participate in the study.

**Blood Sampling**
The blood draw procedures were coincide with the guidelines set forth by the U.S. Department of Health and Human Services Office for Human Protections Research. Blood samples were obtained using Universal Precautions at rest, and approximately 5 ml of venous blood was drawn.

**Genetic Analysis of Blood**

All genetic analyses of participants’ blood were conducted at MyGenetx in Franklin, Tennessee. MyGenetx is a molecular and general chemistry laboratory involved in research concerning pharmacogenetics.

**Results**

*Genes Frequencies:*  
All genotypes and phenotypes frequencies for genes that code for major enzymes systems; CYP450 isoenzymes, involved in drug metabolism are displayed in Table 2. Additional genes detected in the pharmacogenetics testing, play a role in drug response were summarized in Table 3 as well.

*Medication Stratifications:*  
Table 4. displays four classes of drug categories that are most commonly prescribed for children with ASD, showing the percentage from our population that falls under each drug stratification.

**Discussion**  
Our findings of patients’ sensitivity to some of the medications were related to mutation in a specific gene. We focused our interest on the medications that are commonly prescribed for children with ASD, such as anti-depressants, anti-psychotics and anti-ADHD drugs (Muller, Kekin, Kao, & Brandl, 2013), anti-ADHD drugs were
used by 30-85% of children with ASD (Dalsgaard et al., 2013; McPheeters et al., 2011). Methylphenidate which is an anti-ADHD agent was classified as use with caution in 73.3% of the participants; 66.6% are Val/Met variant for the COMT gene and 6.6% are Met/Met variant, this finding is consistent with other research Froehlich et al. (2011); McGough et al. (2009) that observed more beneficial role for methylphenidate among Val/Val variant. The normal function of COMT enzyme; that degrade catecholamines, decreases the bioavailability of the catecholamines in the synapses, thus Val allele responds better to neurostimulant medications such as methylphenidate. Val allele showed more susceptibility to impairment in cognitive functions and exhibit lower performance in memory tasks as a result of decreased dopamine levels (McGough et al., 2009; Meyer-Lindenberg et al., 2006). A study by Kereszturi et al. (2008) showed a significantly higher frequency of the Val/Val allele among ADHD group (28.3%) in comparison to general population (18.7%), in line with these findings James et al. (2006) reported (29%) frequency of the homozygous GG genotype in autistic patients. That was close to the frequency of Val/Val in our sample of 26.6% in children with ASD.

Gadow, Roohi, DeVincent, Kirsch, and Hatchwell (2009) reported that children with ASD carrying COMT Met158 allele have an increased severity of depression. Additionally, this mutation tends to be associated with social phobia and tics, as rated by the teachers of those children.

Another SNP that highly affects the metabolism of drugs was linked to the CYP2C19 gene. Our results showed that the following antidepressants: amitriptyline, clomipramine, doxepin, imipramine and trimipramine, were classified as the following:
1) 26.6% of the participants were classified as use with caution since they carry the 1*/2* or *2/*7 genotypes that have an intermediate CYP2C19 activity. Notably, Medhasi et al. (2016) detected a higher frequency of 33.2% for the CYP2C19*2 allele among Thai children with ASD.

2) 46.6% were classified as use alternatives: 40% are CYP2C19 *1/*17 genotype which is the rapid metabolizer state, 6.6% have the mutated CYP2D6 *4/*5 genotype. To highlight the significance of the CYP2D6 genotype, the patient who had the mutated CYP2D6 had nineteen drugs; that are most commonly prescribed for ASD, under the use with caution category in addition to thirteen drugs under consider alternatives.

Citalopram and escitalopram were classified as consider alternatives in 40% of patients having the CYP2C19*17 allelic frequency, while sertraline was under the use with caution category. Our results parallel other studies’ findings since it highlights the importance of CYP2D6 in metabolizing 25% of all medications and 80% of antipsychotics and antidepressants, and the importance of CYP2C19 gene in metabolizing the most commonly prescribed drugs in patients with ASD (Medhasi et al., 2016). The higher prevalence of having a mutated allele in the CYP2C19 gene among people with ASD adding to the fact that the same gene is implicated in metabolizing the most commonly prescribed drugs for this population stress the need for future investigations in the field.

OPRM1 gene was detected in the pharmacogenetics testing as its mutation causes alteration in the OPRM1 function leading to change in patients’ response to fentanyl and hydrocodone; analgesics from the opioid category used postoperatively and in cancer pain management. Alternatives for hydrocodone should be considered if increasing the
dose was not sufficient to relieve the pain, since the OPRM1 118A>G mutation is associated with increased risk for hydrocodone adverse effects. Similarly, reduced response to fentanyl in patients with mutated OPRM1 was observed and close monitoring during administration of the drug should be ensured as it has a narrow therapeutic index. From our population 33.3% were carrier for one mutation of OPRM1 gene (OPRM1 A118G AG genotype)

Interestingly, the SNPs of OPRM1 gene was studied as a mutation that does not only increase pain sensitivity, but also interferes with increased social rejection sensitivity as well. The similarity in the neuroanatomical and neurochemical factors associated with physical and social pain, in addition to the fact that G variant carriers perceive pain differently from those with the A variant, make the OPRM1 gene a good candidate to be further investigated (Troisi et al., 2012; Way, Taylor, & Eisenberger, 2009). Several research indicate that morphine administration relief distress in infant animals separated from their mother (Way et al., 2009). Although studies on humans are still limited, research signify the link between A118G polymorphism of the OPRM1 gene and fearful of social rejection (Troisi et al., 2012; Way et al., 2009). However, Becker et al. (2014) study on mice model with OPRM1 gene deletion, detected lack of social interaction and increase anxiety among other symptoms related to ASD. Thus, the decreased levels of OPRM1 in early life would negatively impact social reward; consequently, developing different symptoms related to ASD (Becker et al., 2014).

MTHFR genotype frequency in our population in comparison to other research that studied autism is displayed in Table 5. The decrease or increase frequency for the AC or CT and AA respectively, were consistent in our study and in (Marvin Boris & Joseph
Galanko, 2004). Paradoxically, 1) CC genotype frequency were near the normal range in our population while it was significantly decreased in (Marvin Boris & Joseph Galanko, 2004), 2) our results showed decrease frequency of the TT genotype whereas the other study reported significantly higher frequency of the TT genotype in autistic cases. Liu et al. (2011) results were in line with Marvin Boris and Joseph Galanko (2004) as they reported higher frequency of the 677TT genotype in people with ASD, suggesting a link between T allele frequency and ASD. James et al. (2006) signify the role of interaction between MTHFR gene and RFC gene in increasing the risk for autism.

In comparison to the wild type (1298A>C AA/677C>T CC) of the MTHFR gene, all the one heterozygotes or compound heterozygotes mutated genotypes are associated with a decrease in enzyme activity. Although the compromised enzymatic activity for these mutations is not associated with hyperhomocysteinemia, homozygous TT genotype; which is also related to ASD, is associated with hyperhomocysteinemia since the decrease in enzyme activity is around 70%.

Hyperhomocysteinemia is a result of MTFR deficiency, which predisposes to premature cardiovascular diseases and increases hypercoagulability risks. It is treated with vitamin B2, B12 and folate supplementation (Kim et al., 2013; Pramukarso et al., 2015). In the same light, research detected a lack in folate supplementation during pregnancy in mothers of children with ASD; additionally, folate intake has shown to improve clinical symptoms of autism and lower homocysteine levels in this population (Castro et al., 2016; Schmidt et al., 2011). Research investigating the interaction between the genetic and nutritional factors in respect to their association with behavioral and clinical symptoms in children with ASD is still not well established.
Overall, this research study adds to the small literature base of pharmacogenetics and people with ASD. Although, the sample size for this study is relatively small, the consistency of our findings, regarding the prevalence of genotype frequency in ASD population, with other research make it a very relevant information that aim to create a genetic classification for genetic disorders. The main focus of the paper is to relate the genotype frequency with the clinical relevance in ASD children in order to provide better medical care for this special population.
# Chapter Six: Tables

## Table 1. Participant Demographics

<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
</tr>
</thead>
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<tr>
<td>Number</td>
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</tr>
<tr>
<td>Age</td>
<td>7-17 yrs.</td>
</tr>
<tr>
<td>Race</td>
<td>14 Caucasian / 1 Hispanic</td>
</tr>
<tr>
<td>Gender</td>
<td>12 Male / 3 Female</td>
</tr>
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</table>
Table 2. Genotype frequencies for CYP450 family of enzymes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Participants n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>*1A/*1F</td>
<td>Normal Metabolizer- High inducibility</td>
<td>14(93%)</td>
</tr>
<tr>
<td></td>
<td>*1F/*1F</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*1A/*1A</td>
<td>Normal Metabolizer- Possible inducibility</td>
<td>1(6.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2B6</td>
<td>*1/*1</td>
<td>Normal Metabolizer</td>
<td>10(66%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*1/*6</td>
<td>Intermediate Metabolizer</td>
<td>4(26.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*6/*6</td>
<td>Low Metabolizer</td>
<td>1(6.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>CYP2C19</td>
<td>*1/*1</td>
<td>Normal Metabolizer</td>
<td>5(33.3%)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*1/*17</td>
<td>Rapid Metabolizer</td>
<td>6(40%)</td>
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<td></td>
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<tr>
<td></td>
<td>*1/*2</td>
<td>Intermediate Metabolizer</td>
<td>4(26.6%)</td>
</tr>
<tr>
<td></td>
<td>*2/*17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>*1/*9</td>
<td>Normal Metabolizer</td>
<td>14(93.3%)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>Poor Metabolizer</td>
<td>1(6.6%)</td>
</tr>
<tr>
<td></td>
<td>*2/*41</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>*2/*5/*35</td>
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<td></td>
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<td>*2/*4</td>
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<td></td>
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<tr>
<td></td>
<td>*1/*2</td>
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<td>*2/*5</td>
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<td></td>
<td>*1/*35</td>
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<td></td>
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</tr>
<tr>
<td>CYP3A4</td>
<td>*1/*1</td>
<td>Normal Metabolizer</td>
<td>13(68%)</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Intermediate Metabolizer</td>
<td>2(13.3%)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>CYP3A5</td>
<td>*1/*3</td>
<td>Intermediate Metabolizer</td>
<td>4(26.6%)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor metabolizer</td>
<td>11(73.3%)</td>
</tr>
<tr>
<td></td>
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</table>
Table 3. Genotype frequencies for enzymes implicated in drug response in the body

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Participants n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANKK1/DRD2</td>
<td>DRD2:Taq1A GG</td>
<td>Unaltered DRD2 function</td>
<td>9 (60%)</td>
</tr>
<tr>
<td></td>
<td>DRD2:Taq1A AG</td>
<td>Altered DRD2 function</td>
<td>5 (33%)</td>
</tr>
<tr>
<td></td>
<td>DRD2:Taq1A AA</td>
<td>Altered DRD2 function</td>
<td>1 (6.6%)</td>
</tr>
<tr>
<td>COMT</td>
<td>Val158Met GG</td>
<td>High/Normal COMT activity</td>
<td>4 (26.6%)</td>
</tr>
<tr>
<td></td>
<td>Val158Met AG</td>
<td>Intermediate COMT activity</td>
<td>10 (66.6%)</td>
</tr>
<tr>
<td></td>
<td>Val158Met AA</td>
<td>Low COMT activity</td>
<td>1 (6.6%)</td>
</tr>
<tr>
<td>OPRM1</td>
<td>A118G AA</td>
<td>Normal OPRM1 Function</td>
<td>10 (66.6%)</td>
</tr>
<tr>
<td></td>
<td>A118G AG</td>
<td>Altered OPRM1 Function</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>SLCO1B1</td>
<td>521T&gt;C TT</td>
<td>Normal Transporter Function</td>
<td>9 (60%)</td>
</tr>
<tr>
<td></td>
<td>521T&gt;C TC</td>
<td>Intermediate Transporter Function</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>1298A&gt;C AA</td>
<td>Wild Type / Normal MTHFR Activity</td>
<td>4 (26.6%)</td>
</tr>
<tr>
<td></td>
<td>677C&gt;T CC</td>
<td>Wild Type / Normal MTHFR Activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1298A&gt;C AA</td>
<td>One heterozygote mutation(60% of</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>677C&gt;T CT</td>
<td>normal enzyme activity)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1298A&gt;C AC</td>
<td>One heterozygote mutation(80% of</td>
<td>3 (20%)</td>
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<tr>
<td></td>
<td>677C&gt;T CC</td>
<td>normal enzyme activity)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1298A&gt;C AC</td>
<td>Compound heterozygote mutation</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td></td>
<td>677C&gt;T CT</td>
<td>(reduced enzyme activity)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1298A&gt;C AA</td>
<td>Two C677T mutation (homozygous)</td>
<td>1 (6.6%)</td>
</tr>
<tr>
<td></td>
<td>677C&gt;T TT</td>
<td>30% of normal activity</td>
<td></td>
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</tbody>
</table>

Table 4. Drug stratification of the most commonly prescribed medications for ASD
### Anti-ADHD

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Standard Precautions</th>
<th>Use With Caution</th>
<th>Consider Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine (Adderall)</td>
<td>14 (93.3%)</td>
<td>1 (6.6%)</td>
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### Antiaddictives

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<th>Use With Caution</th>
<th>Consider Alternatives</th>
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### Antidepressants

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<td>Escitalopram (Lexapro)</td>
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<td>6 (40%)</td>
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Table 5. Comparison of MTHFR genotype frequency between our sample and Marvin Boris and Joseph Galanko (2004)

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<tr>
<td>CC</td>
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Chapter Seven: Bibliography


Ingelman-Sundberg, M. (2005). Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J, 5(1), 6-13. doi:10.1038/sj.tpj.6500285


Do you have a child with Autism Spectrum Disorder (ASD) who has difficulty sleeping? If so, your child may be eligible to participate in an innovative genetic mapping study.

Western Kentucky University is partnering with MyGenetx to conduct genetic mapping of students with ASD and sleep difficulties.

If you are interested in participating, please read and fill out the attached consent document.

If you participate, *MyGenetx* will give you a drug panel that highlights medicines that may be most effective for your child (value of ~$400), a $25 gift card to a local business, AND you can say you helped move forward the scientific knowledge of ASD.

An open forum question and answer session will be scheduled for anyone interested in learning more about this.
APPENDIX B

CAREGIVER INFORMED CONSENT DOCUMENT

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.

The investigator is available to discuss with you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask him any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the investigator any questions you may have.

If you then decide to participate in the project, please sign on the last page of this form. You will be given a copy of this form to keep.

1. Nature and Purpose of the Project: The purpose of this study is to investigate a possible genetic link between sleep disturbance and Autism Spectrum Disorders (ASD). Prior research in this area suggests there is a genetic link between ASD and sleep disorders. By identifying the genes involved we can identify the most appropriate treatments to improve the lives of those with ASD and their families. Information from this study will also add to the research towards determining the root cause of ASD.
2. Explanation of Procedures:

To be included in the study, your child must meet the following inclusion criteria:

- There is documentation that your child has been identified by a third party professional as having an Autism Spectrum Disorder.
- You have identified that your child currently has or has had in the past disturbed sleep
- Your child is between 7-17 years of age
- Your child is currently on a daily medication

If your child does not meet one of these criteria, he/she will not be included in this study and we will notify you via a letter and/or phone call. Also, we will randomly select participants who meet the inclusion criteria if more than 15 participants sign up. We will notify you via a letter and/or phone call if your child has been selected to participate. If your child is chosen to participate, we will conduct the blood draw at the Suzanne Vitale Clinical Education Complex (SVCEC). All blood draws will be done by a skilled professional trained in phlebotomy from the Exercise Physiology laboratory in the School of Kinesiology, Recreation & Sport (KRS). Also, a professional trained in ASD will be present to help with the blood draw. Blood samples will be obtained using Universal Precautions at rest. Blood draw procedures are as follows:

1. Wrap a tourniquet around your child’s upper arm to stop blood flow.
2. Sterilize the puncture site with alcohol.
3. Insert the needle into the vein
4. Attach the appropriate test tube to the needle. Allow the blood to fill the test tube.

5. Remove the tourniquet to restore blood flow.

6. Place a gauze pad over the site while withdrawing the needle.

7. Apply firm pressure to the site until bleeding has stopped.

An individual vial of your child’s blood will be immediately de-identified to ensure confidentiality. It will be stored in the KRS Exercise Physiology laboratory until it is shipped to MyGenetx in Franklin, Tennessee for genetic analysis. Shipping will be done via United Parcel Service. All genetic analyses of participants’ blood will be conducted at MyGenetx in Franklin, Tennessee. MyGenetx is a molecular and general chemistry laboratory involved in research.

1. Discomfort and Risks: Venipuncture blood collection can cause minor bruising around the sample area and slight discomfort during the sampling procedure. Nausea and fainting can occur. There is a slight risk in the increase in the potential for problem behaviors that may be associated with a blood draw, such as aggression, property destruction or self-injury. If at any time any problem behaviors occur during the blood draw, the procedures will be immediately stopped. Although we will take multiple precautionary steps to protect and safeguard confidentiality, with the transport of data, there is still a very small chance that confidentiality will be breached.

2. Benefits: If you participate in this study, you will be given an individual pharmacogenetics (PGx) report for your child (a $400 - $500 value). The PGx
is a detailed analysis of the effectiveness of prescription drugs based on individual genetics. It will detail which drugs your child metabolizes more effectively than others and can improve treatment.

3. You will also receive a gift card to a local store valued at $25. This gift card will be given to compensate for time travel expenses associated with participating in this study.

4. Finally, the results of this study will potentially allow researchers to identify the underlying genetic components of ASD. This can result in more targeted interventions and prevention efforts. This information will be valuable to all people affected by ASD.

5. Confidentiality: Your child’s blood will be numerically coded for anonymity and stored in a locked office at KRS Exercise Physiology laboratory and in a locked office at MyGentex in Franklin, TN. Any data collected from the blood sample will be kept in a password-protected document on a password-protected computer in the investigator’s office. Any data collected and recorded on hard copy will also be locked and stored in the same location. The data will be kept secure for a minimum of three years after project completion.

6. Refusal/Withdrawal: Refusal to participate in this study will have no effect on any future services you may be entitled to from the University or with the Kelly Autism Program. You are free to withdraw from the study at any time with no penalty.
You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks.

__________________________________________  ____________
Signature of Caregiver               Date

__________________________________________

Signature of Caregiver               Date
APPENDIX C

LETTER TO PARENTS TO DETERMINE COMMUNICATION STYLE AND POTENTIAL BEHAVIORAL PROCEDURES FOR THE BLOOD DRAW

Dear family members and/or legal guardians,

Thank you for agreeing to participate in this study. We will ask your child if he/she wants to participate in the study. We will ask your child using his/her best method of communication. Please check the box next to the method that your child is most likely to communicate whether he/she wants to be in the study.

_____ My child is verbal and can sign his/her name to show whether he/she wants to be in the study.

_____ My child is verbal and can check a box to show whether he/she wants to be in the study.

_____ My child uses sign language and will sign whether he/she wants to be in the study.

_____ My child uses a communication device and will use this to show whether he/she wants to be in the study.
Also, AutismSpeaks has published a guide to drawing blood with children with ASD. They recommend certain supports to help the blood draw go smoothly. We are happy to put in place any additional supports your child may need. If you are interested in any of the supports, please contact the head of this study. Some examples of supports that can you request are:

- Relaxation techniques
- First/Then boards
- Visual schedules
- Reinforcers
- Please list other supports you think may be necessary:

If there are other supports you would like, please let us know and we will try our best to put those in place.

Sincerely,

__________________________________________

___________________
Participant’s name

__________________________________________

Participant’s signature

Date:

__________________________________________

Caregiver name

__________________________________________

Caregiver signature
APPENDIX D

INFORMED ASSENT DOCUMENT
FOR RESEARCH INVOLVING MINORS

Informed Consent Document for Participants who use Communication Devices

This script should be read to the potential participants. Do not change the wording in any way. After you have read the script, ask the participant to use a communication device to state “yes” or “no.”

Your parents have said it is okay for me to ask you if you want to be part of this project. Scientists at Western Kentucky University are looking at genes and autism. As part of the project, the scientists will draw your blood one time. They will also draw blood from 14 other students in Kentucky with autism. They will send all of the blood to a lab in another state. After we draw your blood, we will not know which blood is yours. It will go into a big computer system that will look for ways that the children with autism are similar to one another. Knowing this about children with autism will help scientists better know about autism. If you do not want to do this, it is okay with your parents and it is okay with us. If you say you will do it, but think later that you do not, just tell someone you do not want to do it and it will be okay.
Use your ipad (communication device) to tell me if you want to be in this project. Say yes, if you want to be in the study and let the scientists draw your blood. Say no if you do not want to be in the study or let scientists draw your blood.

The adult obtaining informed consent should mark on the boxes below whether the student would like to participate and sign and date the document.

_______ I do want to participate in the study

_______ I do not want to participate in the study

________________________________________
Participant signature (if applicable)       Date

________________________________________
Signature of the adult obtaining consent    Date
APPENDIX E

INFORMED ASSENT DOCUMENT

FOR RESEARCH INVOLVING MINORS

_Informed Consent Document for Participants who Sign their Names_

This script should be read to the potential participants. Do not change the wording in any way. After you have read the script, ask the participant to check a box and sign his/her name if he/she wants to participate in the study.

_Your parents have said it is okay for me to ask you if you want to be part of this project. Scientists at Western Kentucky University are looking at genes and autism. As part of the project, the scientists will draw your blood one time. They will also draw blood from 14 other students in Kentucky with autism. They will send all of the blood to a lab in another state. After we draw your blood, we will not know which blood is yours. It will go into a big computer system that will look for ways that the children with autism are similar to one another. Knowing this about children with autism will help scientists better know about autism. If you do not want to do this, it is okay with your parents and it is okay with us. If you say you will do it, but think later that you do not, just tell someone you do not want to do it and it will be okay._

_I will pass you this paper and you will put an X next to whether you want to be in this study. If you do, then put an X next to “yes.” If you do not, put an X next to “no.” Then, at the bottom, sign your name and put the date._
Pass the paper to the participant. The adult obtaining consent should then sign and date the paper.

_______ Yes! I do want to participate in the study

_______ No. I do not want to participate in the study

________________________________________
Participant signature (if applicable)  Date

________________________________________
Signature of the adult obtaining consent  Date
APPENDIX F

INFORMED ASSENT DOCUMENT
FOR RESEARCH INVOLVING MINORS

Informed Consent Document for Participants who Check a Box

This script should be read to the potential participants. Do not change the wording in any way. After you have read the script, ask the participant to check a box.

Your parents have said it is okay for me to ask you if you want to be part of this project. Scientists at Western Kentucky University are looking at genes and autism. As part of the project, the scientists will draw your blood one time. They will also draw blood from 14 other students in Kentucky with autism. They will send all of the blood to a lab in another state. After we draw your blood, we will not know which blood is yours. It will go into a big computer system that will look for ways that the children with autism are similar to one another. Knowing this about children with autism will help scientists better know about autism. If you do not want to do this, it is okay with your parents and it is okay with us. If you say you will do it, but think later that you do not, just tell someone you do not want to do it and it will be okay.

I will pass you this paper and you will put an X next to whether you want to be in this study. If you do, then put an X next to “yes.” If you do not, put an X next to “no.”
Pass the paper to the participant. The adult obtaining consent should then sign and date the paper.

_______ Yes! I do want to participate in the study

_______ No. I do not want to participate in the study

________________________________________
Participant signature (if applicable)  Date

________________________________________
Signature of the adult obtaining consent  Date
This script should be read to the potential participants. Do not change the wording in any way. After you have read the script, ask the participant to use sign language to show their willingness to participate. Then you sign the paper to show you have followed these guidelines.

*Your parents have said it is okay for me to ask you if you want to be part of this project. Scientists at Western Kentucky University are looking at genes and autism. As part of the project, the scientists will draw your blood one time. They will also draw blood from 14 other students in Kentucky with autism. They will send all of the blood to a lab in another state. After we draw your blood, we will not know which blood is yours. It will go into a big computer system that will look for ways that the children with autism are similar to one another. Knowing this about children with autism will help scientists better know about autism. If you do not want to do this, it is okay with your parents and it is okay with us. If you say you will do it, but think later that you do not, just tell someone you do not want to do it and it will be okay.*
Use your signs to tell me if you want to be in this project. Show me yes, if you want to be in the study and let the scientists draw your blood. Show me no if you do not want to be in the study or let scientists draw your blood.

Pass the paper to the participant. The adult obtaining consent should then sign and date the paper.

_______  Yes! I do want to participate in the study

_______  No. I do not want to participate in the study

_______________________________  _____________________________
Participant signature (if applicable)  Date

_______________________________  _____________________________
Signature of the adult obtaining consent  Date
APPENDIX H

Survey

The purpose of this survey is to get your personal opinion about some important issues related to pharmacogenomic testing. Please answer all these questions as honestly as you can. All your answers will be completely confidential. If for any reason, you do not feel comfortable answering any specific question, you can decline answering it by not marking an answer choice.

Key definitions:

Pharmacogenetics: Pharmacogenetics is the study of how individuals’ genetic make-up (genes in their DNA) interacts with medication. Although medications are meant to work the same way, medications affect people differently because of their genes.

Pharmacogenomic test: A test (e.g. blood test) to investigate peoples’ genetic make-up (genes in their DNA), in order to find out how the individual metabolizes the medication, and therefor, how well a medication will work for that individual.

1. How much have you heard about pharmacogenetics? (Through media, friends, peers, etc.)
   a. Nothing
   b. A little bit
   c. A fair amount
   d. A lot

2. How familiar are you with pharmacogenetics?
   a. Extremely familiar
   b. Very familiar
   c. Moderately familiar
   d. Slightly familiar
   e. Not at all familiar

3. How would you rate the importance of pharmacogenetics testing?
   a. Extremely important
b. Very important
c. Moderately important
d. Slightly important
e. Not at all important

4. How would you rate the benefit of pharmacogenetics testing?
   a. Extremely beneficial
   b. Very beneficial
   c. Moderately beneficial
   d. Slightly beneficial
   e. Not at all beneficial

5. How likely are you to talk to your doctor about pharmacogenetics?
   a. Extremely likely
   b. Very likely
   c. Moderately likely
   d. Slightly likely
   e. Not at all likely

6. Learning about how my body metabolizes medications could make my medications safer and more effective
   a. Strongly agree
   b. Somewhat agree
   c. Neither agree nor disagree
   d. Somewhat disagree
   e. Strongly disagree

7. I would benefit from learning about how specific genes in the body can affect the metabolism of medication.
   a. Strongly agree
   b. Somewhat agree
   c. Neither agree nor disagree
   d. Somewhat disagree
   e. Strongly disagree

8. I would ask my doctor to use pharmacogenetics results to determine the best drug for me.
   a. Strongly agree
   b. Somewhat agree
   c. Neither agree nor disagree
   d. Somewhat disagree
   e. Strongly disagree

9. I have a good understanding of the healthcare application of pharmacogenetics.
10. I would request a pharmacogenetics test to determine my best choice of medicine in the future.
   a. Strongly agree
   b. Somewhat agree
   c. Neither agree nor disagree
   d. Somewhat disagree
   e. Strongly disagree

11. Have you ever experienced a side effect (bad reaction) from a medication?
   a. Yes
   b. No
   c. Don’t know
   d. I have never taken any medications

12. Have you ever found that a medication did not work for you?
   a. Yes
   b. No
   c. Don’t know
   d. I have never taken any medications

13. How interested are you in doing more pharmacogenetics testing?
   a. Extremely interested
   b. Very interested
   c. Moderately interested
   d. Slightly interested
   e. Not at all interested

14. How comfortable are you talking to your doctor about pharmacogenetics?
   a. Extremely comfortable
   b. Very comfortable
   c. Moderately comfortable
   d. Slightly comfortable
   e. Not at all comfortable

15. How comfortable are you talking to your child’s doctor about pharmacogenetics?
   a. Extremely comfortable
   b. Very comfortable
   c. Moderately comfortable
   d. Slightly comfortable
   e. Not at all comfortable
16. I would ask my child’s doctor to use my child's pharmacogenetics results to determine the best medication for them.
   a. Strongly agree
   b. Somewhat agree
   c. Neither agree nor disagree
   d. Somewhat disagree
   e. Strongly disagree

17. Has your child ever experienced a side effect (bad reaction) from a medication?
   a. Yes
   b. No
   c. Don’t know

18. Have you ever found that a medication did not work for your child?
   a. Yes
   b. No
   c. Don’t know

19. I was hesitant to have my child participate in the research study because it involved genetic analysis.
   a. Strongly agree
   b. Somewhat agree
   c. Neither agree nor disagree
   d. Somewhat disagree
   e. Strongly disagree

20. Do you have anything else that you would like to share about your opinions concerning pharmacogenetics?

21. I agree to contact me for a 7 day period follow up through e-mail
   a. Yes
   b. No

   **If yes, please provide your e-mail address below**

   ____________________________________________________________
   ____________________________________________________________
   ____________________________________________________________
APPENDIX I

Oral Presentation of the Training Session

PHARMACOGENICS: CONSIDERATIONS OF MEDICATION AND ASD

WESTERN KENTUCKY UNIVERSITY IN PARTNERSHIP WITH MYGENETX

AGENDA

4:00-4:15 Meet in CEC conference room and complete surveys
4:15-4:35 Pharmacogenetics training
4:35-4:50 Go over Drug Panel results and ask questions
4:50-5:00 Complete paperwork and collect gift card
DEFINITION OF PHARMACOGENETICS

- Although medications are meant to work the same way, we know that medications affect people differently. Studying these differences is an emerging field of study and is called pharmacogenics.

- Pharmacogenetics is the study of medication-gene interactions (Bowers, 2015).

DEFINITION OF PHARMACOGENETICS

- How a person tolerates side effects of medication and effectiveness of drug.
DEFINITION OF PHARMACOGENETICS

- Although medications are meant to work the same way, we know that medications affect people differently. Studying these differences is an emerging field of study and is called pharmacogenics.

- Pharmacogenics is the study of medication-gene interactions (Bowers, 2015)

DEFINITION OF PHARMACOGENETICS

Gene = Blood Sample
PHARMACOGENICS IN ASD

• If you have met one kid with autism... you have met one kid with autism
  • Difficult to know which medications may work
“KIDS ARE DIFFERENT”

Julian  Jacob

ASD  ASD

Sleep Trouble  Sleep Trouble

“KIDS ARE DIFFERENT”

Julian  Jacob

Self-Injurious Behavior  Prefers talking to adults than kids
Wakes often in the night  Loves Minecraft
No food preference  Difficulty falling asleep
Nonverbal
PHARMACOGENETICS AND ASD

Risperidone and aggression (Litt et al)

PHARMACOGENETICS AND ASD

Risperidone and weight gain (Correia et al)
HOW DO WE DO THIS?

1. Conduct a blood draw
2. DNA is isolated at a lab
3. Unique markers in DNA are identified
4. These markers give clues about how medicines will work, because medicines work differently in different people

CONDUCT BLOOD DRAW
DNA IS ISOLATED AT A LAB

MYGENETX
FRANKLIN, TN

Jim Kendrick
COO, MyGenetx
IDENTIFYING SPECIAL MARKERS

MEDICINES WORK DIFFERENTLY

Medication is metabolized and can have an therapeutic effect or an nontherapeutic effect.
MEDICINES WORK DIFFERENTLY

Medication is metabolized and can have a therapeutic effect or an adverse effect.
We will now walk around to answer specific questions