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Genotyping of TGIF Polymorphisms Associated with Schizophrenia and Psychosis

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GENOTYPING OF TGIF POLYMORPHISMS ASSOCIATED WITH SCHIZOPHRENIA AND PSYCHOSIS

A Thesis

by

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ABSTRACT

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Polymorphisms in Transforming Growth β-Induced Factor (TGIF) have been found to contribute to the risk of development of psychosis and related disorders. Identifying the genes responsible for causing schizophrenia and psychosis will allow for treatment at an earlier stage of development, saving the patient problems, time, and money. The purpose of this experiment is to determine the haplotype of DNA samples obtained from earlier experiments. The DNA samples were collected from people with schizophrenia in Costa Rica, Southern Texas, and Northern Mexico. The samples were tested using 5 single nucleotide polymorphisms (SNPs) and amplified using polymerase chain reaction (PCR). Haplotypes were determined using allelic discrimination. Out of 5 SNPs, three SNPs were shown to exhibit significant association with schizophrenia, while two SNPs show significant association with psychosis.

DEDICATION

The completion of my graduate studies would not have been possible without the love and support of my family and friends. I would like to thank my mother, Blanca J. Vera, and my father, George Vera, for believing in me even when I doubted myself. I would also like to thank my girlfriend, Tracey, for being patient and encouraging me to continue in my studies. It is thanks your inspiration that I was able to accomplish my goal of obtaining a graduate degree. Thank you.

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CHAPTER I

INTRODUCTION

Statement of the Problem

Schizophrenia, psychosis, and other mental disorders affect a significant portion of the population throughout the world. Mental disorders affect people of all races, gender, social status, and ethnicity. Treatment can be found, but often times it is expensive and as such many people are left untreated. These disorders affect people's lives by compromising their relationships with other people. The purpose of this research project is to examine the genetic basis of schizophrenia and psychosis. These disorders are thought to have a genetic component that interacts with environmental factors.

Statement of the Purpose

Identifying the pathways and genes that are most likely to contribute to schizophrenia will provide insight into the pathophysiology of not only schizophrenia, but also associated disorders such as psychosis. New treatments and strategies to combat schizophrenia can be formulated by having a detailed knowledge of the alleles that contribute to the disease.

CHAPTER II

REVIEW OF LITERATURE

Psychosis

Psychosis is an umbrella term used to describe a broad range of mental disorders that include schizophrenia, bipolar disorder, major depression, and other mental disturbances. Psychosis is characterized as a condition where a person loses contact with reality. Delusions and hallucinations are common when dealing with a person with psychosis (Ballas, 2008). A person may be exhibiting symptoms that are similar to schizophrenia, but unless they are diagnosed as having schizophrenia, or another disorder, they can only be classified as having some kind of psychosis (Escamilla, et al., 2007; Chavarria-Siles, et al., 2007). Psychiatrists use the Diagnostic and Statistical Manual of Mental Disorders (DSM) to diagnose a disorder in patients. The DSM provides the major criteria for diagnosing many disorders (Eifert and Sajatovic, 2003).

Schizophrenia

Schizophrenia is a genetic disorder that involves disturbances in emotional and social functioning and cognition (Williams, et al., 2003). It is a common mental disorder that affects 1-2% of the general population (Li, et al., 2004). Schizophrenia affects people's lives by altering their lifestyle and compromising their relationships with other people.

Schizophrenia is common among all cultures and ethnic backgrounds, although, slight increases have been seen in people living in poverty and urban areas (Mueser and Gingerich, 2006). The differences in incidences of schizophrenia can be attributed to biological factors interacting with stress caused by the environment (Mueser and Gingerich, 2006). Environmental factors include anything that is not directly controlled by genes, such as, physical and mental abuse, drug use, diet, pathological infections, living environment, and season of birth (Oh and Petronis, 2008). Treatment can be found, but often times it is expensive and many people are left untreated.

Schizophrenia is caused by a variety of factors. There are several theories that suggest the cortex and the hippocampus may be associated with the disease since those areas of the brain are responsible for abstract ideas, problem solving, and memory (Mueser and Gingerich, 2006). Chemical imbalances involving neurotransmitters such as dopamine have also been linked to the disease (Mueser and Gingerich, 2006). If the flow of a neurotransmitter is interrupted at any time during the release or absorption phase of the cycle then there could be some pyschotic symptoms (Mueser and Gingerich, 2006).

Recent studies have shown that multiple genes of moderate effect may influence the susceptibility of a person developing schizophrenia (Escamilla, et al., 2007). One source of evidence for a genetic component in the disease includes studies showing that having a close relative with schizophrenia may increase a person's chance of developing the disease (Mueser and Gingerich, 2006). There are several theories as to why only some individuals in a family exhibit schizophrenia. A common theory suggests that asymptomatic family members carry the genes that predispose them to the disease, but do not express them or only partially express them (Mueser and Gingerich, 2006). This

would make them carriers allowing them to pass this gene onto their offspring. Aside from environmental risks, expression of schizophrenia may involve multiple interactions of susceptibility genes with each other (Corvin, et al., 2004). Schizophrenia is classified as a multifactorial disorder with environmental factors. As such, schizophrenia does not follow Mendelian principles and a specific inheritance pattern has not been mapped (Yang, et al., 2003).

The symptoms of schizophrenia encompass both physical and psychotic behaviors. Schizophrenic symptoms "can be divided into five categories: psychotic symptoms, negative symptoms, cognitive impairment, mood problems, and behavioral disturbances" (Mueser and Gingerich, 2006). Psychotic symptoms include perceptions that break from reality and most often involve hallucinations or delusions. Hallucinations are false perceptions that a person experiences. Delusions involve having a belief that is not shared by people around you (Mueser and Gingerich, 2006).

Negative symptoms involve an absence of emotional expression, apathy, and the decreased ability to feel pleasure or enjoyment. *Alogia*, or saying very little, has also been a symptom of schizophrenia (Mueser and Gingerich, 2006). Cognitive impairment can involve lack of self care, memory problems, and poor insight into what others are thinking. Mood problems associated with schizophrenia include depression and suicidality, anxiety, anger, suspicion, and rapidly fluctuating moods. Behavioral disturbances are rare, however a person may be dazed or unable to engage in any purposeful behavior. A person may also be catatonic, a state in which a person maintains the same posture for many hours or days (Mueser and Gingerich, 2006).

Bipolar disorder

Bipolar disorder is a mental disorder expressed as manic episodes accompanied by periods of depression. In the past, bipolar disorder was known as manic-depressive disorder or manic-depression, due to the change in disposition from a manic state to a depressed state. In the manic phase, people with bipolar disorder will exhibit irritable or elevated moods, irrational judgement, sleeplessness, and a predisposition towards grandiosity or extravagance (Goodwin and Jamison, 2007). In the depressed phase, the person will experience lethargy, lack of concentration, remorse, and feeling of self-blame. The symptoms may not be recognizable due to variable ranges in intensity between people who are affected with bipolar disorder. Estimates have shown that 2 million Americans may suffer from bipolar disorders (Goodwin and Jamison, 2007). Symptoms for bipolar disorder appear in early adulthood and may continue throughout the course of a person's life. The disorder affects males and females equally, although it is more common in people who have a family history with the disorder (Goodwin and Jamison, 2007).

Schizoaffective disorder

Schizoaffective disorder is a designation used when a person is diagnosed with schizophrenia and a mood disorder, such as bipolar disorder (Eifert and Sajatovic, 2003). People who are subject to schizoaffective disorders experience psychotic symptoms along with severe, long-lasting mood disorder symptoms. In bipolar disorders, psychotic symptoms will occur during an active manic or depressive state, whereas in schizoaffective disorders psychotic symptoms can occur without a severe mood

disturbance. Females are affected at a greater frequency than males, and as with other mental disorders, there appears to be a genetic component (Eifert and Sajatovic, 2003). Schizoaffective and other psychotic disorders are caused by the interaction of several genes with the environment.

Physical mapping

To identify genes and gene location on chromosomes, two methods of mapping genes were developed, physical mapping and linkage analysis. Physical mapping places genes at certain locations on a chromosome to reflect the physical distance between those genes. At low resolution, cytogenetic bands can be used as markers to place genes on a certain region in the chromosome. At a high resolution, physical mapping provides the location and distance of certain genes in base pair units (Nusbaum, et al., 2004). Two methods of physical mapping include radiation hybrid mapping and fluorescent in situ hybridization. Radiation hybrid mapping uses x-rays to denature DNA into smaller fragments. The fragments are inserted into other cell cultures and allowed to grow. If two genes are close together they should be located on the same DNA fragment, conversely, if two genes are far apart they will be located on other DNA fragments. Fluorescent in situ hybridization will insert labeled probes into hybridized DNA allowing for detection using fluorescent microscopy (Nusbaum, et al., 2004).

Linkage analysis

Linkage analysis measures the frequency of two genes remaining close together as they are transmitted from one generation to the next through the use of meiosis. Linkage

analysis is useful because it allows for mapping of genes that can be detected only as phenotypic traits. Linkage analysis uses genetic markers, characteristics located on the same place in a pair of chromosomes, to distinguish individual homologues from each other. One type of marker that can be used is a SNP, or a single nucleotide polymorphism, which is discussed in more detail later in this paper. In order to compare genetic markers, pedigrees will often be created to establish hereditary patterns within families. Studies using siblings, sibpairs, can be used to compare loci between affected individuals and nonaffected individuals from the same family. If one locus is believed to be associated with the disease there will be a greater amount of allele linkage in the affected individual compared to unaffected individuals (Nusbaum, et al., 2004).

Genetic contribution to disorders

The genetic contribution to complex or multifactorial diseases is based on determining what role genetic variation and mutation have in the cause of disorders or diseases. Almost every disease occurs through the interaction of genes with the environment. The goal is to determine how much of a role is played by genes in the disease (Nusbaum, et al., 2004). Diseases with a genetic component can be broken down into three categories: single-gene disorders, chromosome disorders, and multifactorial disorders.

Each of the disorders results from changes to the genome. In the simplest kind of disorder, single-gene disorders, there is a mutant gene (Lewin, 2008). The gene may have a mutation caused by chemical modification of a base or the mutation may arise as a result of a malfunction during replication of DNA. Therefore, if there is a change in DNA

sequence, there may be a change in the gene. Common mutations involve additions, deletions, and insertions of nucleotides. Base pair substitutions include transitions and transversions. Transitions constitute the substitution of one pyrimidine base for another pyrimidine base, or a purine base for another purine base (Lewin, 2008). Transversions involve a purine changing to a pyrimidine, or vice versa (Gibson and Muse, 2002).

Single gene defects

Single gene defects are characterized by individual mutant genes. The single mutation can be present on one or both chromosomes of a pair (Nusbaum, et al., 2004). If the mutation is found on only one chromosome, the corresponding allele found on the homologous chromosome would be normal. The mutation is most often found in the nuclear genome, however, in some cases a single gene defect can be found in mitochondrial DNA. Single gene defects will cause characteristic pedigree patterns and may be found in frequencies as high as 1 in 500 humans (Nusbaum, et al., 2004).

Chromosomal disorders

Chromosomal disorders are due to an excess or deficiency of genes in chromosomes or chromosomal segments. For example, an extra copy of chromosome 21 will produce Down syndrome, even though the genes themselves are not abnormal. Chromosomal disorders are common and are known to affect 7 out of 1000 infants. Chromosomal disorders also account for about half of spontaneous first-trimester abortions (Nusbaum, et al., 2004). An example of disorders caused by an excess of chromosomes are Edwards syndrome, Patau syndrome, Klinefelter syndrome, Triplo-X syndrome, and Jacob

syndrome among many disorders. Turner syndrome is an example of a disorder with a deficiency of the X chromosome (General Biology, 2006).

Multifactorial disorders

Multifactorial disorders are different from other genetic diseases in that, the disorder is not caused by a single error in a gene. Instead, multifactorial disorders are caused by small variations in many genes. When the effects of all of the altered genes are combined, they can predispose someone to a serious disease, mostly in conjuction with environmental factors (Nusbaum, et al., 2004). Multifactorial disorders can reoccur in families, however, the disorders do not show typical mendelian inheritance as would be seen in single-gene disorders. Multifactorial disorders have estimates affecting from 5-60% of the total human population (Nusbaum, et al., 2004). Multifactorial disorders are elusive because they involve more than one gene. There may be many loci linked to a disease, such as schizophrenia, but finding the genes remains elusive due to the large amount of genes involved in the disease. Due to the difficulty in locating genes, markers such as SNPs can be used to pinpoint specific regions in a gene to determine an association between phenotypes. If a significant association is established between many SNPs and a phenotype, the region in which the SNPs are found can be studied in more detail to determine the effect that region has on the phenotype.

Polymorphisms

In the past, alleles were classified according to wild-type or mutant (Lewin, 2008). However, the existence of multiple alleles with varying effects on phenotype alters the

classification that was previously used for alleles. Therefore, the existence of multiple alleles at a single locus is now called a genetic polymorphism (Lewin, 2008). The most common polymorphisms include additions, deletions, and substitutions (Gibson and Muse, 2002). Polymorphisms are naturally occurring variants of alleles that are found in stable concentrations among populations (Gibson and Muse, 2002). An allele that is present in more than 1% of the population is referred to as polymorphic (Lewin, 2008). Polymorphic alleles will possess mutations that may change the phenotype by altering protein function. In addition, wild-type alleles may themselves be polymorphic even if there is no phenotypic change in an organism. To determine whether wild-type alleles are polymorphic, restriction maps can be created that compare differences between genes. Single nucleotide polymorphisms (SNPs) are polymorphisms with a change in a nucleotide at one base pair (Lewin, 2008). SNPs are at the focal point of this study.

Linkage disequilibrium

Most polymorphisms are maintained in a population when a balance is achieved between mutation and genetic drift. In other words, the rate at which mutations are introduced is equal to the rate at which mutations are lost. Most populations contain several haplotypes due to past mutations that have remained in the population through positive selection (Gibson and Muse, 2002). Many of the polymorphisms can be measured by looking at the linkage disequilibrium that exists between alleles. Linkage disequilibrium is defined as the nonrandom association of alleles. In the context of schizophrenia, linkage disequilibrium that exists between two alleles can help with the interpretation of genotype/phenotype studies. Linkage disequilibrium mapping can be

used to look at SNPs that are believed to be associated with schizophrenia. The theory behind determining a candidate gene involves looking at whether a certain SNP is more commonly seen in unrelated individuals that are affected with schizophrenia. If the percentage of SNPs seen is higher than you would expect to see, than the SNP is a potential candidate for further genomic studies (Gibson and Muse, 2002).

SNP effect on phenotype

By default, all polymorphisms are mutations since they involve changing or losing a nucleotide. However, it is only when looking at the result of the mutation in a population that the haplotype can be mapped. Haplotypes are a unique combination of single nucleotide types on single chromosomes at loci. Haplotypes are altered in populations by polymorphisms that affect the normal or wild type phenotypes (Gibson and Muse, 2002). Some of the SNPs may be found in introns resulting in several possibilities (Lewin, 2008). SNPs in introns may upregulate the expression of a gene if they are found in enhancer or promoter regions. If SNPs are located in sequences of short interfacing RNA (siRNA) they may silence the expression of a gene altogether. Alternatively, if a SNP is found in an intron it may have no effect on either expression or translation of a protein if it is removed through RNA splicing immediately after transcription (Lewin, 2008). SNPs that are more likely to be functional will occur in coding or regulatory regions of genes.

In individuals affected by a particular gene, there may be one or more SNPs with mutations that surround the gene responsible for causing the disease (Alberts, et al., 2004). The coinheritance of a disease and a SNP can be studied to obtain inheritance frequencies. If individuals with a disease almost always inherit the SNP, then it is likely

that the gene and SNP are located close together in the same chromosome. If the SNP is located inside of the gene causing the disease, the linkage will be absolute. If the SNP is located outside of the gene causing the disease, then the frequency of the SNP being inherited may be altered (Alberts, et al., 2004).

Case-control studies

One method of measuring familial aggregation is to conduct case-control studies. Affected individuals tend to be clustered in families, familial aggregation. When two related individuals within a family are affected with the same disease, the individuals are concordant. If only one member of the pair of relatives has the disease, they are considered discordant. In a case-control study, patients with a disease are compared to individuals without the disease. Patients without the disease are considered controls, whereas patients with the disease are the cases being studied (Nusbaum, et al., 2004).

The comparison between individuals can take into account many factors: occupations, illnesses, age, ethnicity, and geographical location. Genetic contribution of diseases can compare the frequency that a disease is seen among extended families of cases relative to the positive family histories of controls. Some errors in case-control studies involve ascertainment bias. Ascertainment bias is the difference in reporting of relatives of affected cases when compared to the reporting of relatives of controls (Nusbaum, et al., 2004). Recall bias is also an error that is common in case-control studies. Recall bias is when relatives of patients who are afflicted with the disease are more likely to respond to a questionnaire than are relatives of controls. Choice of controls can also be a problem. Controls should differ from cases only in regards to the presence of the disease or

disorder. Controls should not differ from cases in ethnic background, occupation, socioeconomic status, and if possible gender. Any deviation may result in an important difference that could affect the significance of the results (Nusbaum, et al., 2004).

Associations with case-control studies must be thoroughly examined for interdependence between two factors. Simply because an association is found between a factor and a disease, the factor itself may have nothing to do with the disease. An example of this would be an association found between a disease and ethnic background, when in reality it is not the ethnicity that is contributing to the disease, but rather the diet that is responsible (Nusbaum, et al., 2004).

Genome wide studies

There are many disorders or diseases that contain genetic components: hypertension, diabetes, psychotic disorders, cancer, and coronary heart disease (Visscher and Montgomery, 2009). Genome wide association studies provide an effective method of analyzing thousands of DNA markers that may contribute to the etiology of the disease. Commercial platforms can genotype more than 1 million SNPs using a single chip. Genome wide studies have provided insight into SNP variants common in the population. In addition, genome wide studies have provided avenues for identifying new genes that were previously not associated with a disease. New biological pathways have been discovered in existing genes that are associated with a disease (Visscher and Montgomery, 2009).

SNPs may only play a small role in explaining the development of a complex disease. Genome wide studies have shown differences in genetic architecture that play a role in

the effect SNPs will have in the development of a disease (Visscher and Montgomery, 2009). In some diseases genetic variation may be 50%, however, the variation will be confined to 6 loci. Conversely, some diseases may have genetic variation of 6%, but the variation will be spread out among 50 loci, therefore the genetic component of a disease will influence development in its own way (Visscher and Montgomery, 2009).

Background on previous studies

Escamilla, et al. (2007) conducted a genome wide study on populations of Mexican and Central American origin. The genome scan was performed to determine which chromosomal regions were most closely associated with schizophrenia and psychosis. Dr. Escamilla's study provided insight into chromosomal linkage patterns associated with schizophrenia and other psychiatric disorders in Latin American populations. Previous genome wide studies had been conducted on specific ethnic groups with people from Northern European or Celtic ancestry, however, no study had been performed on Latin American populations. Latinos contain genetic origins from Hispanic, African, and Amerindian ancestors. This diversity in ancestry was thought to cause variations in genes that would affect the development of schizophrenia or other psychotic diseases (Escamilla, et al., 2007).

The subjects were chosen in accordance with guidelines from the declaration of Helsinki and follow recommendations from Institutional Review Boards from the University of Texas Health Science Center at San Antonio and the University of Costa Rica along with all other participating sites (Escamilla, et al., 2007; Walss-Bass, et al., 2006). Families were selected from sites in the Central Valley of Costa Rica (CVCR).

Written consent was obtained from all families that were recruited into the study. The families that were recruited into the study had to contain one subject with a clinical diagnosis of schizophrenia, one sibling with a clinical diagnosis of schizophrenia or a schizoaffective disorder, and two grandparents of Mexican or Central American ancestry (Escamilla, et al., 2007).

Within each family chosen, all members that were affected were attempted to be recruited in order to obtain as accurate a pedigree of the family as possible. If the parents were unable to be recruited, additional siblings were recruited to reconstruct parental genotypes. There were 459 subjects from 99 families chosen to be in the study (Escamilla, et al., 2007). The subjects were screened to determine a lifetime consensus diagnosis. The lifetime consensus diagnosis uses procedures from the fourth edition of the DSM (DSM-IV) along with Research Diagnosis Criteria systems. In addition, all of the subjects were interviewed and psychiatric records were collected on all inpatient and outpatient visits. All of the information for each subject was collected and sent to two diagnosticians. At this time the subject data was deidentified to protect the identities of the subjects. The diagnosticians arrived at a consensus diagnosis for each subject using the DSM-IV criteria (Escamilla, et al., 2007).

Genealogical studies

A genealogical study was performed in order to identify possible relationships between affected subjects who carry the mutation (Walss-Bass, et al., 2006). The ancestors for each of the individuals were determined for the previous six generations. The study identified two parents, four grandparents, 8 great-grandparents, and continued

until all 128 ancestors had been identified. The pedigrees of the subjects confirmed the presence of shared ancestors. Marriage documents, death and baptismal certificates were used as the primary sources of information in developing the pedigrees. Secondary sources of information included the National Archive records which contained testamentary documents, mortuaries, and other documents that could be used to identify the ancestors of the individuals in question (Walss-Bass, et al., 2006). This information was applied to different models used in calculating linkage disequilibrium.

Escamilla, et al. (2007) used two different affected status models. The primary model used was the broad model. The broad model used all subjects that had at some point in their lives experienced persistent delusions, hallucinations, or disorganized behavior or thought processes. The broad model was used because it allowed a larger sample size. The broad model also showed stronger linkage peaks in restrictive phenotype models. The narrow model was used to determine the efficiency of using restrictive diagnostic criteria to obtain more precise results than those obtained from the broad model. The narrow model used only individuals that had been diagnosed with schizophrenia or schizoaffective disorder according to DSM-IV guidelines (Escamilla, et al., 2007).

Loci identification and association values

Three loci, 1pter-1p36, 5q31-35, and 18p11 were identified in the genome wide study as possible locations for genes associated with psychotic disorders. All three loci were identified as areas with the highest linkage in previous studies involving populations with Portuguese and Flemish ancestry. Therefore, it was possible that the 18p11 locus could

contain genes for the predisposition of schizophrenia in Hispanic populations (Escamilla, et al., 2007).

The previous study along with follow-up studies identified several regions throughout chromosomes 1, 5, 6, and 13 that showed some association with the disease (Escamilla, et al., 2007). In addition, three genes were identified as candidates for further study: neuregulin 1 on chromosome 8p11, dysbindin on chromosome 6p22, and G30/G72 on chromosome 13q34 (Craddock, et al., 2005). Transforming Growth β-Induced Factor Gene (TGIF) was one of many genes identified by a genome-wide scan targeting genes that may be associated with Schizophrenia (Chavarria-Siles, et al., 2007; Scavello, et al., 2004). A specific allele for TGIF, D18S63, was found to be in strong association with psychosis (P-value = 0.0005). D18S63 was found within an intron in TGIF. Studies identified four SNPs within the TGIF gene that were found to have strong linkage disequilibrium with D18S63 in patients with psychosis (P-value = 0.011) (Chavarria-Siles, et al., 2007). TGIF1 is located in region 11.3 of chromosome 18 (Figure 1).

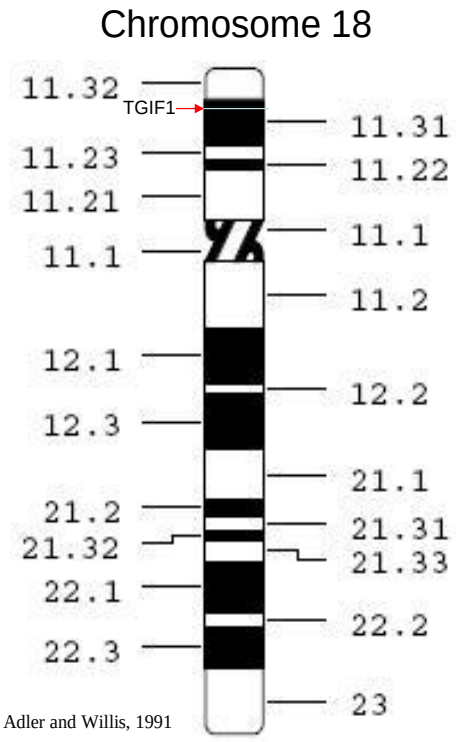

Figure 1: Diagram of Chromosome 18 with Banding Pattern and TGIF1 Labeled. Chromosome 18 is shown with its characteristic banding pattern. TGIF1 is labeled with an arrow according to its location in the 11.31 region of Chromosome 18.

TGIF background

"TGIF is a member of the three-amino-acid loop extension (TALE) superclass of homeobox proteins and acts as an inhibitory transcription factor by repressing transcription of several genes" (Chavarria-Siles, et al., 2007). TGIF is known to regulate neuronal development and survival along with controlling the expression of dopamine receptors in neurons (Chavarria-Siles, et al., 2007). Since TGIF is known to play a role in the development of neurological tissues, we chose TGIF as a candidate gene for further study involving psychosis (Chavarria-Siles, et al., 2007).

CHAPTER III

MATERIALS AND METHODS

Protocol

The procedures used were established by previous studies conducted by Dr. Walss-Bass, Dr. Chavarria-Siles, and other researchers from the University of Texas Health Science Center at San Antonio (UTHSC-SA). The subjects were chosen in accordance with guidelines from the declaration of Helsinki and follow recommendations from Institutional Review Boards from the UTHSC-SA and the University of Costa Rica (Walss-Bass, et al., 2006). Each of the individuals chosen was discharged from the hospital with a diagnosis of schizophrenia (Walss-Bass, et al., 2006). The genomic DNA came from subjects with Hispanic ancestry in Northern Mexico, Southern Texas, and prior subjects originally chosen for the CVCR study (Chavarria-Siles, et al., 2007).

All subjects who showed a history of psychosis were interviewed by a psychiatrist who used the protocol from the Diagnostic Interview for Genetics Studies (DIGS) as the basis to determine whether a person would be selected for the study (Chavarria-Siles, et al., 2007). Additionally, close relatives were interviewed using the protocol Family Interview for Genetic Studies (FIGS) to obtain more information on the subject. Lastly, all psychiatric medical records were obtained from hospitals or clinics to complete the history of the subject (Chavarria-Siles, et al., 2007).

Primers used for the exons were designed by genequest (Walss-Bass, et al., 2006). A Taqman nuclease assay was performed on 1456 samples of genomic DNA used in the study. The DNA was extracted from lymphoblastoid cells of patients who share haplotypes associated with psychosis and schizophrenia (Walss-Bass, et al., 2006). TGIF is about 46,000 base pairs in length and includes 10 exons. Five SNPs were selected that were found either in the gene (TGIF) or in the promoter region of the gene: rs4534950, rs11874742, rs9646489, rs965300, rs7234987 (Table 1; Figure 2).

Table 1: Location and Position of SNPs relative to TGIF1.

Marker	Chr 18 Pos. (bp)	Bps upstream from promoter	Sequence
rs4534950	3,393,231	-18841	ACTTAGCTTAGTGCCTAGCAA ATCTT**C/T**AAGTGCTCAACACG TATGTATTGCT
rs9646489	3,402,186	-9886	ACAGCTTTGTCACTCCTAATG GTTTT**A/G**GACATCTGAGAGG GGCCCTGTGCTA
rs965300	3,402,936	-9136	TTGGGTTTCCTTGTCTAAAAG GACTC**A/G**ATTTCAATCACTTA CTGATGAATAA
rs7234987	3,405,470	-6602	TCCTCAGGCCACCAA**C/T**GGA AGGAGCAAGTCA
rs11874742	3,397,434	-14638	CCACATACATTTTGAGGTTGT GGAGG**C/T**AGATTTTTGTGGA TAAGGAAGGAAT

The position of all 5 SNPs is recorded, along with the number of basepairs upstream from the TGIF1 promoter. A portion of the DNA sequence is shown with the SNP bolded and divided by a backspace to distinguish between allele 1 and allele 2. Information obtained from NCBI SNP database.

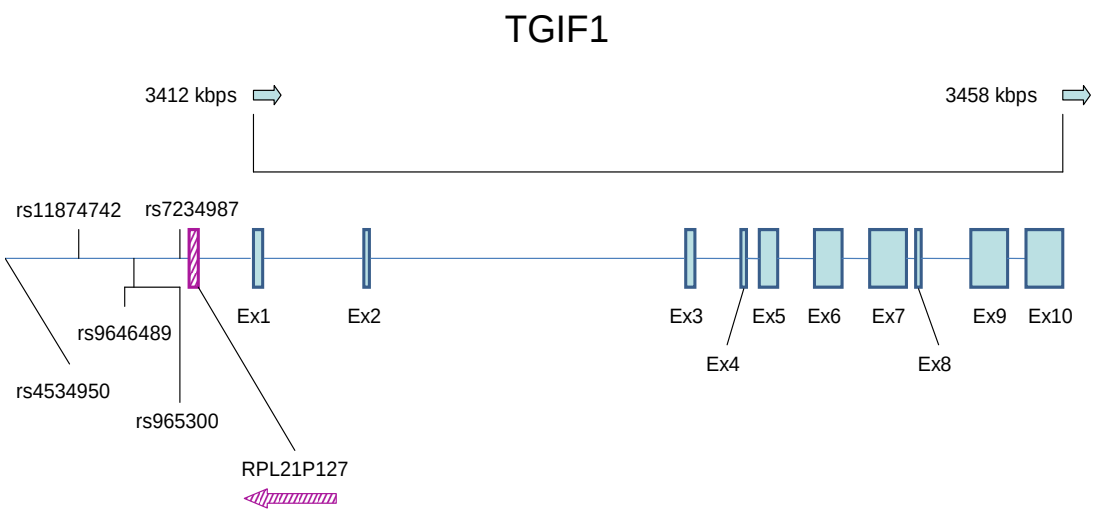

Figure 2: TGIF1 Shown with Distribution of SNPs and Pseudogene. TGIF1 is shown with all 10 exons represented as solid boxes (introns are not labeled, but are implied to be the regions in between the exons). The five SNPs tested for are labeled on the left hand side of the diagram. The pseudogene, RPL21P127, is shown as a box with diagonal lines. The SNPs, gene, and pseudogene are drawn as close as possible to their actual position in Chromosome 18. The line on top of Ex1 and EX10 is a scale showing the approximate length of TGIF1. The solid arrows show the direction of the reading frame for TGIF1, while the arrow with diagonal stripes shows the direction of the reading frame for the pseudogene.
Abbreviations – Ex: Exon; kbps: kilo basepairs.

SNP analysis

The five SNPs span a region of 12,239 base pairs. The SNPs were selected using the TAMAL database (Hemminger, et al., 2006). Once the assay was performed, an allelic discrimination was done using an ABI Prism 7900 Sequence Detection System. The allelic discrimination took the 1456 samples of DNA and compared them against pedigrees obtained for 2439 persons from 721 nuclear families. The genotypes were checked for violations of Mendelian inheritance principles using the PROGENY program. PROGENY was used to determine sibling genotypes (Walss-Bass, et al., 2006). To determine an association between a SNP and the disease, linkage disequilibrium was measured using the family based association test, or FBAT (Laird, 2009).

FBAT

The FBAT is used as the primary method to test linkage disequilibrium as well as association between genes and a phenotype. Family based association is useful for studying the difference between the expected distribution of alleles to offspring versus the actual distribution of alleles in affected offspring. The FBAT combines a variety of different statistical models into a single framework to obtain a consensus model used to test a hypothesis. Some of the models used involve tests with different disease phenotypes, tests with missing information such as parents or siblings, genetic models, sampling procedures, and different null hypotheses (Laird, 2009).

The FBAT has two steps that are required for its use in statistical analysis of genetic samples (Laird, 2009). The first step involves defining a statistic that will be used in showing the association between the trait locus and marker locus. The second step computes the distribution of genetic markers under the null hypothesis. The genotypic data from offspring is treated as random, while the rest of the data will be left unchanged or removed depending on the limitations set by the model. If there is parental data on all of the genetic markers, the null distribution can be obtained by conditional distribution of all family members or by only parental genotypic markers. If parental data is missing, conditional distribution of all family members and offspring must be used. Incomplete parental genotypes are sufficient for the FBAT to perform statistical analysis of genotypic markers (Laird, 2009). Conditional distributions avoid errors due to misspecification, admixture, or stratification of a trait. The formula used to calculate the FBAT statistic is

$$U = S - E[S], \qquad S = \sum_{ij} T_{ij} X_{ij},$$

where U is the FBAT statistic, E is the expected distribution of alleles, and S is the observed distribution of alleles. X_{ij} is the genotype at the locus being tested. T_{ij} is the coded trait which is obtained by substracting a constant from the observed genotypes (Laird, 2009).

The FBAT bi-allelic mode was the model used for the analysis of the SNPs (Walss-Bass, et al., 2006). FBAT was used to determine the degree of linkage disequilibrium between the SNPs and markers forming haplotypes that were observed to be transmitted in higher frequencies to subjects in the CVCR. The analysis of TGIF was performed using the phenotypes of psychosis and schizophrenia (Walss-Bass, et al., 2006). The FBAT provided asymptotic p-values that were used to look at the transmitted marker alleles in affected offspring. The only alleles tested using the FBAT were those alleles found in at least 10 informative families. In addition, an added marker was added that included the additive model and affection status of the trait. The settings for the exam were chosen due to the low frequency of the mutation allele and the low amount of homozygotes present (Walss-Bass, et al., 2006). Significant association of SNPs with schizophrenia or psychosis phenotypes were chosen to be a *P*-value of less than 0.02 for bi-allelic FBAT tests (Chavarria-Siles, et al., 2007).

CHAPTER IV

RESULTS

Population groups

Using information obtained from the National Center for Biotechnology Information (NCBI) SNP database, the allele frequencies obtained in this study were compared to those seen in other population groups (Table 2). The population groups used to compare samples were from Utah residents with ancestry from Northern and Western Europe (CEU), Han Chinese in Beijing (HCB), and Japanese in Tokyo, Japan (JPT) (NCBI, 2008).

Comparison of allele frequencies between population groups

When comparing allele frequencies in this study to allele frequencies seen in other populations, there were similarities and differences depending on the marker and population being used as the comparison. Two SNPs, rs965300 and rs7234987, had similar allele frequencies in the Hispanic group compared to the CEU sample. The difference in allele frequencies between both populations was 0.012 or less. Between the Hispanic group and the CEU sample, the SNPs rs4534950 and rs9646489 had differences in allele frequencies of 0.056 and .193, respectively. The marker, rs11874742, had the largest difference, 0.272, in allele frequencies between both sets of data (NCBI, 2008).

Table 2: SNP Allele Frequencies in Select Populations. Allele frequencies from 4 populations were obtained for all 5 SNPs. Allele frequencies from both alleles is shown on the righthand column. Allele frequencies on the left side of the column correspond to allele 1, while those frequencies on the right side of the column correspond to allele 2. Information for first three populations obtained from NCBI SNP database.

Population	Group	SNP	Allele Frequencies	
			Allele 1	Allele 2
HapMap-CEU	European	rs4534950	C: 0.850	T: 0.150
		rs9646489	A: 0.667	G: 0.333
		rs965300	A: 0.333	G: 0.667
		rs7234987	C: 0.980	T: 0.020
		rs11874742	C: 0.833	T: 0.167
HapMap-HCB	Asian	rs4534950	C: 0.956	T: 0.044
		rs9646489	A: 0.411	G: 0.589
		rs965300	A: 0.589	G: 0.411
		rs7234987	C: 1.000	T: 0.000
		rs11874742	C: 0.852	T: 0.148
HapMap-JPT	Asian	rs4534950	C: 0.867	T: 0.133
		rs9646489	A: 0.511	G: 0.489
		rs965300	A: 0.489	G: 0.511
		rs7234987	C: 1.000	T: 0.000
		rs11874742	C: 0.810	T: 0.190
CR, NM, ST	Hispanic	rs4534950	C: 0.906	T: 0.094
		rs9646489	A: 0.474	G: 0.526
		rs965300	A: 0.321	G: 0.679
		rs7234987	C: 0.992	T: 0.008
		rs11874742	C: 0.561	T: 0.439

Abbreviations – CEU: Utah residents with ancestry from Northern and Western Europe; HCB: Han Chinese in Beijing; JPT: Japanese in Tokyo, Japan; CR, NM, ST: Costa Rica, Northern Mexico, and Southern Texas.

Comparing the Hispanic group to the HCB sample yielded different results than those obtained in the comparison with the CEU sample. Three SNPs were found to have similar allele frequencies in both groups. Three SNPs, rs4534950, rs9646489, and rs7234987, had a difference in allele frequencies of 0.063 or less. In addition, the allele frequencies for rs7234987 were nearly identical in both populations. The SNP, rs965300, had a difference in allele frequencies of 0.268. The largest difference in allele frequencies occurred using rs11874742, which had a difference of 0.291 (NCBI, 2008).

The Hispanic group was also compared to the JPT sample. Three SNPs, rs4534950, rs9646489, and rs7234987, had a difference in allele frequencies of 0.039 or less. Just like in the HCB comparison, the allele frequencies for rs7234987 were nearly identical in both the Hispanic group and the JPT sample. The SNP rs965300 had a difference in allele frequencies of 0.168. As in the previous two comparisons, the largest difference in allele frequencies occurred in rs11874742, which had a difference of 0.249 (NCBI, 2008).

P-values obtained for selected SNPs

Once the FBAT was performed two SNPs were found to be in strong association with schizophrenia and psychosis. The first SNP, rs9646489 had a P-value of 0.003 for schizophrenia. The second SNP, rs965300 had a P-value of 0.0004 for schizophrenia. The P-values for psychosis were 0.005 for rs9646489 and 0.003 for rs965300. In addition, the marker rs11874742 had a P-value of 0.011067, thus showing a borderline association with schizophrenia, however, there was no significant association found for psychosis (Table 3, Table 4).

Table 3: Individual SNP Association Analysis for Schizophrenia. The alleles are numbered and the nucleotide corresponding to the SNP is shown in parentheses. The allele frequencies of each SNP are shown along with the corresponding P-values obtained from the FBAT. The significant P-values are marked with an asterisk.

Marker	Alleles	Allele Frequency	P-value
rs4534950	1(C) 2(T)	0.915 0.085	0.856411
rs9646489	1(A) 2(G)	0.458 0.542	0.003361*
rs965300	1(A) 2(G)	0.323 0.677	0.000458*
rs7234987	1(C) 2(T)	0.993 0.007	0.331621
rs11874742	1(C) 2(T)	0.558 0.442	0.011067*

Table 4: Individual SNP Association Analysis for Psychosis. The alleles are numbered and the nucleotide corresponding to the SNP is shown in parentheses. The allele frequencies of each SNP are shown along with the corresponding P-values obtained from the FBAT. The significant P-values are marked with an asterisk.

Marker	Alleles	Allele Frequency	P-value
rs4534950	1(C) 2(T)	0.875 0.125	0.236486
rs9646489	1(A) 2(G)	0.511 0.489	0.005573*
rs965300	1(A) 2(G)	0.302 0.698	0.003245*
rs7234987	1(C) 2(T)	0.994 0.006	0.074712
rs11874742	1(C) 2(T)	0.619 0.381	0.104893

CHAPTER V

DISCUSSION

Two previous studies had shown some association of different regions on chromosome 18 for schizophrenia and psychosis. The regions with association on chromosome 18 were found to include genes such as Lipin-2 (LPIN2), Myosin regulatory light chain 2 (MRLC2), Myomesin-1 (MYOM1), and Disks large-associated protein (DAP1) (Escamilla, et al., 2007; Chavarria-Siles, et al., 2007). Studies by Escamilla and others, demonstrated a strong association with TGIF and schizophrenia. Our study adds information on 5 SNPs spanning 12239 bps upstream of TGIF. Two SNPs, rs9646489 and rs965300, exhibited strong association with schizophrenia and psychosis. A third SNP, rs11874742, showed association with schizophrenia. The area with the strongest association appears to be located about 9000 base pairs upstream of the promoter, since both rs9646489 and rs965300 were located 9886 bp and 9136 bp, respectively, from the promoter. Both SNPs, rs9646489 and rs965300, are in linkage disequilibrium with each other. This area may have a strong association with either phenotype, making this a prime area to continue searching for other SNPs and regulatory elements that show association to schizophrenia or psychosis. It is likely, that this region contains enhancer elements that are associated with these mental disorders.

Admixture effect

Allele frequencies were similar to frequencies reported on HapMap for North Americans of European descent (CEU), Chinese (HCB), and Japanese (JPT) groups. Some variation was observed for rs11874742. This was not unexpected due to the population containing a mixture of American Indian, African, and European ancestors. CEU, HCB, and JPT groups consist of populations that are more ethnically homogenous compared to the Hispanic group. The admixture in Hispanics exhibits regional variation. For example, allele frequencies from populations in Mexico may be different from those in Costa Rica or Brazil simply because different rates of admixture will alter allele frequencies in those populations. In this study, the Hispanic group contained samples from Costa Ricans from the Central Valley of Costa Rica and people of Mexican descent from Northern Mexico and the Southwestern United States.

Existence of pseudogene

Genomic data places a pseudogene, RPL21P127, 1000 bps away from rs7234987. A pseudogene is a gene that is no longer expressed in a cell. Pseudogenes may contain splice sites and promoters, but they will not code for a protein (Lewin, 2008).The pseudogene, ribosomal protein L21 pseudogene 127 (RPL21P127), is 549 bps in length and is located about 5000 bps upstream of the TGIF gene (Entrez, 2009; Figure 2). Due to the close proximity of the SNPs to the pseudogene, it was initially thought that the SNPs may be influencing the pseudogene instead of TGIF. However, the SNP was found to be located on the minus strand, which would place it downstream of RPL21P127. Therefore, it is unlikely to be associated with the pseudogene (Entrez, 2009).

Proximity of SNPs to enhancer region

The possibility of an enhancer is strengthened by the presence of an enhancer binding site found close to rs965300 (Biobase, 2010). An enhancer is a sequence of DNA that will stimulate transcription. Enhancers are not limited to a certain position on the genome, and can stimulate transcription at different positions and orientation relative to the initiation site (Arnosti and Kulkarni, 2005). The site contains the leucine zipper transcription factor, CCAAT/enhancer binding protein alpha (C/EBPα), which has been shown to play various roles in growth and differentiation. C/EBPα acts as a growth hormone in the pituitary gland where it regulates rates of metabolism in cells by simultaneously controlling gene expression and cell proliferation. C/EBPα regulates tissue differentiation to control energy expenditures in the cell (Liu, et al., 2002).

Future studies

The markers that are being tested for should be present in a large segment of the CVCR population. In addition, the haplotypes should also give an estimate as to what the copy number effect will be on the subjects. For instance, subjects containing a haplotype with two copies of the marker may have a more pronounced psychotic effect compared to subjects containing only one copy of the marker. The polymorphism would also yield conclusive results by comparing the subjects with the polymorphism with an unrelated control group that does not exhibit the polymorphism. This would yield more evidence suggesting a link between the polymorphism and the presence of schizophrenia.

Using case-control studies the identification of other genes could be expanded. Genome wide association studies could take existing data and add samples from other

population groups to confirm the association of certain genes or to identify new genes. Samples could be run against microsatellites or other genetic markers for targeting other alleles as potential candidates for genetic analysis involving schizophrenia or other disorders. Additional studies in other population groups would serve to support the results of these studies. Given the strong association seen with rs9646489, rs965300, and rs11874742; these results should be confirmed in other population groups.

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BIOGRAPHICAL SKETCH

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