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THE IDENTIFICATION OF GENES AND BRAIN PATTERNS IN THE QUANTITATIVE  
TRAIT LOCI OF CHROMOSOME 5

A Thesis

Georgia State University

2018

by

Kimberly Diaz Perez

Committee:

Dr. Jessica Ann Turner, Thesis Advisor

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THE IDENTIFICATION OF GENES AND BRAIN PATTERNS IN THE QUANTITATIVE  
TRAIT LOCI OF CHROMOSOME 5

by

Kimberly Diaz Perez

Under the Direction of Jessica Ann Turner, PhD

ABSTRACT

In previous research, Gupta et al. (2015) analyzed gray matter density as well as volume reductions related to schizophrenia in the region of the insula and medial prefrontal cortex. Sprooten et al. (2015) then identified a set of quantitative trait loci (QTLs), which is a region of DNA associated with variability in these gray matter concentration patterns. The aim of this study is to examine the QTL they found in a region of chromosome 5. We hypothesized that there will be a set of genes in the QTL on chromosome 5 that is related to abnormal brain patterns in potential disorders such as schizophrenia. We identified genes present in the region of the QTL to analyze their function and relatedness to other genes using various software like Ingenuity Pathways Analysis, and Gene Cards. We evaluated their biological functions as well as any related disorders. For the imaging and genetic analyses, the genotypic data contained 9,228 single-nucleotide polymorphisms (SNPs) from shared aggregated datasets. The datasets contained clinical information for 616 subjects (364 controls, 252 cases). Each subject had a corresponding brain image. We identified a set of genes, including SLC1A3, GDNF, C6, C7, and C9, that are possibly related to neurodegeneration as well as brain injury processes. Lastly, we employed the parallel independent component analysis technique (pICA) to incorporate the genetic data with brain imaging to possibly identify an area related to schizophrenia. Some of the genetic variations found corresponded to the genes C7, RPL37, and PTGER4 with a correlation of 0.1012. C7, RPL37, and PTGER4 are involved in the immune system, multiple sclerosis, and neurodegenerative diseases. These genes were correlated with the imaging pattern from the pICA in the regions of the cerebellum, vermis, and mid-temporal lobe. Further analyses are needed to evaluate the correlation obtained from the pICA.

INDEX WORDS: schizophrenia, bioinformatics, genome-wide association studies

## **DEDICATION**

This project would not have been possible without the support of my family and peers. I would like to dedicate this to my parents, Jorge Diaz and Maria Perez, as well as my brothers, Bradley and Jorge.

## ACKNOWLEDGMENTS

I want to give special thanks to Dr. Jessica Turner, Dr. Nora Perrone-Bizzozero, and Wenhao Jiang for being wonderful mentors to me during my time in the Imaging Genetics and Informatics lab. These past two years have been very rewarding to me and I could not have done it without their support and guidance. I want to acknowledge all of the members of the imaging genetics lab, especially Amina Glass, and MRN. Lastly, I would like to acknowledge the IMSD program at Georgia State University, and Dr. Kyle Frantz for guiding me throughout my undergraduate career at GSU.

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## INTRODUCTION

Schizophrenia (SZ) is a mental disorder that affects behavior, emotions, and thoughts, characterized by abnormal behavior. Its cause is still being analyzed continuously in multiple studies. However, SZ possesses genetic, environmental, and brain structural elements that dependently interact to produce the SZ phenotype. Environmental factors that can induce the risk of SZ involves drug use, prenatal stressors, and the current environment of an individual (Moran, Stokes et al. 2016). Another significant component of SZ is the heritability and genotypic variations of the disorder.

There are many genes related to SZ; however, their effect and expression on SZ patients are still being analyzed. Recently, the Psychiatric Genomics Consortium-Schizophrenia Workgroup published a genome-wide association study (GWAS) containing 108 genetic loci that had a high risk for schizophrenia, many of which contain a wide variety of functions in schizophrenic patients (Schizophrenia Working Group of the Psychiatric Genomics 2014). These 108 loci were evaluated based “on a genome-wide significance” (Lencz & Malhotra 2015). Genome-wide association studies (GWAS) are employed to identify genetic changes that are related to disorders. Schizophrenia is considered to be a polygenic disorder, meaning that multiple genes are related to the condition. The heritability of SZ is estimated to be around 80%, and there are approximately 108 genes that are known to contribute to the disorder. Genotypic variations, or single-nucleotide polymorphisms, contribute greatly to the disease susceptibility and the expressivity of the phenotype. GWAS demonstrate that single-nucleotide polymorphisms for schizophrenia are compatible in a large number of regions with common alleles; however, each SNP contributes only a small portion of the risk for the disease (de Jong et al. 2012). However, the accumulation of these combined changes is enough for the disorder to develop, or



at least increase the risk of SZ. These studies have not been as successful as originally hoped, due to the lack of identification of concrete and specific variants responsible for certain phenotypes. Many of the GWAS result in various SNPs that “are in linkage disequilibrium with more than one gene” (Shahar, Eyal et al. 2017). This result contributes to the complexity of the disorder.

As a result, current studies focus on the phenotype and genotype interactions in individuals with schizophrenia. In complex disorders, there may be interactions within a set of genes, so it is very significant to have other study alternatives such as brain imaging data to study the disorder. This result contributes to the complexity of the disorder because there are many genes that may increase the risk for schizophrenia. For this reason, studies about psychiatric disorders such as schizophrenia cannot rely solely on genetic information.

There are differences in the brain structure in individuals affected with SZ compared to controls (Franke, Stein et al. 2016; Wright, Gupta et al. 2016). Brain structure is likely influenced by genetic variations. Structural brain measurements can be reproducible and are heritable (Hibar et al. 2015). Using brain structure analyses could improve the overall analysis of genetic data. One of the brain structures that we could analyze is the gray matter, which is a component of the central nervous system containing multiple types of neurons and synapses with very few myelinated axons. In this case, measuring gray matter could aid in the analysis because it is one of the major components of the brain involved in sensory perception, decision making, emotions, and control of voluntary movement. One of the methods we could use for analysis is by integrating the genetic data with the brain images to determine how the genetic background leads to the phenotype. With this in mind, many studies evaluating the combination of genetic and brain structure are being performed.

## Study of Gray Matter Concentration in Schizophrenia

Our study was done building on the study by Gupta et al. (2015). According to Gupta et al., most patients with schizophrenia have brain differences in that the brain volume, gray matter concentration, and white matter decrease substantially. Gupta et al. used *voxel-based morphometry* was used to identify the gray matter concentration or gray matter volume in the brain. This technique is used to compare the morphological differences between schizophrenia and healthy patients. In this case, gray matter concentration was studied, rather than gray matter volume, because the results prove to be more consistent. This study was one of the largest studies of structural imaging in schizophrenia at the time, comprising 1720 subjects (936 controls and 784 subjects with schizophrenia) from 23 different sites.

Gupta et al. used source-based morphometry (SBM). SBM was used as an alternative to evaluate gray matter concentration on a voxel-wise base, based on independent components, or *sources*. The SBM analysis had gray matter concentration changes grouped into thirty independent spatial components, whereas the voxel-based morphometry analysis identified one large cluster of gray matter concentration loss on the whole brain cortex. The components identified in SBM are sections of gray matter concentration differences that are similar in their covariance across subjects.

Based on the SBM results, seven components reported less gray matter concentration and two components with increased gray matter concentration in patients with schizophrenia. The largest gray matter concentration difference between the two groups was found in the superior temporal gyrus, frontal gyrus, and the insula, which is consistent with other previous studies. Also, it was found that the areas with gray matter loss formed networks of anterior temporal, insular, and medial prefrontal regions, which could serve as phenotypes related to schizophrenia.

## **Study of Gray Matter Density in the Insula and Medial Prefrontal Cortex**

In a study by Sprooten et al. (2015), researchers analyzed gray matter density as well as volume reductions, which were identified by Gupta et al., in the region of the insula and medial prefrontal cortex (mPFC). Sprooten et al. aimed to identify the genetic factors contributing to the loss of gray matter concentration in cases with the disorder. The study highlighted the mPFC and insula as one of the most vital gray matter regions in affected individuals with SZ. Sprooten et al.'s study replicated the findings of Gupta et al., regarding the cases/control differences in the brain patterns.

Sprooten et al. used source-based morphometry (SBM) to derive a gray matter component in the insula-mPFC to analyze the genetic implications. The imaging data included 887 randomly associated T<sub>1</sub>-weighted scans from Mexican-American ancestry with extensive family history, allowing a linkage study of the brain patterns they found. First, they verified that both brain regions were relevant in a case-control sample independent from other studies as a representative sample of the general population. The investigation revealed significant differences in the morphology of the brain in the insula-mPFC component.

The SBM component functioned as a phenotype used in the QTL analysis. A QTL is an analysis of a DNA region that is related to a phenotype variation. The phenotype of the QTL analysis was the gray matter concentration patterns in a set of individuals evaluated. The study showed that the gray matter in the insula and medial prefrontal cortex had high linkage peaks at 12q24 in chromosome 12. The QTL in 12q24 was thought to be heavily involved with gray matter loss related to schizophrenia and other affective disorders. The identified QTL in 12q24 is 10Mb long and contains a region heavily linked to SZ between 113-128 Mb, which included 392 SNPs. However, none of those SNPs were peak-wide significantly related to the phenotype.

Previous studies have not been able to identify any specific variants that may be responsible for the linkage results. Besides the QTL in 12q24, Sprooten et al. also identified other QTLs likely to be related to gray matter development patterns, which may be linked to mental disorders. In summary, this study reiterated that there is gray matter concentration loss in the regions of the insula and mPFC in individuals with schizophrenia. It also identified the QTL in 12q24 linked to this phenotype, as well as other QTLs of smaller effect. The QTL in 12q24 has been extensively studied in unrelated cases and controls; however, it did not show a significant result.

In the current study, we are combining the approaches from Gupta et al. and Sprooten et al. to analyze a QTL that was identified by Sprooten et al. in chromosome 5. The QTL in chromosome 5 contained a relatively high logarithm of the odds (LOD) score, which measures the heritability between genes located near each other. This specific QTL has not been analyzed before by other researchers. The QTL was related to another imaging component, which was the insula-anterior cingulate cortex, which is also thought to be significantly affected and heritable in the brains of individuals with schizophrenia (Penner, Ford et al. 2016).

## **Purpose**

In this study, we evaluate a QTL that could potentially be related to abnormal gray matter concentration patterns in the brain. First, we will identify the genes associated with the QTL and evaluate different combinations of those genes that could dependently affect gray matter. Then, parallel independent component analysis (pICA) will be used to correlate the genotypic data, such as risk-related single-nucleotide polymorphisms, with phenotypic components (Pearlson, G. D., Liu, J., & Calhoun, V. D 2015). We hypothesize that there will be a set of genes in the identified QTL on chromosome 5 by Sprooten et al. that could be related to abnormal brain patterns in potential disorders such as Schizophrenia.

## METHOD

### QTL Selection

From the QTLs identified by Sprooten et al., we selected a QTL with a high logarithm of the odds (LOD) or genetic linkage that has not been analyzed previously by other studies. The LOD is a statistic associating two genes located near each other and their possible inheritance. The genetic linkage is correlated with high predisposition towards mental disorders. The chosen QTL is in the location 32,116,508 BP to 41,273,728 BP in chromosome 5, as shown in Figure 1. The other peaks in the figure correspond to other QTLs from other components in other brain regions such as the insula-medial prefrontal cortex.

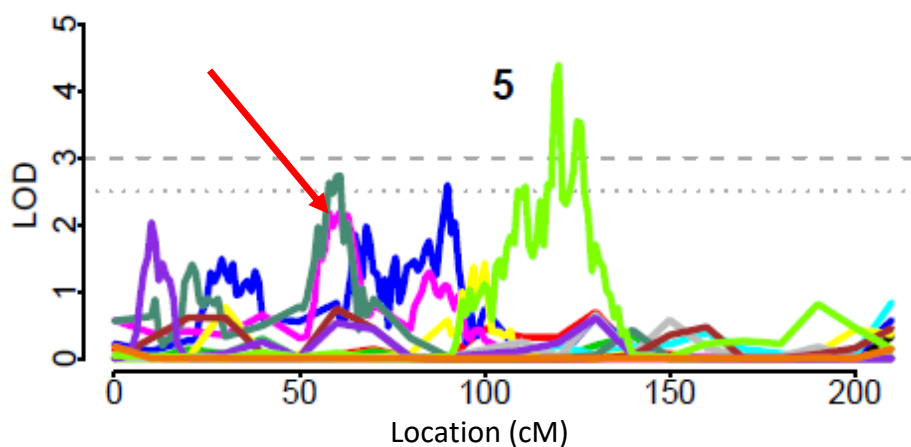


Figure 1. This figure depicts the QTL identified in Sprooten et al. (2015). The QTL we are analyzing corresponds to the first pink peak highlighted with the red arrow. The LOD scores were plotted against the location in chromosome 5 measured in centimorgan.

### Gene Annotation

We used the University of California Santa Cruz Genome Browser (Kent, Sugnet et al. 2002) to identify the genes related to brain atrophy and brain processes. We identified 63 genes within the range of the QTL that were expressed mainly in the brain. We used multiple gene ontology packages to identify the biological functions of these genes as well as their involvement in mental disorders, including Gene Ontology Consortium, Gene Cards, DAVID, and Ingenuity

Pathway Analysis (IPA). The networks were generated through the use of IPA (QIAGEN Inc., <https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis>). Gene Cards (<http://www.genecards.org/>) is a website designed to provide information about specific genes including their location, expression in the body, and their annotations in previous scientific literature. Gene Ontology is a website that provides specific information about the biological pathways of each gene (GO Consortium 2017).

### **Genetic and Imaging Data of Subjects**

The genotypic data contains 9,228 single-nucleotide polymorphisms (SNPs) from shared aggregated datasets including COBRE (Aine, Bockholt et al. 2017) , HUBIN (Nesvag, Lawyer et al. 2008), FBIRN 2 (Segall, Turner et al. 2009), MCIC (Gollub, Shoemaker et al. 2013), Olin (Jamadar, Powers et al. 2013), TOP (Rimol, Hartberg et al. 2010), and NW (Wang, Kogan et al. 2013). The datasets had clinical information for 616 Caucasian subjects (364 controls and 252 cases). The subjects ranged from 13 years to 64 years old. Each subject had a corresponding brain image obtained from a 3 Tesla Magnetic Resonance Imaging scanner.

### **Parallel Independent Component Analysis**

Parallel Independent Component Analysis is a technique used to incorporate the genotypic data, which are the known SNPs, with brain images to possibly identify an area related to schizophrenia (Liu, Demirci et al. 2008). This technique yields clusters of SNPs that are correlated statistically with phenotype images or components. It is a multivariate approach that ideally is used to identify complex genetic factors that are often buried in a large dataset. It is an approach that identifies various components that function independently of one another. However, it is a joint analysis that combines imaging and genetic data to find potential links between the two sets of data (Liu, Demirci et al. 2008; Pearlson, Liu et al. 2015). It results in the

identification of independent spatial patterns and the identification of potential links between the two variables. Using this joint analysis helps evaluate the cross-correlation within each data-type. Using MATLAB (Mathworks, 2011) and the Fusion ICA Toolbox (FIT: [www.mialab.org/software/fit](http://www.mialab.org/software/fit)), we identified ten independent imaging components and five independent genetic components to be analyzed in the pICA. The aim of the pICA is to identify related patterns between the imaging and genetic information of the subjects in the sample. We used the imaging components with a gray matter mask to limit the noise around the brain.

In this study, the genotypic data came from the shared aggregated datasets. The participants will be only cases and controls. The results of the pICA will need further validation to ensure that the results can be replicated and consistent with the original. The technique used is a 10-fold validation, which is ten trials of pICA with a different subset of the population each. As the final step, we performed a permutation and non-parametric test to validate the correlation and the results obtained from the pICA.



## RESULTS

### Gene Identification Results

There were 63 genes located in the QTL region of 32,116,508 BP to 41,273,728 BP in chromosome 5. The 63 genes are expressed in the brain and neuronal cell types (See Table 1).

Each gene was analyzed to identify the location of their expression in the body.

**Table 1. Identified Genes in QTL of Chromosome 5**

Genes in QTL Region of Chromosome 5					
ADAMTS12	CARD6	GUSBP2	NPR3	RANBP3L	TARS
AGXT2	DAB2	IL7R	NUP155	RICTOR	TTC23L
AMACR	DNAJC21	LIFR	OSMR-AS1	RP11-152K4.2	TTC33
BRIX1	DROSHA	LIFR-AS1	OSMR	RP11-113I22.1	UGT3A1
C1QTNF3	EGFLAM	LINC00603	PDZD2	RP11-122C5.1	UGT3A2
CDH6	EGFLAM-AS2	LINC01265	PLCXD3	RPL37	WDR70
C5orf42	EGFLAM-AS4	LMBRD2	PRKAA1	RXFP3	FYB
C6	FYB	MROH2B	PRLR	SKP2	ZFR
C7	GDNF	MTMR12	PTGER4	SLC1A3	
C9	GDNF-AS1	NADK2	RAD1	SLC45A2	
CAPSL	GOLPH3	NIPBL	RAI14	SUB1	

Table 1. Using the UCSC Genome Browser, we identified 63 genes present in the 9 Mb region of chromosome 5.

### Gene Annotation Results

A list of the genes was transferred into the DAVID, Gene Ontology Consortium, and Ingenuity Pathway Analysis. Based on the Gene Ontology Consortium software, there are many pathways associated with the initial gene list (See Table 2). The genes ADAMTS12, PTGER4, DROSHA, RICTOR, OSMR, C7, C6, C9, C1QTNF3, LIFR, and OSMR, are associated with

regulation of inflammatory response, regulation of defense response, and regulation of response to external stimuli. C6, C7, and C9 work co-dependently on the complement system activation. A recent study indicates that a member of the complement family (C4A) is expressed in neurons, where it is involved in synaptic pruning, and significantly increased in schizophrenia (Sekar et al. 2016). The three genes are also involved in prion diseases, especially neurodegenerative diseases. IL7R and PTGER4 are associated with T cell differentiation. GDNF, along with SLC1A3 and DROSHA, were a few of the genes involved in neuronal development and brain injury processes.

**Table 2. Gene Involvement in Biological Process**

Genes	Regulation of Inflammatory Response	Regulation of Defense Response	Multiple Sclerosis	Brain Development	Mitotic Cell Cycle	Complement Activation
ADAMTS12	X	X				
C1QTNF3	X	X				
C6	X	X	X			X
C7	X	X	X			X
C9	X	X				X
CARD6			X			
DROSHA	X	X				
IL7R			X			
MROH2B			X			
NIPBL				X	X	
NUP155					X	
OSMR	X	X				
PTGER4	X	X	X			
RAD1				X		
RANBP3L					X	
RICTOR	X	X				
RPL37			X			
SPK2					X	

Table 2. Using IPA and Gene Ontology Consortium Pathway, we analyzed the pathways of various genes in our initial gene list.

Furthermore, we analyzed the gene involvement in the brain as well as the neuronal network using IPA and the Gene Ontology Consortium (See Table 3). The genes C7, C9, LIFR,

SLC1A3, SUB1, and GDNF are seen to be involved in Huntington's disease, caused by the breakdown of nerve cells in the brain. C6 is involved in the degradation of axons in the neuronal network as well as the breakdown of the myelin sheath, or white matter. Also, SLC1A3, GDNF, and GOLPH3 work dependently to regulate neurodegeneration and syndromic encephalopathy, which is brain injury. From these genes, SLC1A3 is directly involved in the cerebellum injury process.

**Table 3. Gene Involvement in the Brain and Neuronal Network**

Genes	HD	Cerebellum Injury	Degradation of Axons	Neurodegeneration	Syndromic Encephalopathy	Breakdown of Myelin Sheath
C6			X			X
C7	X					
C9	X					
LIFR	X					
SLC1A3	X	X		X	X	
SUB1	X					
GDNF	X			X	X	
GOLPH3				X	X	

Table 3. We used the Gene Ontology Consortium and IPA to evaluate the involvement of the genes in the brain and the neuronal network. Some genes were involved in the same disorders or pathways.

HD = Huntington's Disease

The network was generated through the use of IPA (QIAGEN Inc., <https://www.qiagenbio-informatics.com/products/ingenuity-pathway-analysis>) (See Figure 2). There were eight major pathways that involved two or more genes. The eight pathways were tRNA charging involving TAR and TARS2; Protein Ubiquitination Pathway with the genes DNAJC21 and SKP2; Acute Phase Response Signaling with the genes C9 and OSMR; the Complement System involving C7, C9, and C6; Systemic Lupus Erythematosus signaling with the genes C9, C7, and C8; Neuroinflammation signaling pathway with the genes SLC1A3 and GDNF; G-Protein Coupled Receptor Signaling involving the genes PTGER4 and NPR3; and

cAMP-mediated signaling with the genes PTGER4 and NPR3. C6 and C7 are also involved in deficiencies of C6 pathways and Lymphoblastic leukemia. DAB2, C6, C7, IL7R, MROH2B, and PTGER4 are involved in multiple sclerosis.

**Figure 2. Network of All Genes and their Relatedness**

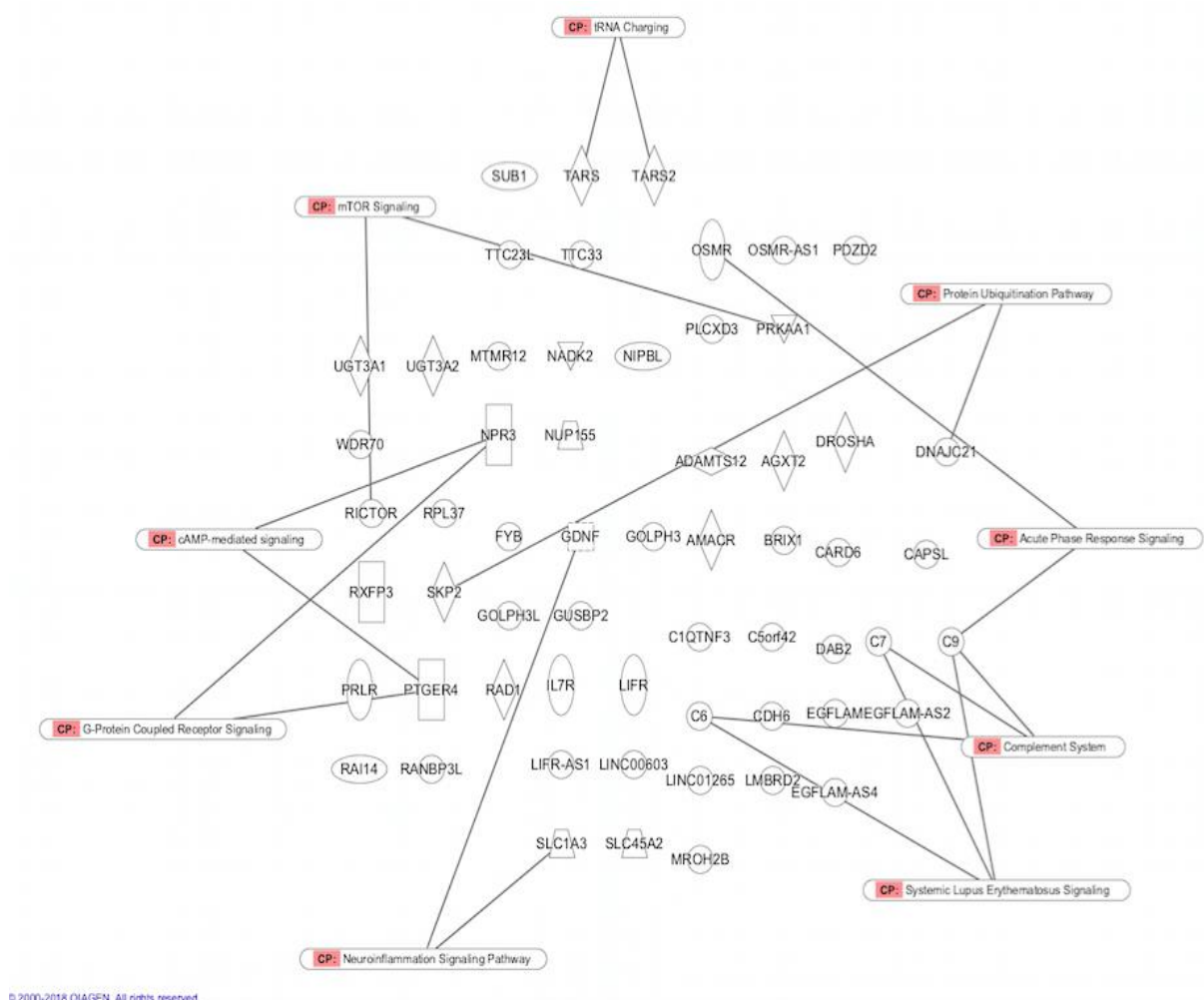


Figure 2. The 63 genes were analyzed on IPA to identify any potential involvement of two or more genes in specific biological pathways or disorders. The connections, or lines, between genes implies a relationship between the genes in different pathways.

### Parallel Independent Component Analysis Results

Based on the pICA results, there was a positive correlation (0.1012) for the pairing of the imaging component #1 and the genetic component #4 (See Figure 3). The imaging component of

the pairing was then further analyzed using MRICron (<https://www.nitrc.org/projects/mricron>). This software allowed us to analyze the brain regions in a three-dimensional view to precisely identify the regions that were expressed on the subjects. The regions that had a prominent z-score (z-score > 2) were regions of the cerebellum, vermis, mid-temporal lobe, anterior cingulum, and inferior parietal lobule. The permutation test resulted in a ratio of correlation values of 0.713.

**Figure 3. Imaging Component Obtained from pICA**

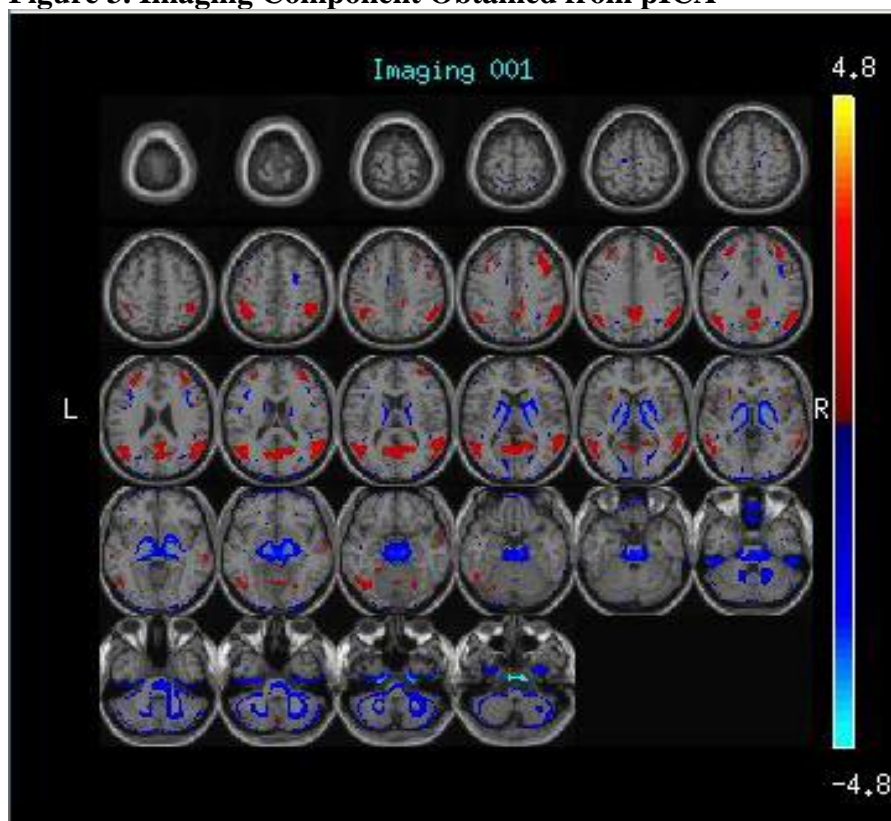


Figure 3. This figure illustrates the most significant imaging component obtained from the pICA. The red regions corresponded to a positive z-score > 2, while the blue regions corresponded to z-scores < -2.

Once we identified the regions of the brain that were expressed in the cases and controls, we analyzed the genetic component to identify any variants that had a z-score higher than 2 (See Figure 4). The region of the QTL with a z-score > 2 corresponded to the position 7,399 to 9,109 on our SNP list. The SNP list contained 597 SNPs with the z-score > 2. Some of the genetic variations that we obtained in our results were rs13167951 and rs1833864, which corresponded

to the gene C6. All the SNPs corresponding to C6 had an approximate z-score of 2. Also, another set of SNPs were identified such as rs10941527 and rs4277953, which belonged to the gene C7. The gene C7 had the largest number of SNPs on the list. There were 25 SNPs that belonged to the gene MROH2B. Another group of SNPs corresponded to the gene PTGER4, which were rs10060234 and rs13186505. Furthermore, there was a set of genotypes corresponding to the gene PRKAA1. Another set of SNPs corresponded to the gene RPL37 and CARD6, which were rs192219 and rs837388 respectively. Lastly, the SNPs rs1644962 and rs249414 corresponded to the gene TTC33. The genes mentioned previously were initially on Table 1 as they are expressed in the brain.

There were also SNPs that did not possess a z-score  $> 2$  on the results obtained from the pICA. An example of these SNPs was rs1389831, which belonged to the gene IL7R. Even though their z-score is not above the significant level, these SNPs may work dependently with another gene, perhaps with a z-score  $> 2$ , to produce a specific phenotype. On the other hand, a large set of SNPs with a z-score  $> 2$  were uncharacterized genes such as LOC105374739 and LOC100506548. Based on the GeneCards website, these uncharacterized loci encode non-coding RNAs that do not have any functional annotation available yet. Even though these genes are unannotated, it is still important to note that a few of these SNPs had a z-score of 4.00 or above. A few examples of these SNPs were rs10055624, rs6879155, rs10941521, and many more (See Table 4 in Appendix). There were also SNPs that did not have any corresponding genes due to the lack of annotation for those SNPs. Some of the unannotated SNPs had a high z-score.

**Figure 4. Genetic Component Result from pICA**

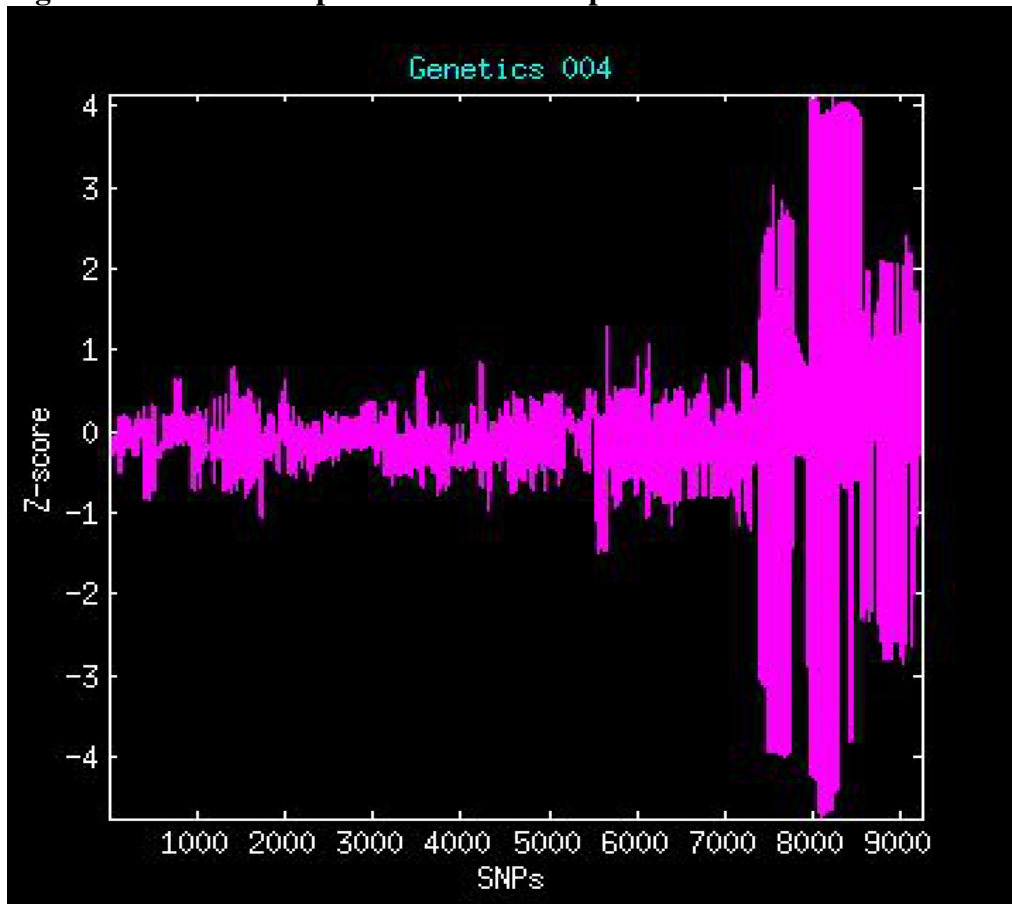


Figure 4. The image above demonstrates the genetic component #4 which had the highest correlation of all pairs. The figure depicts the SNP position or index in our initial SNP list with their corresponding z-score.

Figure 5 illustrates a scatterplot combining the loading coefficients from the imaging and genetic modalities as well as the cases and controls of our population. The imaging loading coefficients are located on the x-axis while the genetic loading coefficients are on the y-axis. The dotted red line corresponds to the cases trend line and the dotted blue line corresponds to the controls trend line. There is a very small and non-significant difference between the correlations in the two subject groups.

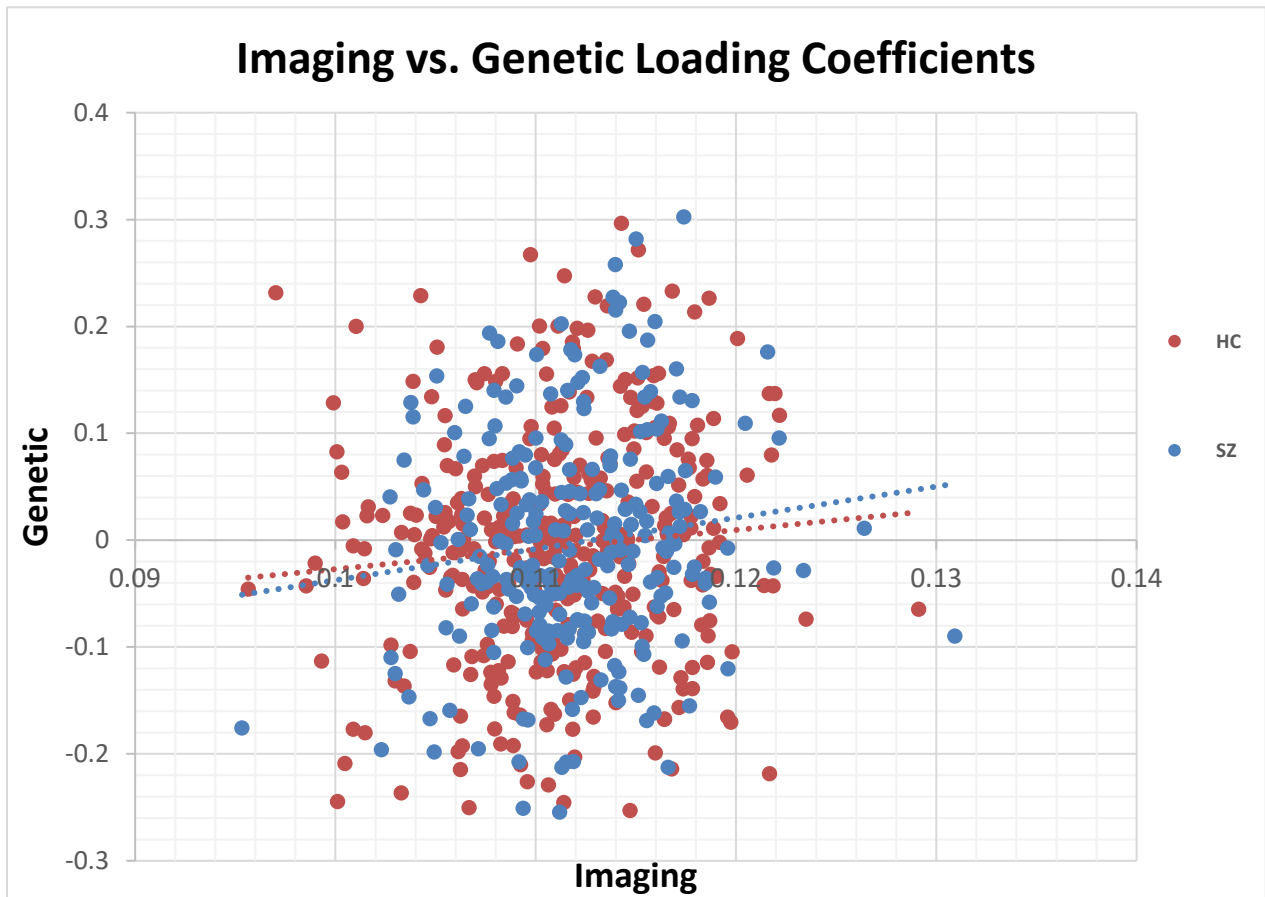


Figure 5. This scatterplot graph illustrates the genetic and imaging loading coefficients for the cases (blue) and controls (red). The imaging loading coefficients are on the x-axis and the genetic loading coefficients are located on the y-axis.



## DISCUSSION

The aim of our study was to identify the brain patterns and genes in chromosome 5 responsible for those brain patterns in a subset of affected schizophrenia patients and unaffected control individuals. The region of study in the genome was established based on the previous QTL study (Sprooten et al. 2015), which identified a set of quantitative trait loci related to abnormal gray matter concentration patterns and volume reductions in the brain. We analyzed a region of chromosome 5, which corresponded to the chosen QTL. The region contained approximately 9 Mb comprising of 9,228 SNPs for 616 cases and control individuals. First, we followed a gene ontology regimen that included evaluating 63 genes that were present in the QTL region. As the second step, we employed the parallel independent component analysis to find the correlation between the genetic and imaging components from the QTL. Lastly, we incorporated the information gathered from the first two steps to evaluate the roles of the genes and SNPs in the imaging component.

The gene ontology analysis consisted of the use of gene ontology programs such as Gene Cards, IPA and the Gene Ontology Consortium. The Gene Cards website helped us evaluate the specific function, chromosome location, and tissue-specific gene expression patterns of the initial gene list. Once we had the information about all 63 genes, the genes were separated based on their expression in the brain, specifically the cerebellum and brain cortex. The genes with the gene expression in the brain were further analyzed. It is worthy to note that the other genes that were not expressed in the brain were not removed completely from our study. The genes were still analyzed; however, they are not very emphasized in our study. There were 30 genes that are expressed in the brain in various expression levels included from our initial gene list. Furthermore, the Gene Ontology Consortium provided information about the biological

pathways of the 63 genes. It also provided information about the interconnectedness between a combination of genes. For instance, there were six genes involved in multiple sclerosis, including DAB2, C6, C7, IL7R, MROH2B, and PTGER4. Also, GDNF, NPR3, PRLR, and SLC1A3 are somehow related to several psychiatric disorders such as schizophrenia. ADAMTS12, PTGER4, DROSHA, RICTOR, OSMR, C6, C7, C9, and C1QTNF3 are involved in the regulation of inflammatory responses in the body and the regulation of response to external stimuli. GDNF and SLC1A3 work dependently to active neuron differentiation pathways. These results contributed to the overall analyses of the gene functions, the pathways they are involved in, and the interactions between a combination of genes.

Furthermore, IPA provided more information about the specific interactions that are involved in the different biological pathways in the brain. Based on IPA, SUB1, GDNF, C7, C9, LIFR, and SLC1A3 work dependently in Huntington's disease. Also, GDNF, C6, and SLC1A3 were highlighted once again in the neurodegeneration process. C6 was also seen to be involved in the breakdown of white matter (myelin sheath) and the degradation of axons. IPA helped us narrow down the genes to a smaller gene list to establish our hypothesis. The processes previously mentioned have a specific interaction with the brain, and so, our hypothesis stated that one, or multiple, genes were involved in the brain patterns of our sample population after the pICA was done.

Once the gene ontology process was completed, we proceeded to evaluate the genetic and imaging information of our sample population of 616 subjects. The pICA technique is intended to correlate the genetic and imaging components to find the most correlated pair of components. Based on the pICA results, the two components that had the highest correlation of 0.1012 were genetic component #4 and imaging component #1. To confirm the validity of the pICA results,

we performed a ten-fold validation test, in which a subset of the population was eliminated in 10 different pICA. The ten different results of the ten-fold validation converged as expected, and the original results were consistent with the outcome of the 10-fold validation. After the 10-fold validation, we performed the permutation test to evaluate the consistency of the correlation of the original pICA. The permutation test resulted in a ratio of correlation values of 0.713. In other words, there is a 29% possibility of obtaining a correlation as large as what we identified by chance. Ideally, the permutation test would result in a ratio of correlations of 0.05. However, our result was 0.29, so the pICA would need to be replicated in the future.

Furthermore, the imaging component consisted of two regions based on the positive or negative z-scores (See Figure 3). The areas in blue corresponded to the negative z-scores while the areas in red or orange corresponded to the positive z-scores. We obtained the absolute value of all z-scores to ensure the regions we are interested in, which are the z-scores  $> 2$ , are highlighted in our overall results. The regions of the cerebellum, vermis, mid-temporal lobe, anterior cingulum, and inferior parietal lobule, were considered to be significant in our results due to their high z-score. The region correlated to the cerebellum is particularly significant because there are genes, such as SLC1A3, in our gene list related to injury to the cerebellum and syndromic encephalopathy.

As for the genetic component, we analyzed the SNPs that had a z-score  $> 2$  and identified the SNPs that had their corresponding gene annotated using NCBI dbSNP database (<https://www.ncbi.nlm.nih.gov/SNP/>). There were 597 SNPs out of the 9,228 initial SNP list with a z-score of 2 or above. From the 597 SNPs, 269 SNPs have not been annotated yet on any gene ontology software. It is worth noting that the z-scores for these SNPs vary from 2 to 4, with a large subset of these SNPs being variants with z-score of 4. Having a high z-score implicates

the expression, or involvement, of these SNPs in the brain patterns obtained in the pICA. Similarly, there are 92 SNPs named with the prefix “LOC”, which are SNPs that have not been annotated yet as well. Some of the SNPs names were LOC105374737 and LOC105374736. These SNPs are in non-coding RNA genes with unknown gene ontology information. The corresponding number after “LOC” corresponds to the gene ID that is used to name SNPs that do not have annotations available. Similar to the SNPs that are not annotated, the z-scores for these SNPs vary greatly. Further studies should be done to determine the function of these SNPs that do not contain any gene information.

Furthermore, there are SNPs with a z-score  $> 2$  that had gene annotation information. For instance, 84 SNPs belong to the gene C7, which is part of the complement system and Huntington’s disease. Most of these SNPs contained a high z-score compared to the other SNPs, which can implicate their substantial involvement in the pICA results. Another subset of the SNPs is in the region of the gene PTGER4, which accounts for 29 SNPs of our list. There are 37 SNPs in the region of C6, which is also part of the complement system. However, the z-score of these SNPs is in the lower 2’s. Similar to the C7 SNPs, most of the SNPs from PTGER4 have a z-score of 4. There are 28 SNPs belonging to the gene TTC33, which have varied z-scores with the majority being z-score values of 4. Also, there are 7 SNPs that are annotated to the gene CARD6, which have a relatively low z-score, and 3 SNPs in the region of the gene RPL37 with a high z-score. Out of the 597 SNPs, 236 SNPs had gene annotation and gene ontology information.

From this information, we analyzed the interactions of these genes and determined the possible biological processes that they are involved in. PTGER4, CARD6, and RPL37 work dependently on the phenotype of multiple sclerosis. Notably, the SNPs rs192219, rs62357601,

and rs13355305 were some of the variations with a high z-score value corresponding to the genes previously mentioned. Another set of genes that proved to be expressed in the imaging component were the C6 and C7 genes. These genes are involved in the complement system and the regulation of inflammatory and defense responses. Their functions related to the immune system suggests that their role in our population sample is highly significant. Also, there is an association of these genes with immune disorders as well as psychiatric disorders such as schizophrenia.

Interestingly, LOC105374737, which is an identified non-coding RNA gene, is heavily involved in schizophrenia and bipolar disorder, as well as the complement system and multiple sclerosis (<http://www.genecards.org/cgi-bin/carddisp.pl?gene=LOC105374737>). There were eight SNPs with a significant z-score that corresponded to the gene LOC105374737.

Additionally, the gene RPL37 is a member of the family of ribosomal proteins and is related to another ribosomal protein gene called RPL10, which is related to microcephaly and abnormal brain morphology (<http://www.genecards.org/cgi-bin/carddisp.pl?gene=RPL10>). Overall, these genes could potentially be working dependently to influence the gray matter patterns seen in the pICA.

Some of the limitations of this study include the lack of consistency of the correlation results from the pICA. The permutation test resulted in a high false alarm rate; and so, if the study is going to be replicated, there is a high probability that the 0.1012 correlation would not be obtained again. Furthermore, a study including parallel independent component analysis with reference needs to be done to establish the relationship between one or a combination of genes and the phenotype (Chen, Calhoun et al. 2013). Using a genetic constraint analysis will help us understand the role of a specific gene in the imaging component of the cases and controls. It will

increase the information about the role of the genes in our list as well as their impact on the affected individuals. Focusing on these two limitations in the future will provide more consistent results to accurately determine the correlation between the imaging and genetic component. It will also aid in evaluating the role of the genes in affected and healthy individuals as well as the differences in the phenotype of both sets of patients.

The aim of this study was accomplished because we identified a set of genes in the QTL region of chromosome 5 and we evaluated the role of these genes in the imaging component of affected and unaffected individuals. We also evaluated the imaging and genetic relationship of the cases and controls. We identified the role of significant genes expressed in the brain such as PTGER4, CARD6, LOC105374737, and C7. Most importantly, our results suggest a role of these genes in the gray matter concentration in the brain of schizophrenia patients, especially in the regions of the cerebellum, vermis, and anterior cingulum. Further studies need to be done to replicate the results and ensure the consistency of the results is maintained.

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## Appendix

**Table 4. Information about Significant SNPs with Z-Score Value > 2**

Position on SNP list (Indices)	Gene	SNP ID	Z-score
7399	Not Annotated	rs13186205	3.0398681
7401	Not Annotated	rs12655997	3.0398681
7402	Not Annotated	rs11744707	2.1762921
7403	Not Annotated	rs12518811	2.1762921
7409	Not Annotated	rs13165828	2.1787496
7418	Not Annotated	rs4957126	3.0685895
7420	Not Annotated	rs4472299	2.1787496
7422	Not Annotated	rs2218465	3.0685895
7424	Not Annotated	rs10043778	2.1787496
7431	Not Annotated	rs6859310	3.0960087
7445	LOC105374736	rs1444999	2.0061693
7446	LOC105374736	rs1445000	2.0061693
7447	LOC105374736	rs10462010	3.1218465
7454	LOC105374736	rs4957127	3.1218465
7456	LOC105374736	rs7733749	2.197945
7459	LOC105374736	rs348600	2.3640292
7461	LOC105374736	rs371958	2.1318409
7463	LOC105374736	rs348599	2.3640292
7465	LOC105374736	rs348597	2.373476
7466	LOC105374736	rs1842074	2.4557597
7467	LOC105374736	rs348596	2.4073236
7468	LOC105374736	rs12697405	3.8059052
7469	LOC105374736	rs348595	2.4557597
7473	LOC105374736	rs12173214	2.4977164
7474	LOC105374736	rs2034185	2.4977164
7476	LOC105374736	rs4957129	2.4977164
7477	LOC105374736	rs36120539	2.4977164
7480	LOC105374736	rs379109	2.5051159
7484	LOC105374736	rs433817	2.4977164
7485	LOC105374736	rs422083	2.4977164
7486	LOC105374736	rs10045016	3.9396459
7491	Not Annotated	rs348605	2.4937597
7492	Not Annotated	rs348606	2.4937597
7495	Not Annotated	rs443583	2.4937597

7496	Not Annotated	rs436695	2.4937597
7497	Not Annotated	rs394213	2.4937597
7499	Not Annotated	rs6897022	2.3243944
7500	Not Annotated	rs2371683	2.4656764
7501	Not Annotated	rs2888122	2.4937597
7506	Not Annotated	rs116716000	2.4937597
7507	Not Annotated	rs114330951	2.4937597
7510	Not Annotated	rs141169420	2.4937597
7513	Not Annotated	rs11746990	2.4937597
7514	Not Annotated	rs11741089	2.4937597
7516	Not Annotated	rs7726744	2.4937597
7517	Not Annotated	rs7707395	3.9329269
7533	Not Annotated	rs12697408	3.9229737
7540	Not Annotated	rs11742533	2.4994258
7542	Not Annotated	rs62359777	2.4994258
7545	Not Annotated	rs7712308	3.9280085
7549	Not Annotated	rs56783346	2.4994258
7550	Not Annotated	rs10044083	2.4994258
7556	Not Annotated	rs2084030	3.0094993
7558	Not Annotated	rs2100001	3.0004762
7590	Not Annotated	rs10473183	3.9544965
7597	Not Annotated	rs7730693	2.480197
7615	Not Annotated	rs7445978	3.9544965
7617	Not Annotated	rs2120855	3.9544965
7620	Not Annotated	rs10473187	2.5828716
7626	Not Annotated	rs12656644	3.9689286
7632	Not Annotated	rs6865484	2.614189
7633	Not Annotated	rs12658567	3.9063868
7650	Not Annotated	rs6880778	2.6086875
7651	Not Annotated	rs10941505	3.9593532
7654	Not Annotated	rs6891952	3.9593532
7658	Not Annotated	rs6883686	2.837838
7660	Not Annotated	rs10473189	3.9678456
7665	Not Annotated	rs6879428	3.989764
7667	Not Annotated	rs6890268	2.6296467
7668	Not Annotated	rs6871591	2.6365331
7669	Not Annotated	rs6891041	3.9678456
7673	Not Annotated	rs10072596	3.9678456
7680	Not Annotated	rs6883302	3.9651164

7681	Not Annotated	rs6883964	2.6365331
7683	Not Annotated	rs12655810	2.6365331
7684	Not Annotated	rs12655827	3.9651164
7686	Not Annotated	rs6888952	2.6365331
7688	Not Annotated	rs6889125	3.9651164
7689	Not Annotated	rs10473191	2.6365331
7691	Not Annotated	rs1445004	2.6365331
7693	Not Annotated	rs7711427	2.6365331
7697	Not Annotated	rs2371720	2.6567058
7698	Not Annotated	rs6873829	2.6916553
7702	Not Annotated	rs6874571	2.711548
7703	Not Annotated	rs114400949	2.6567058
7704	Not Annotated	rs149200362	2.6573012
7706	Not Annotated	rs10473192	2.6567058
7708	Not Annotated	rs7705708	3.9728224
7709	Not Annotated	rs7705462	2.6567058
7711	Not Annotated	rs13189778	3.9700381
7717	Not Annotated	rs7716887	2.6037206
7718	Not Annotated	rs7730306	3.9198372
7719	Not Annotated	rs7730591	2.6037206
7724	Not Annotated	rs1899983	2.6037206
7725	Not Annotated	rs1899982	3.9276118
7726	Not Annotated	rs6879489	2.6037206
7730	Not Annotated	rs6451498	2.6037206
7735	Not Annotated	rs6874500	3.9276118
7737	Not Annotated	rs10042027	3.9400786
7738	Not Annotated	rs6880419	3.9400786
7740	Not Annotated	rs63492863	3.9285697
7743	Not Annotated	rs1445001	2.5761852
7745	Not Annotated	rs6861824	2.5761852
7748	Not Annotated	rs6878213	2.5812838
7750	Not Annotated	rs9292771	2.5937506
7752	Not Annotated	rs4526151	3.9176418
7753	Not Annotated	rs4632848	3.9176007
7754	Not Annotated	rs9292776	3.9176418
7758	Not Annotated	rs10473195	2.5937506
7928	LOC105374737	rs13156302	2.8889842
7954	LOC105374737	rs11745901	2.5125359
7955	LOC105374737	rs17238368	4.0084355

7957	LOC105374737	rs7729332	4.0084355
7959	LOC105374737	rs11955037	4.0084355
7960	LOC105374737	rs11739531	4.2124918
7961	LOC105374737	rs7726047	4.0084355
7963	LOC105374737	rs1395082	4.0084355
7964	Not Annotated	rs7349788	4.0084355
7965	Not Annotated	rs10040364	4.027506
7967	Not Annotated	rs4304113	4.1103382
7969	Not Annotated	rs10055624	4.0453835
7971	Not Annotated	rs11952914	4.0453835
7972	Not Annotated	rs10063864	4.0453835
7973	Not Annotated	rs10063818	4.0700743
7975	Not Annotated	rs6867169	4.0453835
7976	Not Annotated	rs75815217	4.1106462
7977	Not Annotated	rs7714305	4.0453835
7981	Not Annotated	rs6879155	4.0453835
7982	Not Annotated	rs6879329	4.0453835
7984	Not Annotated	rs28862949	4.0203303
7985	Not Annotated	rs183338289	4.0453835
7987	Not Annotated	rs6860233	4.0564231
7988	Not Annotated	rs141096931	4.0564231
7989	Not Annotated	rs143542438	4.0597616
7990	Not Annotated	rs7716982	2.5554468
7991	Not Annotated	rs4957333	4.0644591
7992	Not Annotated	rs6451533	4.2611453
7995	Not Annotated	rs10065874	4.0644591
7996	Not Annotated	rs10065894	4.0644591
7997	Not Annotated	rs6882769	3.9822198
7998	Not Annotated	rs10941521	4.0644591
7999	Not Annotated	rs10473206	4.0366425
8000	Not Annotated	rs10473209	4.0722539
8003	Not Annotated	rs10068069	4.0392889
8004	Not Annotated	rs62358056	4.0590584
8005	Not Annotated	rs10071870	4.0590584
8006	Not Annotated	rs12188666	4.0590584
8007	Not Annotated	rs10057182	4.0170302
8008	Not Annotated	rs10038251	4.0590584
8009	Not Annotated	rs10035697	4.0590584
8010	Not Annotated	rs10043891	4.0590584

8011	Not Annotated	rs10038367	4.0590584
8012	Not Annotated	rs10044538	4.0590584
8014	Not Annotated	rs72747928	4.0590584
8015	Not Annotated	rs11949581	4.0590584
8016	Not Annotated	rs72747930	4.0590584
8017	Not Annotated	rs10941522	4.0590584
8019	Not Annotated	rs10056173	4.1143218
8020	Not Annotated	rs4957334	4.0590584
8021	Not Annotated	rs4957335	4.0590584
8022	Not Annotated	rs4957336	4.0590584
8024	Not Annotated	rs4957337	4.0590584
8025	Not Annotated	rs4957338	4.0590584
8026	Not Annotated	rs4957339	4.0590584
8027	Not Annotated	rs10065482	4.0590584
8028	Not Annotated	rs11744569	4.0590584
8029	Not Annotated	rs12513529	4.0590584
8030	Not Annotated	rs7730020	2.5632605
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8037	Not Annotated	rs10473211	4.0572718
8039	Not Annotated	rs62358800	4.0492359
8040	Not Annotated	rs62356460	4.1044993
8041	Not Annotated	rs62356461	4.0492359
8044	Not Annotated	rs9292785	4.0312987
8045	Not Annotated	rs10473214	4.0492359
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8063	PTGER4	rs10066459	2.4910955
8068	PTGER4	rs45437592	3.9862411
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8155	TTC33	rs29742	4.6857515
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8493	C7	rs2443039	3.4889275
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8496	C7	rs10512750	3.9611886
8497	C7	rs11741315	3.9326047
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8776	MROH2B	rs10512757	2.5887827
8781	MROH2B	rs34305798	2.5808862



8782	MROH2B	rs1423392	2.0558039
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8880	LOC105374739	rs35807549	2.5788175
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8975	C6	rs3805713	2.5540956
8995	C6	rs17260165	2.7099147
9003	C6	rs11749976	2.7169339
9004	C6	rs17260228	2.6876388
9008	C6	rs1025043	2.6876388
9014	C6	rs6883180	2.6129548
9016	C6	rs4642410	2.6191571
9017	C6	rs13167227	2.6123349
9018	C6	rs9332457	2.8581209
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9024	C6	rs13177483	2.8304442
9025	C6	rs4413571	2.0168795
9028	C6	rs7723553	2.0104763
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9031	C6	rs34205640	2.5998779
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9044	C6	rs11957341	2.1543086
9049	C6	rs7443604	2.4000946
9050	C6	rs7443562	2.4000946
9052	C6	rs7448055	2.1543086
9053	C6	rs6451569	2.4000946
9054	C6	rs10941534	2.4000946
9055	C6	rs10941535	2.4000946
9056	C6	rs35179056	2.6208889
9057	C6	rs62361614	2.6067002
9058	C6	rs10447143	2.1543086
9065	C6	rs1822822	2.6248464
9091	C6	rs76520169	2.1928146
9104	C6	rs2921175	2.1859924
9108	C6	rs17260948	2.635451
9109	C6	rs11949807	2.1956727