FAMILIAL CLUSTERING OF SUICIDE AND MAJOR DEPRESSIVE DISORDER: AN OBSERVATIONAL ANALYSIS

by

Nicolette Walano

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This thesis was presented

by

Nicolette Walano

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and approved by

Thesis Director

Lisa Pan, MD, Assistant Professor of Psychiatry, School of Medicine, Attending Physician, Services for Teens at Risk, University of Pittsburgh

Committee Members

David Finegold, MD, Professor, Human Genetics, Graduate School of Public Health, University of Pittsburgh

Andrea Durst, MS, DrPH, LCGC, Assistant Professor, Human Genetics, Licensed Genetic Counselor, Assistant Director, Genetic Counseling Program, Graduate School of Public Health, University of Pittsburgh

Ryan Minster, PhD, MSIS, Assistant Professor, Department of Human Genetics Graduate School of Public Health, University of Pittsburgh

Lisa Pan, MD

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ABSTRACT

Depression is considered the third most important burden of disease globally; further, it is ranked first in middle and high-income countries. It is well understood that depression is has a heritability of 31-43%, but no genetic associations predisposing individuals to depression have been found. A small group of individuals have previously been identified with treatment resistant major depressive disorder (TR-MDD), suicidality and a myriad of metabolic alterations. This is considered a neuropsychiatric inborn error of metabolism that initially presents with psychiatric manifestation. The most common metabolic finding is cerebral folate deficiency. Supplementation of folinic acid has been shown to result in a reduction of symptoms. Understanding this group of individuals, particularly with the knowledge that metabolic disease commonly manifests with psychiatric illness, has implications for the way depression may be diagnosed and treated in the future. This study captured and analyzed the family histories of 36 individuals in an attempt to discern whether the transmission of depression and suicide in these families fits known Mendelian inheritance patterns. By using segregation analysis in addition to an observational analysis this study has assessed autosomal recessive and autosomal dominant inheritance patterns. Observation of family histories showed male-to-male transmission and thus excluded the possibility of X-linked or mitochondrial inheritance in this group. The

observational study identified that 6/36 families (16.7%) met all criteria for autosomal dominant inheritance and no families met all criteria for autosomal recessive inheritance. The just over 30% of families met a few, but not all of the criteria for autosomal dominant inheritance (11/36) and 33 of 36 families or 91.67% met only one criterion for autosomal recessive inheritance. This suggests that these families are transmitting depression and suicidality in a polygenic or multifactorial pattern. The statistical analysis supports this conclusion finding that the families fit a non-Mendelian pattern of inheritance. Understanding the way depression is being transmitted in these families has significant public health relevance as it may inform our understanding and future studies of the genetics of depression, a significant health burden.

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PREFACE

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1.0 INTRODUCTION

1.1 BACKGROUND AND SIGNIFICANCE

1.1.1 Treatment Resistant Depression

Major depressive disorder (MDD) is defined as severe symptoms of depression that interfere with one's ability to eat, sleep, work and enjoy life[1]. MDD is also called unipolar major depression, which differs from bipolar disorder in that people suffering from unipolar major depression are particularly prone to major depressive episodes and have never experienced an episode of mania or hypomania[2]. Many people experience depression, but major depression is an episode of depression that is present every day for most of the day and lasts for at least two weeks[3]. The Diagnostic and Statistical Manual (DSM) 5 defines MDD as:

> "Depressed mood or loss of interest or pleasure in most activities and persisting nearly every day accompanied by four of the following; significant weight loss, insomnia or hypersomnia, noticeable psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or guilt, diminished ability to think or concentrate and suicidal ideation".

The symptoms must interfere with daily functioning and must not be caused by a medication, medical condition or other substance. Persistent depressive disorder is a depressive episode that

lasts at least two years without remission[3]. It is estimated that 30% of depressive people suffer from persistent depression[3].

The ultimate goal of treatment for MDD is remittance; this is considered a full response to treatment and is measured by a behavioral rating scale[4,5]. Partial response may require further treatment or result in relapse or return of significant symptoms of MDD[4,6]. Failure to respond may be a result of such a relapse of symptoms or it may be a complete lack of response to treatment[4,7]. Treatment for MDD may include medication, cognitive behavioral therapy (CBT), electroconvulsive therapy (ECT) or some combination of the three. New methods for treatment include transcranial direct current stimulation and other alternative methods like folate supplementation[8–10]. Typically patients with MDD begin treatment on a selective serotonin reuptake inhibitor (SSRI). Non-response to an SSRI often results in a change to a different class of antidepressant such as selective norepinephrine reuptake inhibitor (SNRI), a monamine oxidase inhibitor (MAOI), or another anti-depressant in the same class[11,12]. There is no proven benefit of one course of treatment over another[12]. Individuals who do not show adequate response to monotherapy will require augmented or optimized treatment; typically patients are prescribed a more traditional class of antidepressant, such as an SSRI, plus another supplemental anti-depressant or alternative medication. These patients may also be undergoing some form of CBT concurrently, thus treatment varies significantly for each person and changes throughout course of treatment depending on individual response[11]. Lithium augmentation and ECT may be recommended after several rounds of non-response or insufficient response to treatment; one algorithm suggests incorporating lithium as the fourth augmentation of treatment, lithium plus a combination of two other anti-depressant classes as the fifth and ECT as the sixth[13].

The current operating definition of treatment resistance in depression is a poor or unsatisfactory response to two adequate (defined as both optimal dose and duration) monotherapy trials of two different classes of therapy[6]. An unsatisfactory response is measured by before and after scores on commonly used rating scales such as the Hamilton Rating Scale for Depression (HRSD)[6]. It is increasingly less likely for an individual to remit after a failed round of treatment, and the number of failed treatments is inversely correlated with the chance of remittance[2,11]. Treatment resistant depression that does not respond to multiple treatment regimens is considered treatment refractory depression and thus, is considered highly resistant depression.

There is a high mortality rate associated with depression, most often resulting from suicide. Many researchers and clinicians rely on the stress-diathesis model to describe the relationship between depression and suicide, i.e. the risk for suicide increases due to high stress caused by the presence of both psychiatric illness and psychosocial adversity[14]. Therefore, among suicidal patients, distinguishing depression from psychosocial adversity can pose a challenge[14]. While over 400,000 deaths per year are suicides, there is currently no reliable way to tell who may be at risk for committing suicide[1]. Among Americans ages 15-34, suicide is the second most common cause of death regardless of gender[15]. There is a 3.4% lifetime risk for suicide among individuals with major depression[16]. Additionally, men are six times more likely to commit suicide than women with a 7% lifetime risk for suicide among men and a 1% lifetime risk for women[16].

1.1.1.1 The Effect of Depression on Family Members

In families with a history of psychiatric disease requests for genetic counseling regarding the psychiatric disorders can arise for a multitude of reasons. Some people are interested in the risks

to future children while others are concerned about the risks to themselves based on a significant family history[17,18]. A diagnosis of depression in a first-degree relative conveys a 31-42% risk for family members to develop depression; however, little research exists regarding how having family members living with depression affects an individual's perceived risk of developing depression themselves. In addition, there is also little information regarding the perception of risks for people related to someone who has committed or attempted suicide.

The impact of living within a family unit where someone is affected by depression or has exhibited significant suicidality has been studied in depth. Families of depressed patients exhibit more impairment in family functioning than families of bipolar or schizophrenic patients[19]. Additionally, they also exhibit more difficulties than families of patients with other types of medical illnesses such as rheumatoid arthritis or heart disease[19]. It should be noted that this impairment of functioning subsides as the patient's episode of depression also remits. This leads to the question of the long-term effect on families of patients with chronic, unremitting depression. Little research has explored this area. Several studies have explored the effects of having a parent experiencing significant suicidality, the majority of which have concluded that having a parent who attempts or completes suicide is a risk factor for developing psychiatric illness, and psychiatric illness develops earlier in life than those patients who do not have a parent who has experienced suicidality[19]. However there is a dearth of information regarding family members' perceived risks to their mental health. Further there is no ability to clinically test affected individuals or their family members to learn information for risk assessment.

1.1.1.2 Diagnosis of Treatment Resistance

Nearly 50-66% of people with depression will not recover fully in spite of treatment with anti-depressant monotherapy and 15% of individuals with MDD will be treatment refractory[11].

Treatment resistance is defined as an episode of depression that displays inadequate response to two adequate rounds of two different classes of antidepressants. Inadequate response is measured using a validated survey method[11,12,20]. The most common method is the Hamilton Rating Scale for Depression (HRSD) where a score below eight is normal, a score between eight and 13 indicates mild depression, 14-18 indicates moderate depression, 19-22 indicates severe depression and a score greater than 23 indicates severe depression. Remission is indicated by the achievement of a normal score on a depression rating scale[5].

In 25% of patients who are treatment resistant optimized or combined treatment will result in a response. Another 50% of patients with treatment resistant major depressive disorder (TR-MDD) will respond to switching therapy and thus remit after a second round of treatment. The final 25% represent those who pose the biggest challenge for clinicians providing treatment[6]. Predictors of treatment response are non-specific and have few clear indications to aid in determining treatment course[7,12,21] For example, demographic predictors of treatment response include being Caucasian, female, well-educated and having a higher income[2,21]. However, the demographic predictors are not consistent across studies[22,2,21]. In addition, some clinical predictors of treatment non-response have also been identified, including comorbidity of a panic or anxiety disorder, high suicide risk, melancholic features and non-response to first anti-depressant treatment in their lifetime[22]. These predictors allow practitioners to understand whom maybe more or less likely to respond to treatment, but not which courses of treatment are most effective.

As the definition for treatment resistance states, one must fail to respond to two adequate trials of two types of anti-depressants to be considered treatment resistant[11,12,20]. However, the term adequate is not well defined. Some researchers state that the adequate time to respond is

between four and six weeks of medication and others define adequate as upwards of eight weeks to response[7]. The definition also states that the dose must be adequate, but there is little consensus on the adequate dose[7].

There is not a single, unified method to establish a diagnosis of TR-MDD[23]. Further, the DSM 5 does not recognize TR-MDD either on its own or as a subtype of depression resulting in a lack of information for clinicians to uniformly identify TR-MDD[3]. The result of this lack of uniformity is a group of pseudo-resistant individuals who have either received inadequate doses, discontinued treatment, have a pharmacogenomic cause for their resistance, are non-compliant or have been misdiagnosed[7].

1.1.1.3 Predictors of Treatment Response

Treatment for depression can involve a number of different types of medications including: SSRI's, tricyclics, MAOI's and ECT[1]. There have been several models for treatment algorithms posed in the literature, many researchers have worked to determine which, if any, of these methods for diagnosing treatment resistance in depressed patients are most effective but there is little consensus on how treatment response should be treated and scored[7,11,14,20]. Studies of treatment algorithms have found them to be beneficial to patients[13].

A significant proportion of patients with depression will fail to respond fully to treatment. Approximately 26-49% of patients with depression will fail to respond to the recommended 6 weeks of anti-depressant therapy[7]. A study by Rush et al. 2006, which seeks to determine the utility of a step-wise treatment model for depression, determined that 32.9% of patients responded to treatment in the first level[11]. The first level of treatment in this study was an SNRI (serotonin-norepinephrine reuptake inhibitor) called citalopram. Participants who did not respond were moved to the next level of treatment where citalopram was either changed or supplemented

with an adjunctive therapy. In the second round of treatment 30.6% remitted. Two subsequent levels altered treatment similarly, the third level achieved 13.6% remission and the fourth level achieved 14.7% remission. Another study found that between 66-95% of individuals treated with up to three rounds of treatment achieved remission[24]. It is important to note that some research has shown that up to 40% of remitting individuals relapse after 15 months (cite). This relapse rate has been interpreted as the persistence of depression and serves to highlight the need for continued treatment even after relief of symptoms[25]. Non-response to the first ever treatment of depression increases the risk for being diagnosed with treatment resistant depression 1.6-fold[22]. Further, remission rates are highest among those who have never been treated for depression (42.7%)[11].

There are other markers for treatment resistance, which can be used to direct the type of anti-depressant to incur the best results. One such marker is the presence of psychiatric comorbidity[20,26]. For example, people with comorbid anxiety are less likely to respond to treatment with SSRI's, tri-cyclic anti-depressants (TCA's) and MAOI's[21]. Atypical depression, which is depression that mimics MDD but exhibits improved mood in response to positive events and pleasure, is more likely to respond to an MAOI than a TCA[27,21]. A diagnosis of less severe depression in addition to early response to treatment are both predictors of treatment remittance[7,21]. Typically, early response to treatment is based on a neuroimaging response to treatment rather than mood improvement[21]. Conversely, patients who are more severely affected or do not show early signs of response are more likely to be deemed treatment resistant[21].

There are also genotype-based predictors of treatment response. There are two categories of genes implicated in treatment response, those that are directly related to monoamine neurotransmitters and genes that are indirectly related. Genes associated with monoamine

neurotransmitters include a variant of the *TPH* gene and an in/del in the promoter of the *SLC6A4* gene called 5-HTTLPR, but there is conflicting data about the role these genes play in treatment resistance[21,28,29]. For instance, *SLC6A4* was not shown to predict treatment response in all groups, but did predict treatment response in patients with anxious depression only[29,30]. Receptor genes for 5-HT, such as *HTR1A*, which are associated with serotonin uptake, were investigated and were found to have a role in response among melancholic depression[30,31]. The gene *TPH2* contains a polymorphism that has been associated with susceptibility to commit suicide, but not with treatment response[30,32]. The *COMT* and *MAOA* genes have also been investigated and have led to conflicting results as to response to treatment resistance[30,33–36].

Genes not associated with the monoaminergic system *BDNF, TREK1, GRIK4* and *FKBP5,* contain variants that have been associated with SSRI treatment outcome[21,30]. The *BDNF* gene, a member of the nerve growth factor super family, has been noted to be under-expressed in depressed states[30,37]. It has also been implicated in treatment response to venlafaxine, but the allele or genotype that conveys risk is still uncertain[30,37]. *GRIK4* has been associated with response to citalopram, but results have been inconsistent[29,30].

The most reliable genes for prediction of treatment response are outside of the nervous system and are instead are the associated with oxidation and reduction of substrates and drugs[30,38]. The cytochrome P450 (CYP450) class of enzymes indicates metabolizer status based on catalytic capacity ranging from poor to ultra rapid[38]. Both *CYP2C19* and *CYP2D6* have both been associated with metabolizer status, in particular metabolizer status associated with *CYP2D6* has been associated with anti-depressant response[30,38,39].

1.1.2 Genetics of Depression

In addition to genetic predictors of response to depression, genetic factors are likely to play a role in the development of depression and the subsequent clustering observed in families[14,40–42]. At this time, the exact role is still a subject of much investigation. Depression is considered a complex disease in which not only genetics, but also shared environments contribute to the heritability[43,44]. Thus many family and twin studies have attempted to determine the extent of these illnesses in families[45–47].

It has been estimated that depression has a moderate heritability of 31-42%[44,45]. The odds ratio for relatives of a proband to develop depression is 3.62 for families that only have depression and no other psychiatric disease in their family history and 2.38 for families who have depression and other psychiatric disease[44]. Some smaller studies have evaluated the heritability of these more homogeneous subsets of depression[48,42]. For example, some studies have determined that patients with dysthymic depression (now called pervasive depressive disorder), which is associated with chronic depressive episodes that last at least two years, were more likely to have a relative with dysthymic depression than those who had major depression[26,42]. In addition to understanding heritability, other studies have attempted to associate environmental factors with major depression. One such study established that factors that increase risk are employment, marital status and alcohol intake[47].

Genetic epidemiology studies and GWAS have both been utilized to determine the specific genetic factors that may be involved in the development of depression[44,49]. In addition, some evidence suggests that there are a number of pathways that lead to a common endpoint of major depression, and many studies have suggested that depression is a heterogeneous grouping of disease. Taken together, attempting to determine the etiology of depression is complex and

requires a homogeneous study population[44]. To elaborate this point, genetics is not simply the concept that 'like class begets like class', in fact, the heredity of complex traits is likely more varied than the heredity of single gene traits[50]. Family studies have been the most effective method for the study of heredity in complex disease until the advent of sequencing the human genome, which has given us significant ability to interrogate the genome for genetic associations of complex diseases[51].

The common disease, common variant hypothesis, which has impacted research on heart disease, diabetes and mental illness, supposes that many susceptibility alleles exist and do not individually convey a highly deleterious function, but synergistically interact to significantly impact the development of disease[52]. As a result, much work is currently being done to tease out the genetic contributions to complex diseases like depression[51,52]. Several identified genes have also been associated with bipolar disorder, schizophrenia and autism[51,53,54]. Even after many genome wide association studies only a handful of candidate genes for depression have been identified and with limited power[55]. Additionally, some investigators are evaluating the role of epigenetics in the development of depression[56].

1.1.2.1 Family Studies of Depression and Suicide

Studies of depression and suicide in families are meant to tease out the extent that these psychiatric illnesses pervade families. Family members of those completing or attempting suicide are more likely to exhibit suicidal behavior than those who are not related to a suicidal family member[57,58]. Additionally, first-degree relatives of suicide victims were more likely to attempt suicide than those without a first-degree relative who committed suicide[19,59,60]. One study has found that there is a 50% increase in suicide attempts in people whose mothers are depressed and attempted or completed suicide than those whose mothers have never attempted[58].

It is also unlikely that the act of suicide is a result of grief as one study compared families of suicide victims to families in which a member died of other causes; the suicide rate was twice as high for family members of suicide victims as their comparators[60]. As a result family history may act as an independent risk factor for suicide regardless of mental illness. However, other risk factors for suicide operate on a population level only, and cannot be used to determine an individual's risk for suicidality. For example, lower quality of life in the month prior to committing suicide is a risk factor for the population that complete suicide, but it is not a threshold which can determine that an individual will commit suicide[61]. Family studies of depression have estimated heritability to be 28-44% and have not found differences between sex, age of onset or illness course[47].

1.1.2.2 Twin Studies of Depression and Suicide

Since monozygotic twins (MZ) share all of their genes and dizygotic twins (DZ) share 50% of their genes, twin studies can help to identify the extent of the impact of genetic factors in a particular trait[45,46,62]. These studies also operate under the assumption that environmental factors are the same or similar for the pair of twins and therefore studying them can be extrapolated to provide information regarding heritability of a particular trait. An estimate of heritability of suicide in 2010 posits that there is a 43% heritability rate[49]. Other studies of suicide in twins have found a concordance rate of 23-38% among MZ twins as compared with a lower rate of 13-17% among DZ twins[62]. This leads to the conclusion that suicide is heritable to some degree and that environmental risk factors interact with this heritable risk for suicidal behavior.

Studies using both methylation analysis and large cohorts of twins have resulted in conflicting evidence regarding the role that environment plays in the development of depression in twins[44–46]. Heritability of depression among twins included in one study was estimated as

38%, and this study also concluded that gender does not play a role in heritability[45]. Other studies have estimated a similar heritability of 37%, but some have estimated heritability to be as high as 70%[44,63]. It should be noted that the highest estimates were based on studies that ascertained participants from inpatient units contributing significant bias to the population studied[63].

1.1.2.3 Linkage and Association Studies

The psychiatric GWAS consortium (PGC) combined eight GWAS studies in a mega-analysis. These studies had identified one candidate gene between them and the subsequent mega-analysis revealed no genome-wide significance and suggested that evaluation of subtypes of major depression may be more revealing[64]. More recently, a more highly homogenous cohort gathered by the CONVERGE consortium of severely depressed Chinese women with melancholic features identified a risk loci at *LHPP* and *SIRT1* as a possible etiologic origin for the development of MDD[40]. Many other possible loci have emerged as possible candidates for depression, but there is conflicting evidence for the associated of these genes with depression[14,43,55,65].

Of particular interest in mental illness has been the *MTHFR* gene. There has been conflicting evidence of its contribution to the development of depression[66–69]. It is a likely culprit of mental illness given its role in brain development and function. Most studies have focused on the two common variants, C677T and A1298C.[68] The *MTHFR* C677T genotype has been associated with increased chance of developing depression, schizophrenia and bipolar disorder[67,68]. In addition, it has been shown to exhibit a deleterious effect on maternal mood both in the prenatal and antenatal periods. Some evidence suggests that the thermolabile variant, C667T contributes to increased risk of depression in some populations, but not all[66–68].

Monoaminergic genes have also been the subjects of investigation for their possible association with depression[55]. The *SLC6A4, 5HTR2A, TH, TPH1, TPH2, COMT* and *5-HTTLPR* genes have all been investigated due to their roles in the synthesis and transport of serotonin and dopamine. Some associations have been found, but none have achieved anything beyond candidate gene status. The *5-HTTPLR* gene has been associated with suicidal behavior and depression related scores on some personality questionnaires. It has also been associated with the development of depression in the setting of adverse or stressful life events. Other such investigations have focused on the brain derived neurotrophic factor, *BDNF*, a neuro-protective protein of which no significant findings have been identified[55].

Several epigenetic mechanisms have also been investigated as a possible genetic predisposition to depression as, theoretically, discordance among twins for depression may be explained by epigenetic modifications[46,56]. In a study of twin pairs several loci were identified through epigenetic interrogation of the genome, *WDR26, CBR3, RPL3,* and *VCAN* were all previously noted to be associated with depression, studies are still investigating the roles of these genes in depression[56]. In addition, maternal mood may affect promoter methylation of the *SLC6A4* gene, a neurotransmitter transporter, and thus predispose children of depressed mothers to a resulting transporter defect that may result in an increased risk for depression or other psychiatric disease[66,70].

There have been many studies attempting to identify single genes that contribute to depression in families. Much of this research has yielded conflicting results and, as of yet, there are no known genetic causes of depression. The lack of consensus regarding the genetics of depression is likely a result of the significant heterogeneity of the depressed population[40,41].

Studies of depression may therefore be unlikely to achieve significant findings if the population is not homogeneous.

1.1.3 Depression in Metabolic Disorders

There are several metabolic disorders presenting with or accompanied by psychiatric symptoms. Psychiatric signs of inborn errors of metabolism (IEM's) may be isolated for years prior to the discovery of the underlying metabolic disease[71,72]. Psychiatric effects of inborn errors of metabolism fall into three classifications; the first represents those in which disorders appear as recurrent attacks of confusion, these are often misdiagnosed as psychosis. The second includes IEM's which have chronic psychiatric symptoms that appear in adolescence and early adulthood, and the third includes disorders which present with intellectual disability and behavioral or personality changes.[71,72] Depression is a feature of several IEM's including Phenylketonuria (PKU), Fabry disease and Maple Syrup Urine Disorder (MSUD); and persists even with treatment.[73–75]

1.1.3.1 Other Metabolic Disorders

PKU, a genetic disorder of phenylalanine metabolism, is easily treated through controlled protein intake[76]. As a result of its treatability, it warranted screening for all newborns as a seminal disorder for the newborn screening panel[77]. However, untreated PKU can result in psychiatric and neurocognitive deficits, poorly controlled PKU exhibits similar psychiatric outcomes[74,78–81]. Similarly, patients with Fabry disease, an X-linked lysosomal storage disorder, exhibit psychiatric symptoms typically in the form of depression and anxiety. Many patients experience these symptoms even when on regular treatment with enzyme replacement therapy[73]. Fabry

disease in women is often missed, and since women are X-linked carriers and random X-inactivation can result in a much milder presentation than men, they often present with only depressive symptoms[73].

1.1.3.2 Cerebral Folate Deficiency (CFD)

Any neurological syndrome associated with low levels of methylenetetrahydrofolate (MTHF) in the cerebrospinal fluid (CSF) in the presence of normal folate metabolism outside of the nervous system is considered cerebral folate deficiency[82]. Symptoms of CFD include epilepsy, cerebellar atrophy, behavioral changes, sleep disturbances, psychomotor retardation, unrest, irritability and cerebellar ataxia[83–85]. CFD is not a well-defined metabolic syndrome, but rather the common result of different genetic, metabolic and unknown processes. However it is treatable, and investigation of CSF MTHF can determine aberrant levels and thus, treatment[86].

Folate promotes the synthesis of purines and thymidine, the conversion of homocysteine to methionine as well as the formation of the active methyl group donor, s-adenosyl methionine (SAM), which is used in the transfer of methyl groups as well as in the methylation of DNA[87]. Depleted levels of folate in the central nervous system reduce the turnover of the serotonergic and dopaminergic pathways[87]. The proposed mechanism is a dysfunction in the transport mechanism that affects the ability of the choroid plexus to shuttle folate across the blood-CSF barrier[88,89]. Folinic acid, the biologically active form of folate, can be transported across the blood-CSF barrier and is often used to treat patients with CFD with sufficient clinical response, including restored muscular function and relief of neurologic symptoms[84,88,90].

At least five inherited disorders of folate transport are known and lead to general folate deficiency; methylnetetrahydrofolate reductase (MTHFR) deficiency, dihydrofolatereductase deficiency, proton coupled folate transport (PCFT) deficiency, FR-alpha and Kearns-Sayre

Syndrome (KSS)[91,84]. Mutations in *FOLR1*, which produces the folate receptor protein, have been associated with childhood CFD[84,90]. Other patients produce excess folate receptor antibodies leading to reduction of the binding ability of folate[84]. The mechanism for other forms of CFD, such as secondary CFD, is unknown and is a subject worthy of investigation[91,90].

A novel approach to psychiatric disease has been posited in a paper published by Pan and Vockley (2013) in which a previously undefined category of IEM's, neuropsychiatric IEM's, is described. Patients in this category of IEM's present with psychiatric symptoms prior to or without the onset of physiologic symptoms[72,92]. This may be a result of stress as individuals with undefined category of IEM often exhibit crisis during such times. As a result, this group of patients is inadequately treated and is unable to achieve relief from their depressive symptoms[72]. The patients represent a heterogeneous group of IEM's, which can be diagnosed through a battery of testing that ultimately may identify a treatment in addition to a cause for depression. The following were included in the testing for all participants:

1. Blood: amino acids, acylcarnitine profile , lactic

   acid, ammonia, lysosomal WBC enzymes with mucopolysaccharide and lipid panel, transferrin electrophoresis for glycosylation defects, chromosome micoroarray analysis, Fragile X, serotonin, dopamine, norepinephrine, folate, B12, cytochrome P450 testing, pharmaceutical levels when applicable.

2. Urine: organic acids, amino acids, purines and pyrimidines, urinalysis.

3. CSF: (in participants providing CSF) amino acids, glucose, lactate, homovanillic acid, biopterin, neopterin, 5-hydroxyindoleacetic acid (5-HIAA), 5-hydroxytryptophan (5-HT), 5-methyltetrahydrofolate (5MTHF), norepinephrine, dopamine, 3,4-Dihydroxy-Phenylacetic Acid (DOPAC)[93].

Of 33 individuals with TR-MDD, 19 (57.57%) were identified to have a CSF metabolite abnormality. The most commonly seen IEM in this group (n=12/19, 63.1 %) was cerebral folate deficiency (CFD). Others were identified with a myriad of disorders of metabolic origin, including GTP-cyclohydrolase deficiency and metabolic profile similar to glutaric academia type II[93]. For patients with CFD, folinic acid was prescribed as a supplement to their existing medications, the addition of which resulted in improvement of depressive symptoms for all individuals. For participants with other findings, a full genetic work-up was recommended in order to more fully understand the diagnosis.

Folate in Depression

Up to one-third of depressed patients are folate deficient, and some receive folate supplementation in addition to anti-depressants[9]. Folate supplementation has been known to reduce depressive symptoms, and many forms of folate have been shown to be tolerated, but there is little understanding regarding who will respond to folate supplementation[10]. The research by Pan et al. (2016) poses a model for beginning to understand who may be responsive to such supplementation while also identifying other metabolic aberrations in depressed patients. However, it should be noted that folate supplementation for blood folate deficiency is not the same as folinic acid supplementation for CSF folate deficiency. Folic acid cannot cross the blood brain barrier until it is reduced[87,90]. Folinic acid is the reduced form of folic acid; it can cross the blood brain barrier into the choroid plexus and be incorporated into multiple metabolic processes, including the conversion of homocysteine to methionine[87].

1.1.4 Genetic Counseling and Testing

Genetic counseling is defined by the NSGC as:

> "The process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease. This process integrates: 1. Interpretation of family and medical histories to assess the chance of disease occurrence or recurrence. 2. Education about inheritance, testing, management, prevention, resources and research. 3. Counseling to promote informed choices and adaptation to the risk or condition.[94]"

Genetic counselors meet with patients and their families to discuss the role genetics may play in their health. Often genetic counselors discuss testing with their patients and can help patients understand the results of their genetic tests. Many times genetic counselors also help patients process and come to a personal and integrated understanding of the risks of developing disease. Genetic counseling often involves a discussion of diseases caused by a single gene, like inborn errors of metabolism, which are commonly caused by a mutation in both copies of a gene and directly cause illness. Genetic counseling for common or complex disease involves discussing how genes and environment interacts to contribute to the development of disease. In addition to sharing information about risks to family members, genetic counselors allow patients to explore their feelings regarding these risks[52].

1.1.4.1 Psychiatric Genetic Counseling

Similar to genetic counseling for complex diseases, psychiatric genetic counseling often involves a discussion of how genetic and environmental influences work synergistically to foster the development of psychiatric disease. By creating a space for patients to explore their feelings

regarding risks, inheritance and the etiology of psychiatric disease, genetic counselors can foster a more integrated understanding of psychiatric disease and complement psychotherapeutic efforts to resolve anxiety regarding genetic contributions to disease[95,96]. However, unlike other areas of genetic counseling, there are currently no tests available to let us know which individuals may be at risk for developing psychiatric conditions[96].

The Psychiatric Genetic Counseling Session

Hippman et al. has published a trial to assess the impact of genetic counseling on patients with bipolar disorder (also schizophrenia and schizoaffective disorder) in which genetic counseling explored the current understanding of the etiology of complex disease in addition to sharing risks to relatives and the analysis of each individual's family history[97]. Patients were also given material to take home and visual genetic counseling aids were provided. Patients who were provided this information reported increased knowledge and understanding of risks than the group that did not receive genetic counseling. Changes in perception also included increased optimism after genetic counseling[98].

In particular, Peay et al. 2008 suggests that during these sessions genetic counselors would do well to address the uncertainty within the current understanding of the etiology of psychiatric disease[17]. Peay also suggests that the genetic counseling session should address the client's specific concerns, i.e. family planning, and should balance the recurrence risks with the adversity of living with such psychiatric disease. Peay places emphasis on exploring the client's own understanding about their particular concerns and addressing any areas that are inconsistent with current knowledge[17]. In addition to being informative these sessions can relieve anxiety for many people[17,95]. Challenges to psychiatric genetic counseling arise when determining risk and relaying this information to patients because much of the current information regarding risks and

heritability was not published with the intent of clinical use. Further, there are no genetic tests available for providing more accurate diagnostic or risk estimates[17]. When gathering this information genetic counselors are cautioned to be aware of the phenotype definition, the diagnostic criteria, and the method used to ascertain diagnosis in probands and relatives[17,18].

1.1.4.2 Neuropsychiatric IEM's and Genetic Counseling

Much of psychiatric genetic counseling focuses on schizophrenia, bipolar disorder and autism spectrum disorder; while there is no clinical test available for any psychiatric condition, except autism spectrum disorder, the genetics of all these conditions are much more clear than the genetics of depression[97]. However, in light of this newly described group of inborn errors of metabolism which present with neuropsychiatric features, the role of genetic counseling may be crucial for patients to understand their illness[72].

Genetic counselors are well versed in metabolic disease both with and without psychiatric manifestations and have been working with this population for many years to the great benefit of the affected individuals and their families. Additionally, research regarding psychiatric genetic counseling has made clear that there is a need for genetic counselors to work with individuals with psychiatric disease[17,18,95–97]. Since this newly described group of individuals are both affected with metabolic disease and, as a result, psychiatric illness, genetic counseling is crucial to the healthy incorporation of complex medical information into a patients' understanding of their illness. More information regarding this unique group of individuals will assist genetic counselors and other professionals to counsel these patients regarding risks, management and even, in the future, available testing options.

This study aims to address the lack of information regarding risk assessment for people with TR-MDD and suicidality who are part of a newly identified category of IEM's presenting

with psychiatric manifestations. By studying the pattern of inheritance in families with a defined neuropsychiatric IEM as opposed to others with unknown metabolic status it may create new avenues for research on this particular population. This may ultimately lead to more accurate and personalized risk information as well as a clinical test for psychiatric disease. Because of the distinct metabolic findings in this population, this study represents a unique opportunity to study a newly defined group of individuals with treatment resistant depression and thus may elucidate previously inaccessible information that can be translated to the population of depressed individuals as a whole[93]. We hypothesize that the families of probands present in the study are exhibiting an autosomal dominant mode of inheritance of depression and suicidality.

1.2 SPECIFIC AIMS

1.2.1 Specific Aim 1

Use a segregation study to analyze the family histories to assess if inheritance is segregating in an autosomal dominant manner. This analysis will also assess if there is a difference between genders indicating reduced penetrance in men. In addition it will compare patient's family history with CSF metabolic profiles that are indicative of a cerebral folate deficiency, those with CSF findings indicative of another metabolic disorder, those with another identified metabolic finding and those without.

1.2.2 Specific Aim 2

Observational analysis will determine if there is an observable pattern of inheritance in this population of individuals. Characteristics of dominant inheritance will be used in this analysis. For comparison autosomal recessive inheritance will be assessed to confirm that families inheritance in families is not conforming to any known autosomal patterns of inheritance. In addition, a simple segregation analysis will be performed to determine if there is a difference in pattern of inheritance between genders.

2.0 MATERIALS AND METHODS

The recruitment, subsequent interviews and analysis of participants in the Metabolomics of Early Suicide Attempts (MESA) and Metabolics of Treatment Resistant Depression (MTRD) were approved by the University of Pittsburgh Institutional Review Board (IRB PRO11120375 and IRB PRO14060600). Letters of approval are found in Appendix A. The informed consent can be found in Appendix B. This study is performed under this IRB approval; no modifications were made to perform this analysis.

2.1 DATA SOURCE

The Metabolomics of Early Suicide Attempt (MESA) study at the University of Pittsburgh Medical Center is ongoing and aims to identify abnormalities of neurotransmitters or other biomarkers for inborn errors of metabolism in young people with treatment resistant depression with suicidality and discern novel and more effective treatment and diagnostic options through metabolomic analysis of cerebrospinal fluid. The Metabolomics of Treatment Refractory Depression (MTRD) study at the University of Pittsburgh Medical Center is ongoing and is the adult arm of the MESA study and aims to translate the metabolomics findings from MESA to adult onset treatment resistant depression. Treatment resistance is defined in this study as failure of at least three maximum dose medication trials continued for at least six weeks. This differs

from other definitions of treatment resistance to refine the phenotype of TR-MDD in this population; by failing three courses of treatment an individual is less likely to ever respond to treatment and thus is more likely to be truly treatment resistant. Affected participants were recruited through referral from the inpatient treatment units, the electroconvulsive therapy service by a treatment provider known to them, and from the Services for Teens at Risk (STAR) registry.

The MTRD study collects detailed, directed metabolic studies including profiling of neurotransmitter metabolites as well as interrogation of intermediary energy, amino acid, and carbohydrate metabolism with acylcarnitine, organic acid and amino acid profiling. Functional testing is performed to study individual enzymes through key metabolic pathways as indicated by the metabolite profiles. Finally, non-directed profiling of CSF and blood for a broad array of metabolites will be performed using ultra-high pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Participants are provided with the option to opt out of the lumbar puncture portion of the study but still undergo other study related evaluations.

At the time of recruitment an IQ test, Beck Depression Inventory (BDI), Suicidal Ideation Questionnaire (SIQ) and neurological test are performed. A self-report questionnaire is also administered to participants regarding their previous and current medications and diagnosis. Inclusion for the study occurs when a participant has met the following criteria:

- Participants must be between 14 and 54 years of age
- Diagnosis of major depressive disorder (MDD) with at least one suicide attempt
- Must have been on at least three failed drug trials for treatment of MDD

This study aims to determine if there is a possible metabolic cause for depression, and participants identified to have a metabolic or other genomic alteration are prescribed treatment

and are evaluated by the Medical Genetics Department at the Children's Hospital of Pittsburgh, of UPMC. Some participants may also be asked to participate in whole exome analysis on a research basis to rule out novel, related genetic alterations.

Family history was taken at intake via a three-generation pedigree; a trained M.D., study coordinator or genetic counseling intern took the pedigree for each visit. Pedigrees were extended beyond three-generations as participants reported information and included all psychiatric disease as well as major medical problems. Affected individuals on family histories included in this analysis were reported by the participants as having experienced depression, major depression, a suicide attempt or suicide completion. Family history was not confirmed via medical records. Participants were included in this analysis if they completed a family history interview by February 15, 2016. Those completing entry into the study, or a family history interview after this date were not included in the analysis.

2.2 DATA ANALYSIS

Pedigrees were constructed and drawn using Progeny Clinical Hosted (Progeny Genetics LLC, Delray Beach, FL) and were coded for analysis. Coding included the identification of affected family members who were those affected with depression, major depression or experienced suicidality. Family members explicitly identified as under the age of 10 were excluded, as prevalence of depression is typically low until the early teens. Additionally, individuals adopted into families were excluded for simplicity of analysis, except in the case of an identified founder who was adopted. Comorbidity of other psychiatric illness was not exclusionary. Individuals were added in order to run the analysis, as all individuals who are not founders must have two

parents according to this analysis. As a result, 108 individuals were added with null data as parents. Data was input into an excel file and was converted into a .csv (comma separated values) file for analysis by S.A.G.E. See Figure 4 for a sample pedigree file.

2.3 OBSERVATIONAL ANALYSIS

In addition to the statistical analysis, an observational analysis was completed. Pedigrees taken for the study were edited for clarity and ease of assessment, and a trained genetic counseling student assessed the observed inheritance patterns. This was done by observing whether or not each family history fit known Mendelian patterns of inheritance. The observational analysis captured two patterns of Mendelian inheritance, autosomal dominance and autosomal recessive inheritance. The decision to capture autosomal recessive inheritance in the observational analysis was two-fold. First, the majority of metabolic disorders are inherited in an autosomal recessive manner. Second, male-to-male transmission was observed in the pedigrees precluding both X-linked and mitochondrial inheritance. Autosomal dominant inheritance was included based on the appearance of the family histories. We hypothesized that the depression and suicide in these families is inherited in an autosomal dominant manner. The following criteria are used for assessment of autosomal dominant and autosomal recessive inheritance:

Autosomal dominant:

- Affected people in every generation
- Each affected individual has an affected parent
- There is a 50% risk of inheriting the trait
- Unaffected individuals cannot pass on the trait

- Men and women are at the same risk to be affected
- Both men and women can pass the trait on to their children

Autosomal recessive

- The chance to be affected is equal for men and women
- Individuals have a 25% chance to be affected when both parents are carriers
- Recessive disorders appear sporadic
- Siblings are typically the only affected individuals in a three-generation pedigree

Families were assessed based on the number of criteria met for each of these inheritance patterns. Autosomal recessive inheritance was assessed for comparison. In the autosomal dominant population counting affected individuals in the sib-ship of the proband assessed risk. For sib-ships that were not clearly discernable as 50% risk, a conservative approach was taken. For example, in a sib-ship containing three individuals with two affected individuals the sib-ship was not considered at 50% chance of being affected.

Further analysis of sib-ships of the probands were analyzed using the Hardy-Weinberg equilibrium formula $(O-E)^2/E$ to assess whether inheritance in these sib-ships is occurring in an autosomal dominant manner. This analysis assumes that there is an allele in the family and that an affected parent is passing on the trait.

2.4 STATISTICAL ANALYSIS

Data was analyzed using the Statistic Analysis for Genetic Epidemiology Software version 6.3 (S.A.G.E.) for a segregation analysis. In order to run the analysis a .txt file was created specifying parameters for the analysis. The program first determined the likelihood that

transmission occurred in a Mendelian pattern; this was then used to assess likelihood ratio. In addition to determining the likelihood that inheritance is occurring in a Mendelian vs. non-Mendelian pattern, the parameter file indicated that the analysis also include gender as a covariate to determine if there was a difference in the effect of gender on affected and unaffected individuals. This file also specified that the program evaluate traits based on patients' metabolic findings. These were bivariate and include presence or absence of metabolic alteration in CSF, presence or absence of CFD and presence or absence of a possible metabolic alteration in blood. Because family members were not tested for metabolic alterations, the analysis assumes that they were of unknown status and thus that it was possible for them to be affected. Additionally, this analysis ran descriptive statistics regarding gender distribution and family information such as number of singletons, founders, etc. Unfortunately, this was unable to be included in the analysis due to the small sample size. Finally, participants' status as proband was included to account for ascertainment bias. For parameter file please see Figure 5.

The software for the segregation analysis operates under specific assumptions. In particular, families must meet all criteria for autosomal dominance to be considered dominant; this program does not consider penetrance, other types of inheritance or anticipation in its analysis. If phenotypic heterogeneity is present in a group it decreases the power for the study overall. Additional decrease in the power of the study occurs when a group contains families that may not be inheriting a trait in an autosomal dominant manner.

In addition to the use of S.A.G.E. to perform segregation analysis, examinations of the segregation ratios among the subships in the study was performed. Three tests were conducted: (1) all participants in sibships, (2) all female participants in subships and (3) all male participants in sibships. In all three tests, a segregation ratio of 0.5 (autosomal dominant mode of

transmission) was taken as the null hypothesis, and a chi-squared goodness-of-fit test was performed to test the alternative hypothesis that the mode of transmission is not autosomal dominant. This analysis does not account for ascertainment bias nor can it account for other covariates that would affect penetrance or phenocopy

3.0 RESULTS

3.1 DEMOGRAPHICS

The segregation analysis consisted of 36 families containing 872 individuals. Thirty-seven of these individuals were probands. There was one affected sib-ship in this analysis, and these siblings are fraternal twins. The analysis included 423 male individuals (48.5%) and 407 females (46.67%) 42 individuals were of unknown gender (4.8%) (Table 1). Additionally, there were 8 (22.2%) male probands and 28 (77.78%) female probands in this analysis. While there were 34 complete pedigrees included, 45 constituent pedigrees were identified. There were no consanguineous mating pairs identified, as a result no marriage rings or marriage loops were included. Twenty individuals across 13 families were identified to have multiple mates. In total there were 122 affected female and 67 affected males in all families in this study.

Table 1 Family demographics

Gender	Total N	Probands	Total Affected
Male	423 (48.5%)	8 (22.2%)	67
Female	407 (46.67%)	28 (77.78%)	122
Unknown	42 (4.8%)		N/A
Total	872		189

3.2 OBSERVATIONAL ANALYSIS

Very few pedigrees fit all the criteria for autosomal dominant inheritance. In fact, only six of 36 pedigrees fit all criteria (See Table 3). For an example pedigree meeting all autosomal dominant criteria see Figure 1. Females are predominantly affected in these families with 32.4% of females and 19% of males affected, suggesting that there is some possible reduction of penetrance in males. Additionally, many affected males did not have children, making it difficult to assess whether men were able to pass the trait on to their children. There were 68 affected women with children in these families and 30 affected men with children, this is proportional to the number of affected individuals of both genders, 122 and 67 respectively.

When disregarding the criteria that males must pass on the trait, 11 out of 36 families met either four or five of the criteria for autosomal dominant inheritance, see Figure 2 for an example family. No families met all of the criteria for autosomal recessive inheritance; in fact, only one family met three of the five criteria for autosomal recessive inheritance. Thirty-three out of 36 families met either 0 or one criterion for autosomal recessive inheritance. The majority of the families who met one criterion (n=20/36) were those who had both affected males and females in the family. See Figure 3 for an example family history.

A segregation analysis of sib-ships belonging to the probands determined that for all individuals in the sib-ships and for males only the null hypothesis that autosomal dominant inheritance was occurring could not be rejected ($p = 0.275$ and 0.273 respectively). However, for females in the sib-ships the null hypothesis could be rejected for the alternative hypothesis that autosomal dominant inheritance was not occurring ($p = 0.029$). Please see Table 2 for more information on this analysis.

Table 2: Simple Segregation Analysis with Gender

		Observed	Expected	$(O-E)^2/E$	χ^2	*p*
All Individuals	Affected	47	42	0.595	1.19	0.275
	Unaffected	37	42	0.595		
Females	Affected	35	27	2.37	4.741	0.029
	Unaffected	19	27	2.37		
Males	Affected	12	15	0.6	1.2	0.273
	Unaffected	18	15	0.6		

This population is metabolically heterogeneous, thus it is likely that there are several possible inheritance patterns being observed here. Of the 14 participants who were identified to have cerebral folate deficiency, there are five, 35.7%, with families meeting four or five criteria for autosomal dominance. Nine of these affected individuals, 64.2%, are women. It is also possible that this metabolic alteration is observed as a result of polygenic inheritance, which could not be tested for in this model. The probands were categorized into three groups; affected with no metabolic finding, affected with CFD and affected with another metabolic finding. There was no significant difference between age and gender in any group using a one-way ANOVA and Fisher's exact test respectively. See Tables 3 and 4 for reference.

Table 3: Comparison of Age and Presence or Absence of CFD

CFD	N	Mean	Grouping	Difference of Levels	*p*-value
1	15	1.4	A	1-1	0.2048
0	11	1.18	A	2-0	0.6193
2	11	1.09	A	2-1	0.0758

Table 4: Comparison of Gender and Presence or Absence of CFD

CFD	N	Mean	Grouping	Difference of Levels	T-Value	*p*-value
1	15	26.91	A	1-1	-0.61	0.8147
0	11	25.067	A	2-0	1.32	0.3936
2	11	22.64	A	2-1	-0.81	0.7012

Table 5: Observational Analysis Scoring

	Family 1	Family 2	Family 3	Family 4
Autosomal Dominant Criteria Met	3	4	0	1
Autosomal Recessive Criteria Met	0	0	1	2
Cerebral Folate Deficiency	No	Yes	Yes	No
Sex	F	F	F	M
	Family 11	Family 12	Family 13	Family 14
Autosomal Dominant Criteria Met	4	2	1	2
Autosomal Recessive Criteria Met	0	0	1	1
Cerebral Folate Deficiency	No	No	Yes	No
Sex	F	M	M	F
	Family 21	Family 22	Family 23	Family 24
Autosomal Dominant Criteria Met	1	6	6	4
Autosomal Recessive Criteria Met	1	1	1	1
Cerebral Folate Deficiency	Did not complete	No	No	No
Sex	F	F	F	F
	Family 31	Family 31	Family 32	Family 33
Autosomal Dominant Criteria Met	3	3	4	3
Autosomal Recessive Criteria Met	1	1	0	1
Cerebral Folate Deficiency	Yes	Yes	No	Yes
Sex	M	F	f	F

Table 5: Observational Analysis Scoring **cont.**

Family 5	Family 6	Family 7	Family 8	Family 9	Family 10
5	3	0	4	4	1
3	2	0	1	1	1
Yes	No	No	Yes	Yes	Yes
F	F	F	M	F	M
Family 15	Family 16	Family 17	Family 18	Family 19	Family 20
0	1	0	1	6	5
1	1	0	1	1	1
Did not complete	Yes	Yes	Yes	No	No
F	M	F	F	M	M
Family 25	Family 26	Family 27	Family 28	Family 29	Family 30
6	3	2	4	5	0
1	1	1	0	1	1
Did not complete	Yes	No	No	Yes	No
F	F	F	F	F	F
Family 34	Family 35	Family 36			
0	4	5			
1	1	1			
No	No	Did not complete			
F	F	F			

3.3 SEGREGATION ANALYSIS

The program S.A.G.E.'s sub-program SEGREG was used to assess the segregation of depression and suicide in these families[53]. The parameters for the analysis directed the program to run a likelihood ratio test to determine if the families fit an autosomal dominant mode of inheritance, compared to polygenic inheritance of disease. The parameters also specified that the program evaluate trait for this mode of inheritance based on the metabolic findings in our probands. As a result of the small sample size of the metabolic phenotype, this was ultimately not used in the analysis.

For this analysis the following is assumed:

H_0 = Transmission is occurring in an autosomal dominant manner

H_A = Transmission is not occurring in an autosomal dominant manner

This assumption deviates from the original hypothesis as a result of the nature of the segregation analysis. Because autosomal dominance is a known pattern, the segregation analysis can determine whether the families assessed fit or do not fit the pattern; however it cannot do the reverse.

The probability that transmission occurs when a parent has the AA genotype is 0.00000508, for the AB genotype probability is 0.70935667 and for the BB genotype probability is 0.00000444. The likelihood ratio calculated the difference between what was observed and what was expected for dominant inheritance. This calculation assumes that expected is 1.0 for the AA genotype, 0.50 for the AB genotype and 0.00 for the BB genotype. A test statistical *D* was calculated as two times the difference in the log-likelihoods. The log-likelihood of the null

hypothesis is -361.897, and the log likelihood of the alternative hypothesis is -347.015, thus D=29.725. D has a chi-squared distribution with three degrees of freedom because there are three fixed differences between the two models; τ AA, τ AB and τ BB where τ is the transmission probability of A given the parental genotypes AA, AB or BB, respectively. The result of the likelihood ratio test is $P(\chi^2(3) \geq 29.725) = 1.58e\text{-}06$. At $\alpha = 0.05$ the null hypothesis that these families are exhibiting autosomal dominant inheritance was rejected in favor of the alterative hypothesis, that inheritance is not autosomal dominant.

Simple segregation analysis of the segregation ratio of the sibships in the study showed a segregation ratio of 1.19 for all sibships (P = 0.275 for test of deviance from the expected ratio of 0.5,), 4.741 for female sibships (P = 0.029), and 1.2 for male sibships (P = 0.273). The null hypothesis of autosomal dominance was rejected in favor of the alternative hypothesis for female sibships and not rejected for males or all sibships.

4.0 DISCUSSION

In spite of the many associated biologic factors with depression, there is no known cause for depression, no diagnostic test and few indications for who may develop chronic, treatment resistant depression[21]. It is often recurrent and accompanied by significant morbidity and mortality[14]. Suicide is highly correlated with depression but there is no reliable method to determine who is at risk[14,99]. This study represents a small, metabolically heterogeneous group of participants with treatment resistant major depression and suicidality. It attempts to describe the patterns of inheritance in this unique cohort and ultimately add to the growing knowledge surrounding this newly described group of neuropsychiatric IEM's[72,93].

The majority of individuals in this group are affected by cerebral folate deficiency, traditionally thought of as an autosomal recessive disorder[90,91]. It is important to note that these individuals are not phenotypically identical to those that are homozygous for FOLR1 mutations, which results in severe neurological disease presenting with ataxia, seizures, and developmental delay among other symptoms[84,90,91]. However, individuals in this study are not affected with biallelic FOLR1 mutations and thus results in a conundrum for discerning the cause for CFD in this population.

Current understanding of the etiology of depression describes it as a multifactorial disorder in which both genetic and environmental factors contribute to the development of disease[2,44,47]. The segregation analysis performed by S.A.G.E. ultimately supports this

conclusion since no Mendelian inheritance pattern was identified through the analysis. Upon observation, many of the families also did not meet all of the criteria for dominant inheritance, further ruling out the possibility of a single gene cause for the depression and the underlying metabolic alteration. Previous GWA studies of depression have been unable to identify anything more substantial than candidate genes in very large cohorts of individuals[30,49].

In simple segregation analysis, the null hypothesis, that autosomal dominance was occurring, could not be rejected for two of the three groups of the simple segregation analysis performed on the sib-ships of probands; males and all individuals even though it was rejected for females. It is important to consider that the alternative hypothesis, that autosomal dominance was not occurring, was not rejected for males and all individuals because the small sample sizes of those groups reduce the power to detect such a deviance from the expected. Because this segregation study assumes that an affected parent is present and passing on the trait it is important to consider adoption studies. One of which found that maternal depression, but not paternal depression, increases risk of depression in adoptees[100]. Additionally, the T-tests performed on this group of individuals determined that males and females who were affected were significantly different from one another. As a result it may be important for future studies to be more particular about the number of individuals in each gender for a more cohesive group and for interpretation.

Evidence gained from this study allows for the consideration of a more complex pattern of inheritance within this unique subgroup of individuals. In cases of other types of metabolic diseases with unknown genetic origin, it has been suggested that many subtle genomic changes together may lead to the manifestation of disease[92]. These genomic changes are largely not understood at this juncture; they may be epigenetic changes, or multiple heterozygous mutations

within a pathway or related pathways. It is likely that a similar mechanism is resulting in this specific group of patients with TR-MDD with metabolic alterations[93].

Many studies have determined that depression is difficult to study because groups of participants are typically highly heterogeneous[41]. While the cohort for this study is also heterogeneous, we have taken advantage of a unique group of individuals identified by recent studies of depression that use metabolomics to determine if there is an underlying metabolic alteration that may be contributing to the development of depression to discern whether inheritance in this population aligns with the findings of previous studies of depression. This metabolic difference has identified a quantitative measure that can be utilized not only for treatment options but can optimize the ability to study depression[93]. Studies of this sub-group may translate into a quantitative test for depression, biomarkers for treatment-resistance or remain a unique, but alternative method for identification of personalized treatment options for those who are treatment-resistant with major depressive disorder. Future application of studies of neuropsychiatric IEM's may eventually help to create testing options for affected individuals and their families.

4.1 PUBLIC HEALTH SIGNIFICANCE

The World Health Organization (WHO) predicts MDD will be the second leading cause for disability by the year 2020, thus placing significant burden on the public[101]. Approximately 10-15% of US adults have depression; women are 1.5-3 times more likely to be affected than men, with this difference beginning in adolescence[65,101]. The prevailing theory regarding why women experience depression at higher rates than men involves the complex interaction between

environmental experiences and biologic vulnerabilities which differ between men and women[102]. Depression can occur at any age, but the most affected age group is within the 25-34 year range, with 30.1% of affected individuals falling in this age range[103]. It is estimated that 14 million people suffer from depression and roughly half of them will seek some form of treatment[3,104,]. Some proposed reasons for many individuals with depression not seeking treatment involve financial and situational circumstances as well as perception and stigma surrounding mental illness. The majority of people with major depression feel that they could handle or treat the depressive episode themselves[104]. Some people do not perceive their condition as an illness; others feel that it is a normal response to events in their lives[104]. Additionally, some experience financial barriers to seeking treatment[104].

By identifying the cause of depression within this group it may not only affect the individuals in this cohort by determining a treatment in addition to a cause, but could also decrease the cost of treatment and the length of time individuals are non-responsive to treatment in the larger population of depressed people. In addition, learning who may be at risk for developing depression in this population would increase the quality of life and general health for these individuals and their family members. Further, by using a personalized treatment method the cost of treating depression would decrease. The ability to identify biologic causes for depression and suicidality may also affect the stigma associated with mental health issues and depression. Therefore this study and subsequent studies of this group of individuals may also increase the amount of individuals who seek treatment or who identify their depression as worthy of seeking treatment. Further, identification of inheritance and depressive etiology in this population may decrease the cost of treatment overall reducing the public health burden of depression.

4.2 LIMITATIONS

Limitations in the ascertainment of family history are present in this study. Family history information is patient reported and, other than in the case of the patient, was unable to be confirmed through medical records in family members. In addition, stigma that surrounds reporting of mental health issues may contribute to the limitations of self-reported family history, many people are unable or uncomfortable discussing mental health with others. As a result, much of the family history information could be either missing or unknown. Additionally, men seek treatment significantly less often than women, limiting the ability to study the population completely. Because the reason for difference between men and women to be seek treatment is unknown there are a number of possible causes that could be investigated; it may be a result of social factors, there may be reduced penetrance specifically affecting the likelihood for males to be affected or there may be biological factors associated with gender involved in the development of depression.

The ability to perform the analysis is also limited by the fact that the CSF analysis was only collected for the proband. Therefore, metabolic and genetic information was unknown for the majority of individuals in this study. This limited the ability to thoroughly phenotype affected individuals in the segregation analysis beyond reported mental health phenotypes. Furthermore, the size of the analysis limits the ability to determine significance in both analyses in this study. Finally, one individual performed the observational analysis. Having an independent review of observed patterns by more than one person may have been beneficial for the reliability of this study. The sample size in this study was small and therefore lends little power to the study interpretation. In particular, the sample size of men in the study was quite small leading to the inability to reject either the null or alternative hypothesis in this group.

4.3 FUTURE STUDIES

Future studies should all be conducted to look at the unique metabolic groups individually. This would result in more homogeneous groups of individuals and would lend higher reliability to these analyses. CSF analysis of probands and their affected and unaffected family members would better guide the phenotypic identification of truly affected and unaffected individuals. A study of this magnitude would be difficult to undertake, but would be more comprehensive in the determination of a phenotype for affected individuals. Studies of this cohort may also benefit from analysis of genetic information in addition to a segregation study. Currently whole exome sequencing is being analyzed for some participants to determine if there are subtle genomic changes that may be affecting the development of the phenotype. Additionally, a survival analysis may be helpful in clarification of risks to family members within this specific population.

Since this population is newly identified, an outcomes analysis of mental health functioning using the Hamilton rating scale for depression, a questionnaire that is taken at each visit, may be beneficial to understand how receiving or not receiving a biological cause for depression has affected coping. Further, a genetic counseling outcomes scale has been created and validated by McAllister et al. which explores patients experiences coping with clinical genetics services and may be useful for those participants receiving genetic counseling services in this population[105]. It may be beneficial to understand how their results were received and what they understand about their diagnosis. Since this study is unique in it's exploration of metabolic alterations in treatment resistant major depression it may also be helpful to explore whether there is a need for genetic counseling for all individuals participating in this research.

5.0 CONCLUSION

This is the first family history study of a newly defined population of individuals with TR-MDD and associated metabolic alterations. While the study did not identify any new or unique patterns of inheritance in this sub-group, it is beneficial to understand that there is little observable difference in the inheritance in this population as compared to other populations of individuals with depression. This knowledge taken in the context of our understanding that depression and other psychiatric manifestations are common in metabolic disorders, which have an underlying genetic cause, allows for a reconceptualization of TR-MDD with suicidality not as a disorder of unknown etiology, but as a result of multiple small metabolic alterations culminating in this common phenotype. Thus it may be possible to translate the metabolomic studies that have been used to identify this population to the larger population of individuals with depression and may ultimately change the way depression is tested for, treated and conceptualized.

APPENDIX A: FIGURES

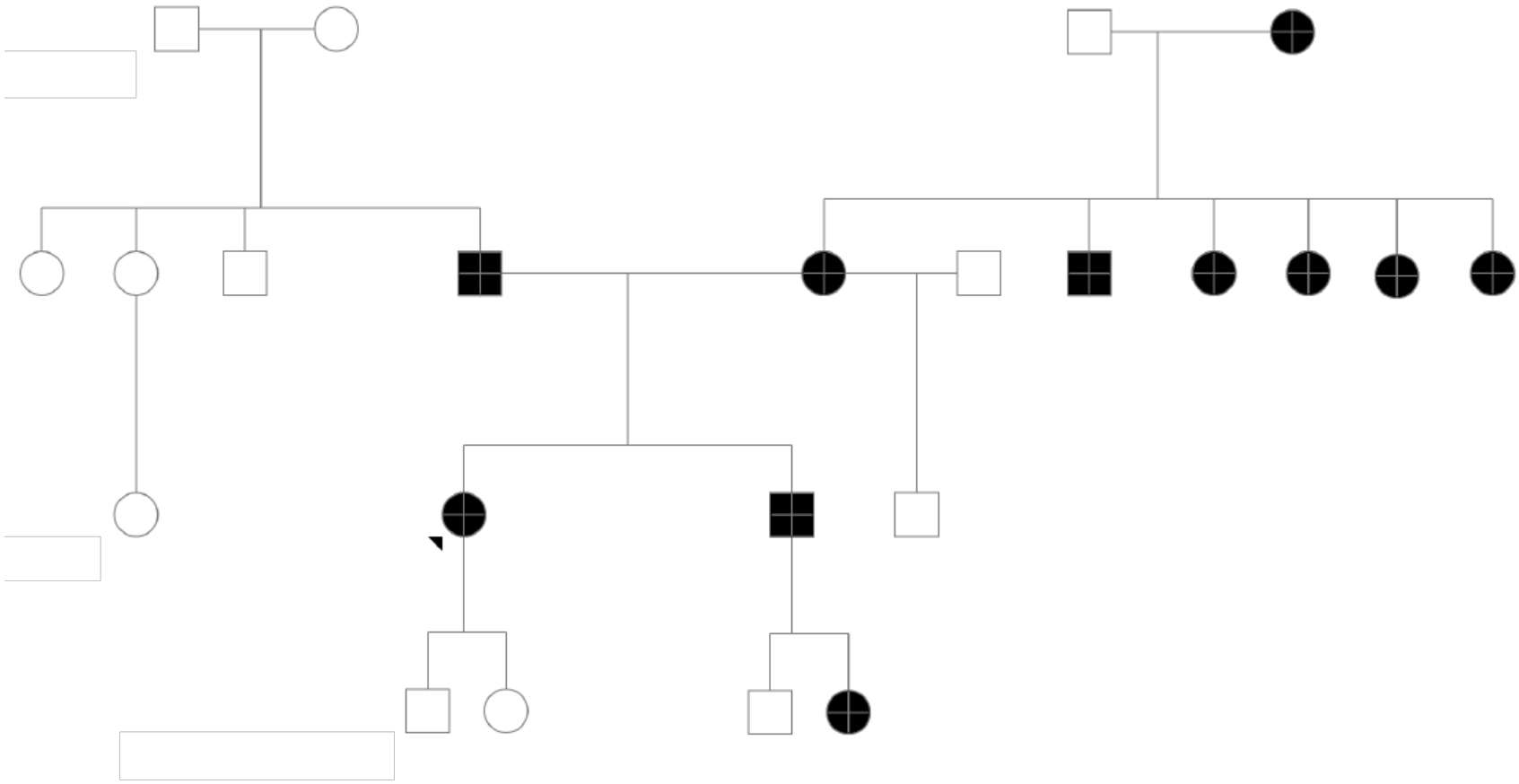

Figure 1. Autosomal Dominant Family History

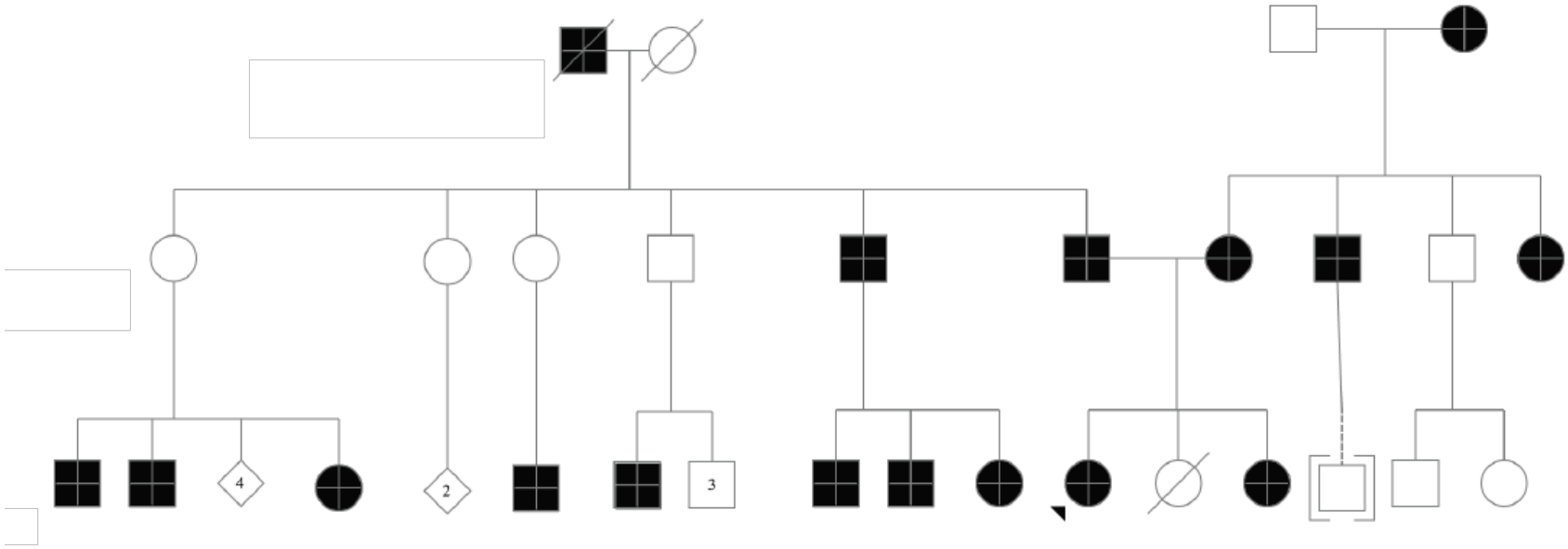

Figure 2. Family History Meeting 4 of 6 Criteria for Autosomal Dominant Inheritance

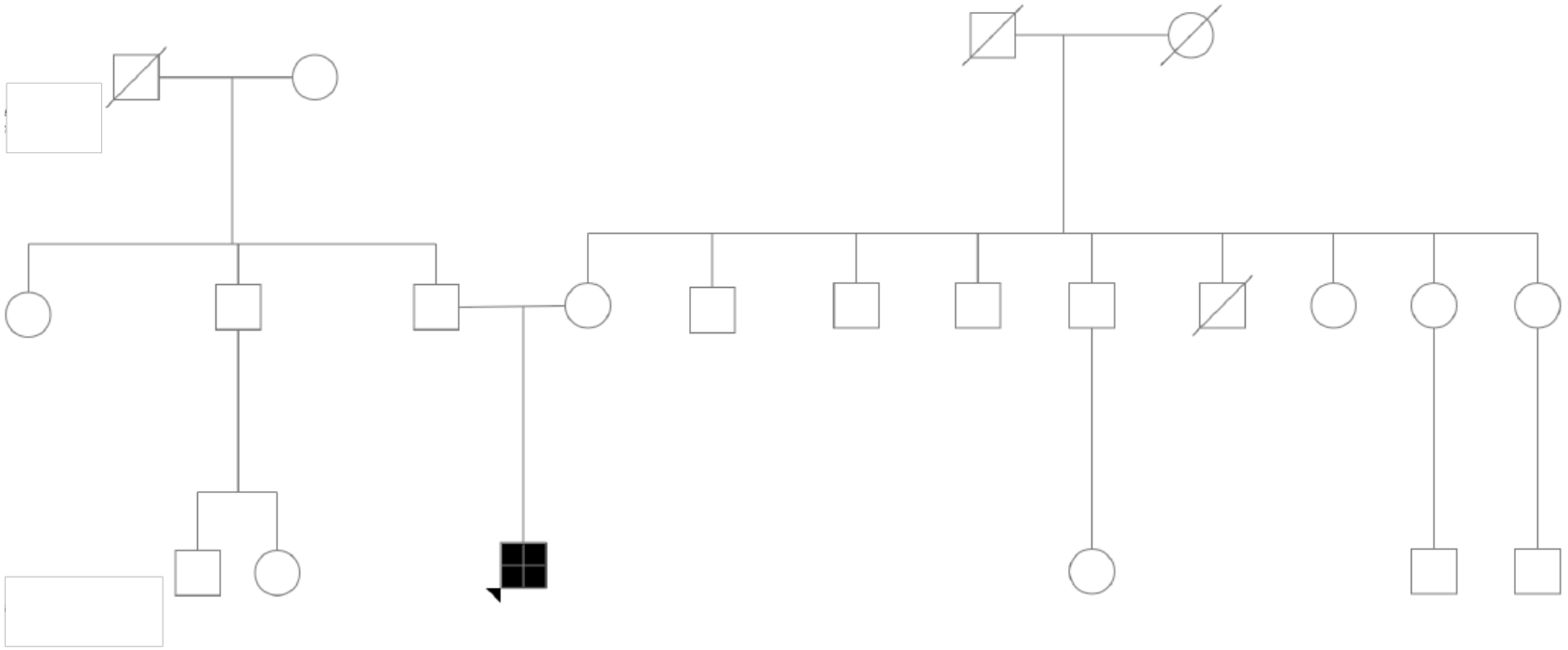

Figure 3. Autosomal Recessive Family History

P_ID	Proband_AFI	Proband_CSI	Proband_CFI	Proband_OT	AFF	Gender	P1	P2	ID	CSF	CFD	OTHER
301	1	0	0	0	Yes	F	3012	3013	3011	N	N	N
301	0	0	0	0	Yes	F	3014	3015	3012	U	U	U
301	0	0	0	0	No	M	3016	3017	3013	U	U	U
301	0	0	0	0	No	F	30123	30122	3014	U	U	U
301	0	0	0	0	No	M	0	0	3015	U	U	U
301	0	0	0	0	No	F	0	0	3016	U	U	U
301	0	0	0	0	No	M	0	0	3017	U	U	U
301	0	0	0	0	No	F	3014	3015	3018	U	U	U
301	0	0	0	0	No	F	3014	3015	3019	U	U	U
301	0	0	0	0	No	F	0	0	30112	U	U	U
301	0	0	0	0	No	U	30112	3013	30114	U	U	U
301	0	0	0	0	No	M	0	0	30115	U	U	U
301	0	0	0	0	No	F	3012	30115	30116	U	U	U
301	0	0	0	0	No	M	0	0	30117	U	U	U
301	0	0	0	0	No	U	3016	3017	30119	U	U	U
301	0	0	0	0	No	M	0	0	30120	U	U	U
301	0	0	0	0	No	F	3014	30120	30121	U	U	U
301	0	0	0	0	No	M	0	0	30122	U	U	U
301	0	0	0	0	No	F	0	0	30123	U	U	U

Figure 4. Sample Coded Family History

```
segreg, out = "walano.out"
{
  trait = AFF,binary
  ascertainment
  type_suspect=1
  {

  psf_indic=PROBAND
  }
}
{
  trait = AFF,binary
  ascertainment
  type_suspect=0.75
  {

  psf_indic=PROBAND
  }
}
{
  trait = AFF,binary
  ascertainment
  type_suspect=0.50
  {

  psf_indic=PROBAND
  }
}
pedigree
{

  pedigree_id=I_ID
  individual_id=ID
  sex_field=GENDER,male=m,female=f,missing=u
  parent_id=P1
  parent_id=P2
  trait=AFF,binary,affected=Yes,unaffected=No,missing=U
  individual_missing_value=0
  trait=CSF,binary,affected=Yes,unaffected=No,missing=U
  individual_missing_value=0
  trait=CFD,binary,affected=Yes,unaffected=No,missing=U
  individual_missing_value=0
  trait=OTHER,binary,affected=Yes,unaffected=No,missing=U
  individual_missing_value=0
}
```

Figure 5. Parameter Input File

APPENDIX B: IRB APPROVAL

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Ground Level
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Lisa Pan, MD

From: IRB Office

Date: 1/29/2016

IRB#: MOD14060600-04 / PRO14060600

Subject: Metabolomics of Early Suicide Attempt (MESA).

The University of Pittsburgh Institutional Review Board reviewed and approved the requested modifications by the expedited review procedure authorized under 45 CFR 46.110 and 21 CFR 56.110.

Modification Approval Date: 1/29/2016
Expiration Date: 8/17/2016

The following documents were approved by the IRB:
We would like to add the following individuals to our study team: Dr Michael

Morowitz, and Shilpa Argade. They will be assisting us with analyzing the blood, urine, and CSF samples that we have collected.

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least one month prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Lisa Pan, MD

From: Aviva Katz, MD, Vice Chair

Date: 9/15/2015

IRB#: REN15070250 / PRO11120375

Subject: Metabolomics of treatment refractory depression and early onset suicide attempt

---

At its full board meeting on 8/19/2015, the University of Pittsburgh Institutional Review Board, Committee G, reviewed the Renewal for the above referenced research study and approved it pending minor modifications. Your responses to these

comments have been reviewed and the research submission, in its currently modified form, adequately addresses the concerns of the IRB and is therefore approved.

The risk level designation is Greater Than Minimal Risk.

Please note the following information:

Approval Date: 9/14/2015
Expiration Date: 8/18/2016

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

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Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

APPENDIX C: MTRD ADULT CONSENT FORM

CONSENT FOR AN ADULT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY-
Affected Adult

TITLE OF PROJECT: Metabolomics of Treatment Refractory Depression

INVESTIGATORS:

Lisa Pan, M.D.	David Brent, M.D.	Gerard Vockley, M.D.,Ph.D.	Marion Hughes, M.D.
3811 O'Hara St.	3811 O'Hara St.	4401 Penn Ave.	200 Lothrop St.
Pittsburgh, PA 15213	Pittsburgh, PA 15213	Pittsburgh, PA 15224	Pittsburgh, PA 15213
(412) 246-5597	(412) 246-5596	(412)-692-7746	(412)-647-8666

RESEARCH COORDINATOR:

AnnaMaria Tomlanovich, B.S.
3811 O'Hara St
Pittsburgh, PA 15213
(412) 383-8226

SOURCE OF SUPPORT: Brain and Behavior Research Foundation, NARSAD

We are conducting research to understand the metabolic basis for depression that is not responsive to known treatments. The purpose of this study is to identify potential metabolic causes for depression that is treatment resistant. There are many known causes of depression. However, in some individuals, there may be an unidentified underlying metabolic disorder even though a precise diagnosis is unknown.

In this study we will be studying genetic and metabolic material from your blood and cerebrospinal fluid (CSF). Cerebrospinal fluid is a clear colorless fluid that is continuously produced and absorbed and that flows around the surface of the brain and spinal cord. This research study will use new techniques to study metabolic pathways in your cells that might cause a health problem if there is a change. These techniques are called metabolic profiling.

Your genetic material is a substance within the body, such as DNA and RNA, which is passed down from parents to children and can affect what types of diseases people have. Metabolic measures used in this study will measure products of pathways coded by your DNA and RNA. In this study we will be studying genetic material and metabolites from your blood and CSF. This research study will use new techniques to measure genetic and metabolic profiles that might cause a health problem if it contained a mistake.

We are inviting you to participate in this study because of significant depressive symptoms. Because your depression has been difficult to treat, there is a small possibility that we may find an underlying contribution to your symptoms.

As part of this study, you will complete the following procedure. You will be asked to participate in an initial study visit, which will include a psychiatric interview and completion of self report forms asking questions about depression, anxiety, and suicidal ideation. A brief measure that estimates verbal and spatial intelligence will also be administered. Tubes of blood will be drawn and a urine sample will be collected in the clinical lab at the Bellefield clinic by the lab personnel after the clinic visit. The entire initial visit is estimated to last one hour, and will not exceed two hours. In addition, we will schedule you for a second appointment to have an additional blood draw and a lumbar puncture to obtain cerebrospinal fluid for testing. The total amount of blood collected over both visits will be 80 ml or less of blood, equivalent to 16 teaspoons, and approximately 1/5 of what is given during blood donation. Lumbar puncture involves the insertion of a needle into the fluid within the spinal canal. The time for the lumbar puncture is estimated at one hour. While the procedure is brief, you will be asked to rest immediately following the lumbar puncture to reduce the risk of headache. Because the metabolic disorders that may contribute to depression are often specific to the cerebrospinal fluid, the cerebrospinal fluid sample has the most likelihood of revealing a metabolic contribution to depression.

There will be no need for specific follow-up appointments or outpatient visits related to this research study until we have the result of the research study which will be disclosed to the you during a third clinic visit regardless of being positive or negative. Positive results would include any finding suggesting a difference in metabolism. Positive results will be confirmed in a lab a laboratory that meets Federal standards established under the Clinical Laboratory Improvements Amendments (CLIA) to insure that the results are not in error before being disclosed. At this third visit, we will also repeat a psychiatric interview. This third and visit is estimated to last 30 minutes. Only positive results of the research study that leads to a diagnosis which can be treatable will be included in the medical records with the permission of the participant. An additional visit may be scheduled to collect one 2 ml tube of plasma; this procedure is identical to receiving a regular blood draw. After your blood is drawn and collected, we will separate the plasma from the blood. This visit will take place at Bellefield clinic and should only last approximately 30 minutes. A 6 month follow-up appointment will be scheduled for only those participants who come up positive for an inborn error of metabolism. At this 6 month visit, you will be asked to fill out the same questionnaires relating to depression, anxiety, and suicide ideation as you completed in the initial visit. This 6 month follow-up visit is estimated to last between 30-60 minutes.

We are also requesting your authorization or permission to review your medical records to record past, current, and future medical information from UPMC facilities. We will obtain information concerning your diagnosis, psychiatric records, diagnostic assessments, health and family history, and results of any physical exams, tests of urine, blood, tissues, and any other tests, including results of genetic tests. We will use this information to help us understand the results of the tests performed as part of this study. This identifiable information will be made available to members of the research team, for an indefinite period of time. The University of Pittsburgh Research Conduct may monitor this study and as the result of this monitoring may have access to your identifiable information.

We are also requesting your permission to re-contact you in the future regarding participation of your family/relatives in this study. You may refuse to be re-contacted in the future. Your decision will not affect your relationship with the University of Pittsburgh or the UPMC, nor will you lose any benefits that you might be eligible for because of this decision

Results of the research study will be disclosed to you during a clinic appointment with appropriate psychiatric and genetic counseling and plans for clinical follow-up and testing. After the research study and verification studies are completed, your DNA sample will be stored indefinitely for future molecular studies and to compare to future planned whole genetic and metabolic studies. This will be done by the same researchers of this study. Upon participation in the research study and when stored, these samples will be given a case number and the code linking the name to this number will be maintained separately with very limited access to research team. The blood samples will be labeled with a case number and the code linking the name to the number will be maintained separately with very limited access to the research team.

We expect that the results of the research study will be available in six months due to the data to be analyzed. In some cases patients might get the results earlier than that.

There are a number of possible risks, side effects, and discomforts associated with participation in this research study. The risks of each procedure are infrequent.

- **CSF sampling by lumbar puncture (LP)**: The lumbar puncture includes minimal risk for bruising, bleeding and infection. There is a risk of back discomfort or pain following the LP. There is an infrequent risk of headache following the lumbar puncture due to leaking of fluid. This risk is minimized by the use of fluoroscopic guidance. Rarely nausea, vomiting, and dizziness can accompany headache. In the event of a brain tumor or mass in the brain, removal of CSF can lead to compression of the brainstem.

  There is a very rare potential risk of excessive bleeding or leakage of cerebrospinal fluid at the procedure site. This would be treated with the use of an epidural blood patch. An epidural blood patch involves your own blood being injected into the dural space (which contains the cerebrospinal fluid), which is an effective treatment in the event of severe or refractory cases of bleeding.

  Participation in this research study involves exposure to radiation from fluoroscopic guidance for LP. The amount of radiation exposure that you will receive from this procedure is approximately 0.1 rem (a “rem” is a unit of radiation dose) to your lower back with minimal exposure of other body areas. For comparison, radiation workers are permitted, by federal regulation, a maximum annual radiation exposure of 20 rems to the most sensitive organs of their body. There is no known minimum level of radiation exposure that is recognized as being totally free of the risk of causing genetic defects (abnormal cells) or cancer. However, the risk associated with the amount of radiation exposure that you will receive from this study is considered to be low and comparable to everyday risks

Blood draws: Brief discomfort, bruising, slightly prolonged bleeding, infection at the site, scar noted at the site, the clotting of blood around the site, and dizziness or fainting are

- infrequent. There is a possibility of obtaining life-altering results. Care will be taken to avoid these potential risks and discomforts.
- **Blood draw plasma collection:** The risks are identical to the regular blood draw. We will be collecting one 2ml tube of blood from each participant. After the collection is complete, we will separate the plasma from the blood. There are no risks associated with separating plasma from the collected blood.

- **Because your genetic information is being used in this research study,** there is a rare risk that that information could become accessible to people other than members of this research team. **Breaches in confidentiality** involving genetic information could impact future insurability, employability, or reproduction plans, or have a negative impact on family relationships, and/or result in paternity suits or stigmatization. To minimize these risks, genetic information (as well as medical information) will only be recorded in files marked with case numbers, not your name.

- **There is also a possibility of learning life-altering results**. We will provide appropriate counseling, support and referral to appropriate treatment.

- **There is a risk of boredom or discomfort** related to the completion of the self reports and assessments.

- **There is a risk of breach of confidentiality** with regard to medical record review.

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies and group health plans to use genetic information in making decisions regarding your eligibility or premiums. GINA also makes it illegal for employers with 15 or more employees to use your genetic information when making decisions regarding hiring, promoting, firing, or setting the terms of employment. This new Federal law does not protect you against genetic discrimination by companies that sell life, disability, or long-term care insurance.

If we learn of any new information about study risks that could cause you to change your mind about continuing to participate in the study, we will notify you promptly.

Benefits of participation in this research study: If a specific genetic disorder or inborn error of metabolism is identified, treatment may be available based on the information. It is possible that a specific inborn error of metabolism is identified, but not guaranteed. Participation in this research may help patients with depression that is difficult to treat in the future.

None of the procedures you receive during this research study (research blood draws, lumbar puncture or sample analysis) will be billed to you or your health insurance. If you get a bill or believe your health insurance has been billed for something that is part of the study, notify a member of the research team. However, you or your insurer will be billed for all other usual care services, including routine surgery, blood draws for clinical/routine care, follow-up care, or testing done for clinical/routine purposes.

You will be paid up to $150 for your participation to compensate you for your time and travel. You will also be compensated $10 at each visit for transportation expenses. Although it is possible that your biological samples may lead, in the future, to new inventions, discoveries or

products that may be sold, licensed, or patented, there are currently no plans to share with you any money or other rewards that may result from the development of those new products.

If you believe that the research procedures have resulted in an injury to you, immediately contact Dr. Pan or a member of the Research Team (see first page). Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

To protect your privacy and maintain the confidentiality of information we obtain from you and from your medical records, we will maintain all information in a secure location. This research study will involve the recording of current and/or future identifiable medical information from your hospital and/or other (e.g., physician office) records. The information that will be recorded will be limited to information concerning your psychiatric, metabolic and genetic assessment. All paper records that could identify you will be stored in locked file cabinets, and all electronic records will be stored in password-protected files. Your identity on these records will be indicated by a case number rather than by your name, and the code linking your name to this number will be maintained separately with very limited access to research team members. Although we will do everything in our power to protect your privacy and the confidentiality of your records, just as with the use of your medical information for health care purposes, we cannot guarantee the confidentiality of your research records, including information that we obtained from medical records. However, **no third party, including relatives, personal physicians or insurance companies, or other researchers will have access to your identifiable information, with one exception**. Authorized representatives of the UPMC hospitals may have access to research data and documents and identifiable information only for the purpose of (1) filling orders made by the researchers for hospital and health care services (e.g., laboratory tests) associated with the research study, (2) addressing correct payment for tests and procedures ordered by the researchers, and/or (3) for internal hospital operations (e.g., quality assurance). Also, authorized representatives from the University of Pittsburgh Research Conduct and Compliance Office (RCCO) will have access to these files but only for the purpose of monitoring the conduct of the study.

Your doctor may also be involved as an investigator in this research study, but you are not under any obligation to give consent to participate in any research study offered by your doctor. Before agreeing to participate in this research study, or at any time thereafter, you may wish to discuss participation in this study with another health professional, to obtain a 'second opinion' about study participation. You may also contact the University 'Research Participant Advocate' 1-866-212-2668 for additional information.

Your participation in this research study is completely voluntary. Whether you participate/not participate in this research study will have no effect on your current or future relationship with the University of Pittsburgh, UPMC or its affiliated health care providers or health care insurance providers. **If you decide that you no longer wish to continue to participate** after you have signed the consent form, you should contact Dr. Pan or her colleagues. Your blood samples and DNA will then be destroyed if they are not in the midst of being analyzed. You may also withdraw, at any time, your authorization to allow the research team to review your medical records, but if you do so, you will no longer be permitted to

participate in this study. Any information obtained from you up to that point will, however, continue to be used by the research team. Your decision to withdraw from this study will have no effect on your current or future relationship with the University of Pittsburgh or with UPMC or its affiliated health care providers or health care insurance providers. However if withdrawal takes place, no information regarding results will be returned to you and your DNA sample will be destroyed so that no additional future testing can be performed. Results from the study obtained prior to withdrawal will still be analyzed to the extent possible.

VOLUNTARY CONSENT:

All of the above has been explained to me and all my current questions are answered. I understand I am encouraged to ask questions and voice concerns or complaints about any aspect of this research during the course of it, and that those questions, concerns, or complaints will be answered by the researchers listed on the first page of the form. I understand that I may always request that my concerns be addressed by a listed investigator. I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss any issues; obtain information; offer input; or discuss situations in the event that the research team is not available. By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____

Participant's Name (Print)

_____ _____

Participant's Signature Date

CERTIFICATION of INFORMED CONSENT:

I certify that I explained the nature and purpose of this research study to the above-named individual(s). I discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future issues that arise. I certify that no research component of this protocol was begun until after this consent form was signed.

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

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