12-2011

Effect of Gender on the Association of Single-Nucleotide Polymorphisms with Bipolar Disorder

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A thesis

presented to

the faculty of the Department of Biostatistics And Epidemiology

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Public Health in Epidemiology

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by

Jerald Eric Mullersman

December 2011

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ABSTRACT

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Bipolar disorder is a relatively common form of mental illness that depends strongly on genetic inheritance for expression. The author of this study has sought to evaluate whether the gender of subjects influences which genetic variants are associated with the disease. A portion of the cases from a previously published study were analyzed using PLINK software and the association of single-nucleotide polymorphisms was evaluated separately for all cases, for female subjects alone, and for male subjects alone. The results obtained for male subjects alone reached higher levels of statistical significance than when both genders were evaluated together or when female subjects were evaluated alone. The most significantly scoring polymorphisms were distinctly different for the 2 genders. In particular, a site downstream of the ion exchanger *SLC24A3* and upstream of the Rab5-interacting protein *RIN2* gene on chromosome 20 (rs6046396) yielded very high significance in men (p=3.91 X 10^{-9}).

I am truly grateful to my wife and companion of many years, Bette Mullersman, for her support and love. This thesis is dedicated to her.

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CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

Some Historical Perspectives on Mental Illness

Perspectives of the Ancients

Mental illness has a long history (Bennett, 2007) that begins with ancient civilizations where the afflicted were generally viewed as being troubled by demons or evil deities. During the Greek classical period, Hippocratic physicians developed a system of thought that attributed differences in behavior to the influences of "humors" and made many keen observations concerning a variety of psychiatric conditions including mania, depression, and phobias. One of these humors, black bile (melancholia), was associated with sadness, and it is from this that we derive the word "melancholy". Galen and other physicians of the classical Roman era elaborated upon these earlier Greek formulations.

The limited insights of the Greeks and Romans were, however, largely forgotten during the long Middle Ages and superstitious fears were ascendant during that period. With the advent of the Enlightenment, many thinkers applied observation and reason to the evaluation of the mentally ill once again. The efforts of Enlightenment thinkers led ultimately to the foundations of modern psychiatric thought (Bennett, 2007).

Classification of Serious Mental Illnesses

In 1899 a German psychiatrist, Emil Kraepelin, classified serious mental illnesses into two broad categories, manic-depressive psychosis and dementia praecox (Ebert & Bär, 2010). This division of these serious and relatively common mental illnesses into two groups is referred to as the Kraepelinian dichotomy and has been a long-standing principle of the classification of these disorders. The Swiss psychiatrist Eugen Bleuler refined the concept of dementia praecox and

introduced the concept of the schizoid personality. Ultimately, this led to changes in thinking about the nature of dementia praecox and its being renamed as schizophrenia (Ebert & Bär, 2010).

The treatment of schizophrenia and depression through the use of drug- or electric shock-induced convulsions had some limited success during the early 20th century (Payne & Prudic, 2009). Such success encouraged a more biological view of these diseases and promoted further investigation of their causes and treatments.

The Development of Neuroscience and Psychopharmacology

The introduction of early antipsychotic medications like chlorpromazine (López-Muñoz et al., 2005) for the treatment of schizophrenia and lithium (Shorter, 2009) for manic-depressive disease met with reasonable success despite problems with toxicity. This led to investments by the pharmaceutical industry (Shen, 1999) and the development of funding by research organizations like the newly established National Institute for Mental Health (Grob, 1996) to promote greater understanding of the biological underpinnings of normal and abnormal brain function. Through such understanding, it was anticipated that diagnosis and treatment could be improved significantly.

Diagnostic Criteria for Schizophrenia, Bipolar Disorder, and Related Illnesses

The classification of mental illnesses and the criteria by which they are diagnosed have evolved over many years. A key standard in this diagnostic classification in the United States is the *Diagnostic and Statistical Manual of Mental Disorders*, which has been maintained by the American Psychiatric Association. The current, revised fourth edition of this manual is referred to here as DSM-IV-TR (American Psychiatric Association., 2005). According to DSM-IV-TR

schizophrenia is viewed as a psychosis, a condition that is dominated by distorted perceptions of reality and disordered thinking. A medical practitioner arrives at a diagnosis of schizophrenia through eliciting a history of persistence of two or more psychotic symptoms (hallucinations, delusions, disorganized speech, gross disorganization and/or so-called negative symptoms) and social dysfunction in the absence of other plausible medical or psychiatric explanations. In contrast, manic-depressive illness, which has been renamed bipolar disorder and is designated as BD here, is classified as a disorder of mood in DSM-IV-TR. There is a spectrum of conditions related to BD, but they all involve cycling from periods of elation, agitation, or irritation to periods of depression. The type I condition, BD-I, is the most severe and involves mania, a condition of elation, agitation, or irritation with a greater scope and intensity of symptoms. The type II condition, BD-II, requires that the patient suffer from hypomania, which is similar to but less intense than mania. Cyclothymic disorder, in turn, is similar to but less severe than, BD-II. According to the paradigm delineated by the Kraepelinian dichotomy, schizophrenia and BD should be considered to be clearly distinct clinical entities. However, this distinction has eroded over the years and DSM-IV-TR includes entities like schizoaffective disorder that are chimeric categories that include features of both a psychosis and a mood disturbance. New formulations are being considered for inclusion in DSM-V that would place BD-like illnesses and schizophrenia-related conditions on a spectrum that are related to each. Such an approach has some support from genetic studies (Lake & Hurwitz, 2007), and the genetic overlap between BD and other psychiatric conditions is discussed further below.

Prevalence and Societal Burden of Bipolar Disorder

Mental illnesses are, in general, relatively common diseases that produce considerable disability in sufferers and have a significant deleterious impact on economies world-wide (Organisation mondiale de la santé, 2008). Ferrari and colleagues recently completed a systematic review of published data for the prevalence of BD (Ferrari, Baxter, & Whiteford, 2011). The average point prevalence was found to be 0.741% for the entire spectrum of bipolar disorders. This was felt to be an underestimate of the true prevalence due to the tendency of many sufferers to be nonresponders to survey requests. Difficulties with clinical definitions and the varied approaches to the inclusion of less serious conditions in the counting of subjects complicated the development of accurate estimates. They confirmed that males and females have very similar rates of occurrence and that prevalence was quite similar throughout the world.

Fajutrao and colleagues have recently completed a systematic review of the evidence concerning the burden of BD in Europe (Fajutrao, Locklear, Priaulx, & Heyes, 2009). They found tremendously large costs related to unemployment, chronic medical care, and suicide attempts. In the UK alone, they estimated the annual national cost of BD to be £4.59 billion. Shippee and colleagues performed a comparative analysis of the impacts in the US of unipolar depression versus BD (Shippee et al., 2011). While unipolar depression is much more common than BD, patients with BD experienced poverty and social dysfunction at a much higher rate than those with unipolar depression.

The relatively high prevalence of BD and its disabling effects on patients pose a challenge for society. This motivates a continuing desire to develop a better understanding of the disease's etiologic factors so that better diagnostic and therapeutic modalities can be developed. Recent studies have been directed toward examining the genetic and other factors that promote the

expression of BD. The next sections provide a brief summary of the genetic studies of BD that have led up to the efforts that are described here and provide a context for understanding the significance of the strategies employed in this work and for assessing the potential impact of the current findings on the field.

<div align="center">Evidence for Genetic Predilection Toward Mental Illnesses</div>

The advances in genetics during the 20th century and the advancing appreciation of the biological character of some mental illnesses naturally led to assessments of the inheritance of psychiatric diseases. Early studies relied heavily upon the study of families where mental illness seemed to recur frequently in several branches of a family tree. Studies of twins and first-degree relatives have typically demonstrated heritability of schizophrenia and BD in the range of 60% - 90% (Edvardsen et al., 2008; Kieseppä, Partonen, Haukka, Kaprio, & Lönnqvist, 2004; Lichtenstein et al., 2009). In order to provide a suitable context for the research being reported here, a short history of genetic approaches that have been applied to understanding the inheritance of BD is provided.

<div align="center">The Search for Genes that Underlie Psychiatric Illnesses</div>

Early Studies of X Chromosome-Linked Inheritance

Because of the relatively high frequency with which X-linked recessive traits manifest and because of the ease with which the inheritance of these traits can be comprehended, the human X chromosome quickly accrued a number of genetically mapped markers. Some anecdotal observations of apparent association of certain X-linked conditions with BD led to protracted efforts to map genetic susceptibility to BD on the X chromosome. In a report by Mendlewicz and colleagues, they detailed efforts to evaluate linkage between the gene for glucose-6-

phosphate dehydrogenase and BD and traced the history of such efforts back as far as 1969 (Mendlewicz, Linkowski, & Wilmotte, 1980). There followed a controversy over the next 15 years wherein various authors made claims and counterclaims of linkage between BD and markers on the X chromosome (Baron et al., 1993; Baron, Rainer, & Risch, 1981; Baron et al., 1987; Berrettini et al., 1990; Bocchetta, Piccardi, Martinelli, Quesada, & Del Zompo, 1999; De Bruyn et al., 1994; Del Zompo, Bocchetta, Goldin, & Corsini, 1984; Mendelbaum et al., 1995; Risch & Baron, 1982). This series of reports illustrates some difficulties that have persisted, even after the development of more sophisticated technologies, with the replication of results. This problem with replication of findings in subsequent studies likely stems from several sources including the effects of random error, confounding, and variation in the genetic structure of different populations (Cichon et al., 2009; Craddock & Sklar, 2009).

Development of Genome-Wide Evaluations of Genetic Susceptibility

With the advent of the polymerase chain reaction (PCR) technology, a new era of analysis was ushered in. PCR made it possible to evaluate a large number of microsatellite markers that could be distinguished as sequence-tagged sites with repeat lengths varying between alleles. A large number of linkage analyses were performed, some focusing just on candidate genes or chromosomal regions and others seeking to detect signals across the entire genome (Dick et al., 2003; Lambert et al., 2005; Logue et al., 2009; McAuley et al., 2009; Park et al., 2004; Ross et al., 2008; Saunders, Zhang, Copeland, McInnis, & Zöllner, 2009; Zandi et al., 2007). A substantial problem with the results obtained from early linkage scans was the breadth of the signal peaks that arose from them. The peaks were often millions of base pairs wide and encompassed chromosomal regions that contained a large number of genes. While they were

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quite sensitive and focused attention on a set of genes, it has often been challenging to narrow these broad regions down to the specific genetic variants that are causal.

More recently microarrays that can determine the status of hundreds of thousands of single-nucleotide polymorphisms (SNPs) have largely supplanted the determination of microsatellite alleles. The breadth of the regions identified by linkage scans has fallen, as larger numbers of markers have been increasingly employed. The evaluation of SNPs can support the examination of candidate genes, chromosomal regions, or genome-wide surveys. They also permit the ready conduct of studies with a variety of designs. Both family studies and case-control designs can be performed. Several genome-wide association studies (GWAS) of BD have been performed in recent years (Baum et al., 2008; Curtis et al., 2011; Hattori et al., 2009; Lee et al., 2011; Oedegaard, Greenwood, Johansson, et al., 2010; Sklar et al., 2008; Smith et al., 2009). While these studies have identified some interesting candidates for genes underlying BD, there have been challenges in replicating findings between studies. This replication problem appears to arise from several sources including population differences, small effect size of most BD genes, and various kinds of confounding. The current status of the effort to understand the genetics of BD has been recently reviewed (Barnett & Smoller, 2009; Byerley & Badner, 2011; Craddock & Sklar, 2009; Gershon, Alliey-Rodriguez, & Liu, 2011; Goes, Sanders, & Potash, 2008; Hamshere et al., 2011; Piletz, Zhang, Ranade, & Liu, 2011). Askland and colleagues have undertaken a pathway-oriented analysis of BD GWAS research and have concluded that a major fraction of the genes implicated in BD are related to the function of various ion channels and elements of the synaptic transmission apparatus (Askland, Read, & Moore, 2009).

The Challenge of Common Disease Genes with Small Individual Effects

There is now considerable evidence that BD is caused in most individuals who suffer from this ailment by a set of relatively common genetic variations that each have a relatively small effect size. The detection of such small-effect genes requires relatively large numbers of cases and depends upon controlling confounding as much as possible. There is increasing evidence that BD overlaps with a number of other psychiatric conditions in terms of genetic etiology. While most of the documented genetic commonality has been demonstrated for BD and schizophrenia (Carroll & Owen, 2009; Lichtenstein et al., 2009; Palo et al., 2007; Tang, Thornton-Wells, & Askland, 2011; Wray & Goddard, 2010), there have also been indications of shared genetic underpinnings between BD and autism (Carroll & Owen, 2009), BD and substance abuse (Johnson, Drgon, McMahon, & Uhl, 2009), and BD and unipolar depression (McGuffin et al., 2003). This speaks to the complexity of all these diseases and suggests that they may share traits that are not readily apparent. For instance, while BD does not display any gender preference in its expression overall, that does not mean that individual contributing loci, such as some that it shares with unipolar depression (which shows a clear female propensity), would not demonstrate a gender-specific effect in an appropriate analytical context such as gender stratification of an association study.

Improving the Power to Perceive Genetic Variants

In order to improve the ability to detect causal genetic variants with as much sensitivity as possible, great care must be taken in ensuring the integrity of the genotyping data, to improve the homogeneity of the study population, and to increase the size of the sample (Anderson et al., 2010; Iles, 2011; Rodriguez-Murillo & Greenberg, 2008).

The Effect of Gender on the Expression of Bipolar Disorder

Although the overall prevalence of BD in both genders is comparable, epidemiologists have sought to determine whether specific aspects of the expression of the disease vary between the genders. Results have varied between studies and this topic is somewhat controversial. A recent comprehensive review finds little evidence for differences in the nature of the expression of BD in the two genders (Diflorio & Jones, 2010). However, two more recent studies both conclude, independently of each other, that women with BD spend more time in a depressed state than men do (Altshuler et al., 2010; Nivoli et al., 2011). This is interesting, because unipolar depression demonstrates a female preponderance.

There has been increasing interest in epigenetic mechanisms of control of gene expression in the brain (Meaney & Ferguson-Smith, 2010). These epigenetic mechanisms involve methylation and hydroxymethylation of cytosine residues, primarily at CpG dinucleotides, and post-translational modification (e.g., acetylation) of histones in the core of nucleosomes. Evidence regarding the role of epigenetic processes in psychiatric disorders has been reviewed (Connor & Akbarian, 2008; Tsankova, Renthal, Kumar, & Nestler, 2007). This sort of gene regulation has also been found to play an important role in coordinating the gender-specific development of the brain (Matsuda et al., 2011; McCarthy & Arnold, 2011; Nugent & McCarthy, 2011). Given the role of epigenetics in both normal sexual differentiation of the brain and in psychiatric problems, it is perhaps not surprising that epigenetics has emerged as one of the important mechanistic paradigms for understanding how gender may impact the expression of mental health disorders (Jessen & Auger, 2011; Mill et al., 2008).

Another type of mechanism for the control of gene expression in the brain that has received increased attention in recent years involves the action of noncoding RNAs. For instance, the

altered expression of microRNAs has been implicated in both schizophrenia and BD (Forero, van der Ven, Callaerts, & Del-Favero, 2010; Miller & Wahlestedt, 2010; Moreau, Bruse, David-Rus, Buyske, & Brzustowicz, 2011; Xu, Karayiorgou, & Gogos, 2010). Interestingly, noncoding RNAs, as a broad category, have been implicated in the gender-dependent processes underlying susceptibility to psychiatric disease (Qureshi & Mehler, 2010).

Given that researchers have discovered gene control processes that intersect with both the expression of gender differences in the brain and the development of psychiatric diseases, it is probably not surprising that genetic alterations have been found that show a gender-specific predisposition toward mental health disorders. For instance, the Reelin gene (*RELN*) has been found to have a gender-specific association with both BD (Goes et al., 2010) and schizophrenia (Liu et al., 2010; Shifman et al., 2008). The mechanisms by which Reelin demonstrates this gender-specific effect are being investigated. Ovadia and Shifman reported alterations in the pattern of mRNA splicing (Ovadia & Shifman, 2011) that may explain these effects. There have been conflicting reports regarding the role of DNA methylation on Reelin gene function (Connor & Akbarian, 2008). Gender has also been found to modulate the effect of a variant of the serotonin transporter gene on the expression of mania (Rucci et al., 2009).

CHAPTER 2

RESEARCH QUESTION AND STUDY APPROACH

Statement of the Research Question Being Posed by the Current Study

Likely because of the essentially equal prevalence of BD in men and women, the role of

gender has not been evaluated in genome-wide studies of the association between BD and

genetic markers. However, as has been delineated above, there are known mechanisms by which

gender-specific control of the expression of psychiatric illnesses like BD could occur, and some

specific examples of gender-specific effects of genetic variation on the development of BD have

already been detected. The author of the current study has sought to examine explicitly whether

stratification on gender in a genome-wide association study could detect one or more genetic

variants that contribute to the expression of BD under conditions where evaluation of the same

data set without such stratification would be unable to detect the influence of such variants.

Description of the Scientific Approach Used in the Current Study

A secondary analysis of data generated for a study previously published by Smith and

colleagues (Smith et al., 2009) was performed. In order to improve the odds of detecting genetic

variants at a level that would be considered statistically significant, some new elements of study

design were adopted as compared to the study by Smith and colleagues. A genetically more

homogeneous study population was selected. Only the European-American (EA) subjects from

the data set were chosen for study and were then evaluated using principal component analysis in

order to identify and eliminate outliers with respect to ancestry. Also, only subjects with a

diagnosis of BD type I (BD-I) were considered in this study. Finally, and most germane to the

primary hypothesis of the study, the association analysis was performed in a manner so that the

effect of gender could be determined.

CHAPTER 3

METHODS

Data Sources

Data for Cases and Controls from a Study of Bipolar Disorder

Data from the study of Smith and colleagues (Smith et al., 2009) were obtained via the database of Genotypes and Phenotypes (dbGaP) that is managed by the National Center for Bioinformatics (NCBI). Per dbGaP, the use of this data set, designated phs000017.v3.p1, does not require Institutional Review Board approval ("dbGaP | phs000017.v3.p1 | Whole Genome Association Study of Bipolar Disorder," n.d.). Out of 653 EA cases in the data set, 2 outliers were identified by principal component analysis (Price et al., 2006) using the HelixTree software suite (Golden Helix, Bozeman, MT) and removed. Of these cases, 605 of them carried the BD-I diagnosis and were evaluated in the current study. This data set also provided 1034 EA controls. Genotyping data was derived from the Affymetrix 6.0 microarray and included 729,454 SNPs.

The collection of the DNA samples and the associated phenotypic information was described by Smith and colleagues in a supplement to their report (Smith et al., 2009). Cases were selected from individuals who had been identified over an 18-year period. The subject accrual was performed in five successive efforts referred to as "Waves". The first two Waves involved four sites and focused on large pedigrees that each contained multiple individuals affected by mental illness as well as a BD-I proband. Waves 3 and 4 focused on smaller pedigrees and involved 11 sites. Wave 5 mainly included only individual, unrelated cases that were collected for the purpose of conducting large-scale association studies. The GWAS study performed by Smith and colleagues selected 175 unrelated EA subjects from Waves 1 and 2, 396 subjects from Waves 3 and 4, and 430 subjects from Wave 5. Subjects were evaluated using the Diagnostic

Interview for Genetic Studies tool, which was revised between Waves 4 and 5 in order to increase the amount of information collected. All indicators were scored by a senior clinician who was typically a psychiatrist. Of the 1,041 subjects who were initially selected, 40 were excluded because of uncertainty regarding the diagnosis. Controls were gathered by a separate process that relied upon a self-administered questionnaire. Individuals were selected as controls if their questionnaire indicated that they did not suffer from major depression and if they denied having a history of psychosis or BD.

Data for Controls from a Study of Schizophrenia

A corresponding study of schizophrenia was conducted by the same group of researchers from whom the BD data were obtained. In order to increase the power of our study, we sought to include controls from the schizophrenia study in our analysis. These control data were also obtained via dbGap (phs000021.v3.p2) and are also not subject to approval by an Institutional Review Board, per the dbGaP website ("dbGaP | phs000021.v3.p2 | Genome-Wide Association Study of Schizophrenia," n.d.). Most of the 1442 EA controls from this data set were identical with control subjects in the BD data set. Consequently, only 362 controls were added to our study from the schizophrenia study data.

Of the 1,396 controls used in this study, 754 (54.0%) were female and 642 (46.0%) were male. Of the 605 cases used in this study, 313 (51.7%) were female and 292 (48.3%) were male.

Data Analysis

Association Study Using PLINK

Allelic association analyses (Sasieni, 1997) were performed using PLINK version 1.07 (Purcell, n.d.; Purcell et al., 2007) with the command line "--assoc" flag set.

Evaluation of Identified Single-Nucleotide Polymorphisms

The quality of the high-scoring SNPs was evaluated in terms of compliance with Hardy-Weinberg equilibrium (HWE), minor allele frequency (MAF), and genotyping call rate. SNPs were removed under the following circumstances: HWE p-value $< 10^{-5}$, MAF $< 1\%$, or call rate $< 95\%$. A very conservative per test significance level of 7.2×10^{-8} was chosen (Dudbridge & Gusnanto, 2008). At the same time, we used a less stringent criterion of "suggestive association" with a cut-off of $\alpha = 10^{-5}$.

Sequence positions reported in this study refer to Build 36.3 of the human genome sequence. The relationships between some closely spaced SNPs with respect to linkage disequilibrium were investigated using the software program Haploview, version 4.2 (Barrett, Fry, Maller, & Daly, 2005). Measures of linkage disequilibrium between SNPs relied upon data available from The International HapMap Project ("The International HapMap Project," 2003). The localization of SNPs to specific regions of chromosomes and to the vicinity of known genes was aided by the National Center for Bioinformatics (NCBI) through use of a SNP database that is accessible via the Internet under the moniker dbSNP ("dbSNP Home Page," n.d.).

CHAPTER 4

RESULTS

Association Analysis of All Cases

An association analysis of all cases was performed as a point of reference. The results are

shown in Table 1.

This analysis used substantially fewer cases than that of Smith and colleagues (Smith et al.,

2009). Consequently, it was not entirely surprising to see that the significance of the highest

scoring SNPs was lower than that seen in their study. Only 9 SNPs yielded nominal p-values at

or below 10^{-5}, the level at which some researchers consider results to be potentially significant.

SNP rs1825828 was one of the highest-scoring markers detected by Smith and colleagues in their

analysis of the EA subpopulation. When they analyzed the data with the help of a genomic

control method, they obtained a nominal p-value of 2.9 X 10^{-7} for this SNP, a value that is nearly

10-fold lower than the p-value of 2.49 X 10^{-6} that was obtainable in this study. This ability of

the current study to detect some of the same markers as the original study in this nonstratified

group of subjects, albeit at a lower level of significance, gives support to the overall validity of

the current analysis and provides a benchmark against which the results obtained from the two

gender strata can be appropriately judged.

Table 1

SNPs Showing Strongest Association with BD-I, When Testing Against All Cases

SNP	[a]Chr	Position (bp)	[b]MAF	[c]HWE p-value	[d]OR (95% CI)	p-value
rs4964010	12	26689656	0.38	0.86	0.69 (0.59-0.80)	1.11×10^{-6}
rs12692245	2	239320476	0.42	0.80	1.40 (1.22-1.60)	1.45×10^{-6}
rs1825828	3	97873650	0.29	0.0015	0.68 (0.58-0.80)	2.49×10^{-6}
rs2893420	1	99736275	0.33	0.20	0.70 (0.60-0.82)	5.70×10^{-6}
rs17111920	14	26476732	0.01	0.74	3.07 (1.83-5.14)	7.36×10^{-6}
rs10922013	1	194294495	0.25	0.80	0.68 (0.57-0.80)	8.21×10^{-6}
rs4128254	2	239310782	0.41	0.86	1.36 (1.19-1.56)	9.06×10^{-6}
rs6901039	6	26108864	0.28	0.92	1.39 (1.20-1.61)	9.13×10^{-6}
rs1106634	8	20110329	0.12	0.02	1.52 (1.26-1.84)	1.00×10^{-5}
rs14380	3	42553583	0.14	0.20	1.48 (1.24-1.76)	1.16×10^{-5}
rs12194046	6	113095902	0.10	0.47	1.55 (1.27-1.89)	1.27×10^{-5}
rs11585894	1	99744018	0.32	0.08	0.72 (0.61-0.83)	1.42×10^{-5}
rs1965290	3	124770496	0.44	0.09	1.35 (1.18-1.55)	1.42×10^{-5}
rs1956817	14	26377654	0.005	0.88	3.69 (1.96-6.94)	1.44×10^{-5}
rs9458298	6	161884928	0.23	0.69	1.40 (1.20-1.63)	1.51×10^{-5}
rs12195005	6	161875995	0.22	0.61	1.40 (1.20-1.64)	1.52×10^{-5}
rs802568	7	145590176	0.02	0.01	2.28 (1.55-3.35)	1.55×10^{-5}
rs13430905	2	133728463	0.15	0.97	0.62 (0.50-0.78)	1.67×10^{-5}
rs8129319	21	19284492	0.18	0.35	0.65 (0.54-0.79)	1.68×10^{-5}
rs13388370	2	155345758	0.01	0.79	3.01 (1.78-5.11)	1.73×10^{-5}
rs12209107	6	161864009	0.22	0.74	1.40 (1.20-1.63)	1.73×10^{-5}
rs1012873	3	180994713	0.05	0.49	1.82 (1.38-2.40)	1.86×10^{-5}
rs11239177	10	44443385	0.50	0.32	0.74 (0.65-0.85)	1.88×10^{-5}
rs17119815	11	115934773	0.19	0.02	1.43 (1.21-1.69)	1.88×10^{-5}
rs7791106	7	78375618	0.12	0.20	0.60 (0.47-0.76)	1.89×10^{-5}

[a]Chr, chromosome number; [b]MAF, minor allele frequency; [c]HWE p-value, p-value for test of Hardy-Weinberg equilibrium; [d]OR (95% CI), odds ratio and its 95% confidence interval

Association Analysis of Female Cases

Results from an association analysis of female cases alone are shown in Table 2. This analysis also demonstrates relatively few higher-scoring SNPs, in this case only 5 SNPs with p-values less than 10^{-5}.

It should be noted here that the initial output of the association analysis of the female cases did contain one result that was nominally at a level of high statistical significance. This result was for SNP rs5932307, which lies on the X chromosome at position 126,993,095 base pairs. However, the p-value for the test of Hardy-Weinberg equilibrium was, at 3.32×10^{-180}, far less than 10^{-5} and this was, therefore, regarded to be an invalid result for the purposes of this study because of the deviation from HWE (Sasieni, 1997). This SNP is in an intergenic region on the long arm of the X chromosome (Xq25) approximately 19.5 kb downstream from the *ACTRT1* gene that encodes an actin-like protein (Heid et al., 2002). This region of the X chromosome does not correspond to any of those identified in a recent review of the genetics of bipolar disorder as candidate chromosomal regions for this disorder (Serretti & Mandelli, 2008).

Three other X chromosome SNPs (rs6525223, rs17265313, and rs5965550) were also considered invalid due to violation of HWE and are not shown in Table 2. The SNPs yielded p-values ranging between 1.71×10^{-5} and 3.41×10^{-5}. All three SNPs lie close to each on the long arm of the X chromosome (Xq12) in the oligophrenin 1 gene (*OPHN1*), which encodes a Rho GTPase-activating protein. Deletion of *OPHN1* leads to an X-linked syndrome that causes mental retardation, thought to be through disruption of its role in maturation of synapses (Nadif Kasri, Nakano-Kobayashi, Malinow, Li, & Van Aelst, 2009). The Xq12 chromosomal region has also not been previously associated with a high risk of bipolar disorder.

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Table 2

SNPs Showing Strongest Association with BD-I, When Testing Against Female Cases

SNP	[a]Chr	Position (bp)	[b]MAF	[c]HWE p-value	[d]OR (95% CI)	p-value
rs2367911	7	81624156	0.02	0.44	2.98 (1.85-4.82)	3.13×10^{-6}
rs7291885	22	17009153	0.05	0.62	2.34 (1.61-3.39)	4.36×10^{-6}
rs2931018	18	74610846	0.48	0.55	1.53 (1.27-1.85)	7.25×10^{-6}
rs1825828	3	97873650	0.29	0.0015	0.60 (0.47-0.75)	7.98×10^{-6}
rs7825634	8	91425419	0.02	0.53	3.28 (1.89-5.70)	8.38×10^{-6}
rs1992045	8	59003478	0.06	0.19	2.70 (1.48-2.90)	1.37×10^{-5}
rs2373842	7	88589572	0.23	0.69	1.60 (1.29-1.98)	1.41×10^{-5}
rs2189061	7	88577308	0.23	0.54	1.59 (1.28-1.97)	1.99×10^{-5}
rs6980880	8	123856242	0.44	0.02	0.66 (0.54-0.80)	2.41×10^{-5}
rs11986500	8	62113757	0.10	0.96	1.82 (1.38-2.42)	2.53×10^{-5}
rs1020605	2	12314886	0.08	0.27	1.87 (1.39-2.51)	2.56×10^{-5}
rs16934680	11	17721489	0.17	0.66	1.64 (1.30-2.07)	2.64×10^{-5}
rs17152884	8	11018996	0.01	0.72	3.99 (1.99-8.03)	2.82×10^{-5}
rs470422	13	105452539	0.25	0.79	1.55 (1.26-1.91)	2.98×10^{-5}
rs11651919	17	30090490	0.50	0.80	1.49 (1.23-1.80)	3.17×10^{-5}
rs17111920	14	26476732	0.01	0.74	3.95 (1.97-7.95)	3.28×10^{-5}
rs1992044	8	59003462	0.06	0.38	1.98 (1.42-2.75)	3.69×10^{-5}
rs3852489	10	92769203	0.42	0.60	0.67 (0.55-0.81)	3.87×10^{-5}
rs4076005	2	192410652	0.48	0.50	1.49 (1.23-1.80)	3.91×10^{-5}
rs4665788	2	21041972	0.25	0.45	1.54 (1.25-1.89)	3.98×10^{-5}
rs2167201	4	139749099	0.46	0.01	1.48 (1.23-1.79)	4.04×10^{-5}
rs9505426	6	831884	0.04	0.59	2.31 (1.53-3.49)	4.22×10^{-5}
rs4142729	2	39076419	0.07	0.17	1.98 (1.42-2.76)	4.81×10^{-5}
rs3094356	5	9801246	0.44	0.63	0.67 (0.56-0.82)	4.82×10^{-5}
rs6036025	20	21987868	0.08	0.73	1.87 (1.38-2.54)	4.91×10^{-5}

[a]Chr, chromosome number; [b]MAF, minor allele frequency; [c]HWE p-value, p-value for test of Hardy-Weinberg equilibrium; [d]OR (95% CI), odds ratio and its 95% confidence interval

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Association Analysis of Male Cases

The results for the association analysis of the male cases are shown in Table 3. These results are strikingly different from those of the analyses that examined all cases or just female cases. One SNP, rs 6046396, yielded a nominal p-value of 3.91×10^{-9}. This appears to be significant on a per test basis. Also, there are 18 other SNPs with p-values less than 10^{-5}. Most of these highly significant SNPs are located in regions on chromosomes 6, 8, 14, 15, and 20.

The considerably stronger association results for the male cases is especially interesting, given that there are only about half the cases here as were evaluated in the "all cases" analysis. This suggests the possibility of male-specific effects that are masked when both genders are evaluated together or when females are studied separately.

The highest-scoring SNP, rs 6046396, and the other two SNPs on chromosome 20, rs4813377 and rs4813376, are located in an intergenic region, downstream of the gene *SLC24A3* and upstream of the gene *RIN2* (Figure 1). Analysis of the CEU (Northern European ancestry) data provided by The International HapMap Project using the Haploview software program shows considerable linkage disequilibrium between the SNPs on chromosome 20 (Figure 2). There are three high-scoring SNPs in a region of chromosome 14. While the highest-scoring SNP, rs11158205, lies within an intron of the gene *KIAA0586*, the other two SNPs, rs7155496 and rs12587974, are in an intergenic region approximately 100 kb downstream of *KIAA0586*. The minor allele of all three of these SNPs on chromosome 14 displayed a modest protective effect relative to BD. The next highest-scoring SNPs, rs4276463 and rs9343877, are located close to each other on chromosome 6 in an intergenic region between genes *PHIP* and *HMGN3*. There are also nine SNPs listed in Table 3 that lie on chromosome 15 within the gene *LASS3*.

Table 3

SNPs Showing Strongest Association with BD-I, When Testing Against Male Cases

SNP	[a]Chr	Position (bp)	[b]MAF	[c]HWE p-value	[d]OR (95% CI)	p-value
rs6046396	20	19800503	0.26	0.46	1.89 (1.52-2.33)	3.91×10^{-9}
rs11158206	14	58017118	0.31	0.16	0.58 (0.46-0.73)	2.48×10^{-6}
rs4276463	6	79937274	0.09	0.55	2.18 (1.57-3.05)	2.71×10^{-6}
rs9343877	6	79922723	0.07	0.43	2.31 (1.60-3.31)	3.86×10^{-6}
rs2654596	15	98798517	0.27	0.30	1.63 (1.33-2.02)	4.10×10^{-6}
rs2587742	15	98752615	0.27	0.19	1.63 (1.32-2.01)	4.14×10^{-6}
rs2587784	15	98862970	0.26	0.00	1.66 (1.33-2.05)	4.20×10^{-6}
rs2243973	14	85665903	0.05	0.64	2.61 (1.71-4.00)	4.70×10^{-6}
rs2587814	15	98788816	0.27	0.22	1.62 (1.31-2.00)	5.83×10^{-6}
rs2654642	15	98839754	0.31	0.14	1.60 (1.30-1.96)	6.47×10^{-6}
rs7012730	8	135131419	0.03	0.30	3.06 (1.84-5.09)	6.55×10^{-6}
rs4813377	20	19799466	0.16	0.89	1.79 (1.39-2.31)	6.56×10^{-6}
rs1106634	8	20110329	0.12	0.02	1.85 (1.41-2.42)	7.31×10^{-6}
rs7155496	14	58132929	0.36	0.06	0.61 (0.49-0.76)	7.72×10^{-6}
rs16905065	8	135146666	0.05	0.96	2.41 (1.62-3.59)	8.56×10^{-6}
rs4813376	20	19799455	0.14	0.44	1.80 (1.39-2.34)	9.04×10^{-6}
rs17629030	4	171304652	0.05	0.05	2.36 (1.60-3.49)	9.07×10^{-6}
rs12587974	14	58120895	0.30	0.08	0.59 (0.47-0.75)	9.75×10^{-6}
rs2585243	15	98752202	0.27	0.26	1.60 (1.30-1.98)	9.76×10^{-6}
rs1503474	15	98820261	0.33	0.34	1.57 (1.29-1.93)	1.01×10^{-5}
rs10503391	8	8692796	0.09	0.50	0.40 (0.26-0.61)	1.03×10^{-5}
rs2587741	15	98752634	0.27	0.40	1.60 (1.30-1.97)	1.14×10^{-5}
rs17068296	8	3817488	0.02	0.37	3.71 (1.98-6.98)	1.34×10^{-5}
rs2587813	15	98790270	0.27	0.17	1.59 (1.29-1.96)	1.42×10^{-5}
rs1017706	6	77479216	0.14	0.02	1.80 (1.38-2.36)	1.52×10^{-5}

[a]Chr, chromosome number; [b]MAF, minor allele frequency; [c]HWE p-value, p-value for test of Hardy-Weinberg equilibrium; [d]OR (95% CI), odds ratio and its 95% confidence interval
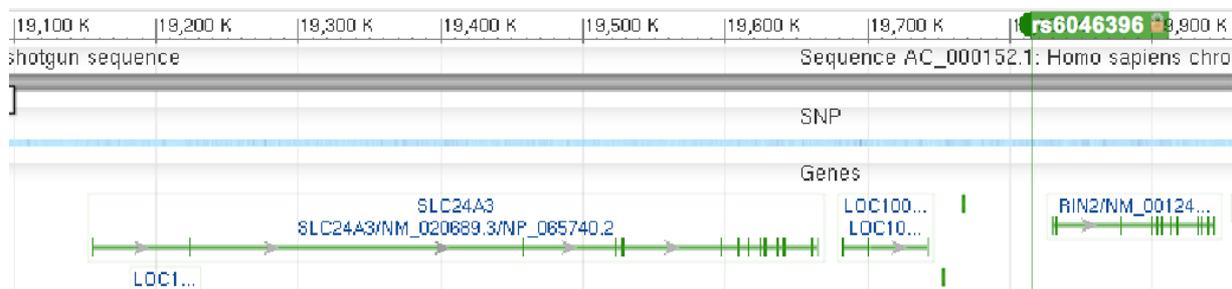
Figure 1. Map Showing the Location of SNP rs6046396 on Human Chromosome 20
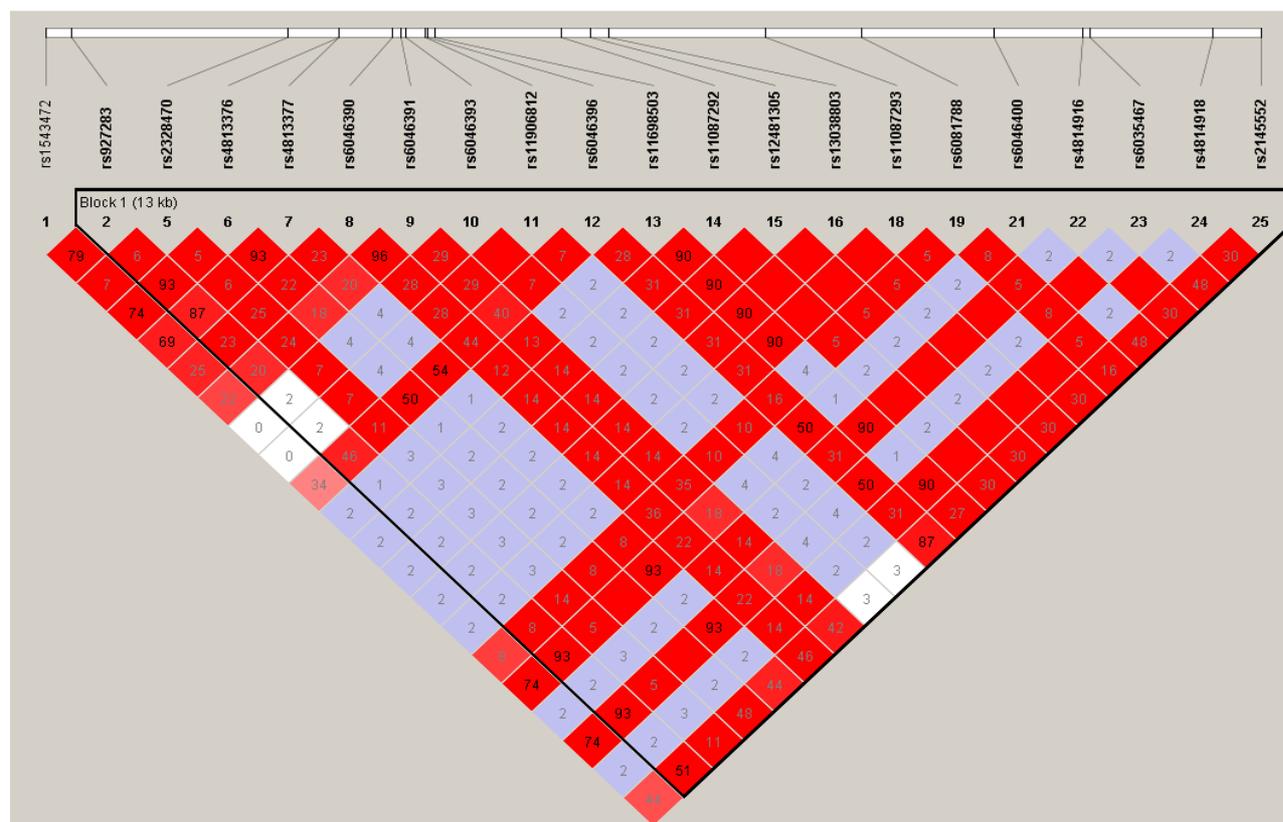

Figure 2. Linkage Disequilibrium Structure of SNPs Within a 13-Kilobase Block at 20p11. The numbers indicate the r^2 values between the corresponding pairs of SNPs. The linkage disequilibrium data derive from the CEU (Northern European ancestry) portion of The International HapMap Project data.

CHAPTER 5

DISCUSSION

The identification of the SNP, rs6046396, on chromosome 20 is, by far, the most interesting finding from this study. Given the origin of the data set being examined here, a key consideration is to review whether this SNP was observed by Smith and colleagues in their GWAS study with this data set (Smith et al., 2009). They did, in fact, detect this SNP in a portion of their analysis. It yielded a nominal p-value of 4.93×10^{-7} in a side analysis performed on a subset of their data that they referred to as the "Pritzker non-overlap data set". They derived this data set by removing a portion of their data, the part that had been obtained from the Pritzker Neuropsychiatric Disorders Research Consortium. Interestingly, this SNP did not score as well when the entire data set was examined by Smith and colleagues and became more significant only when they evaluated the smaller "Pritzker non-overlap data set". Significantly, that corresponds to the situation in the current study. This SNP only became significant when a portion of the data (the male cases) were examined in the current study. This SNP was also observed with a p-value of 8.62×10^{-5} by Wang and colleagues (Wang, Liu, & Aragam, 2010) when they performed a genome-wide meta-analysis with the same data set used here in combination with corresponding data obtained from schizophrenia patients.

A careful review of the literature suggests that the presence of this susceptibility locus for BD on the short arm of chromosome 20 has been detected several times before in linkage scans. Detera-Wadleigh and colleagues detected a signal (p=0.05) at D20S0604 in a study of 97 NIMH pedigrees (Detera-Wadleigh et al., 1997). Radhakrishna and colleagues studied a large Turkish pedigree with apparently dominant BD and located evidence of a susceptibility locus on chromosome 20, based on markers D20S604, D20S470, D20S836, and D20S838, with the

highest 2-point LOD score being 4.34 (Radhakrishna et al., 2001). Haplotype analysis placed the BD locus between the microsatellite markers D20S186 and D20S109. A replication set of 56 NIMH pedigrees that was evaluated by Willour and colleagues confirmed the locus on chromosome 20 with a peak between markers D20S162 and D20S604 (Willour et al., 2003). Very recently, Oedegaard and colleagues performed a genome-wide linkage analysis of BD and comorbid migraine (Oedegaard, Greenwood, Lunde, et al., 2010). They detected a region of the short arm of chromosome 20 where susceptibility to BD and migraine seemed to be coincident, with a peak at approximately 49 cM (about 19 Mb).

The three high-scoring SNPs on chromosome 20 that were detected in this study are in an intergenic region between *RIN2* and *SLC24A3*. Because they lie upstream of *RIN2*, one might expect that they are in an area related to the control of transcription of *RIN2* and that *RIN2* is the gene involved in BD at this locus. However, a consideration of what is known about these two genes may be worthwhile in assessing which gene is, in fact, involved in BD. The *RIN2* protein has been found to interact with the GTPase Rab5 (Saito et al., 2002). The Rab GTPases serve as regulators of endocytosis, and the disruption of their normal function has been implicated in a number of disease processes. However, *RIN2* is expressed at relatively low levels in the brain and the only central nervous disease with which Rab5 has been associated is Alzheimer's disease (Agola, Jim, Ward, Basuray, & Wandinger-Ness, 2011). Furthermore, deficiency of *RIN*2 has been found to result in a syndrome typified by macrocephaly, alopecia, cutis laxa, and scoliosis (Basel-Vanagaite et al., 2009; Syx et al., 2010). Thus, based on several lines of evidence, *RIN2* does not seem a very likely candidate for the genetic effect associated with these SNPs. However, Schubert and colleagues have recently published a review of evidence that implicates dysfunction of clathrin-coated vesicles in the pathogenesis of BD and schizophrenia (Schubert,

Föcking, Prehn, & Cotter, 2011). Because Rab5 is a key control component of this pathway, the *RIN2* gene product is, likewise, possibly involved via its action on Rab5. There is a need for more experimental work to cement this still tenuous connection. In contrast to this, *SLC24A3* is a more appealing prospect. It is a potassium-dependent sodium/calcium exchanger found at high levels in the neurons of the thalamus, the hippocampal CA1 area, and layer IV of the cerebral cortex (Altimimi & Schnetkamp, 2007; Kraev et al., 2001). Ion channels of this type also play important roles in controlling vascular tone through actions on smooth muscle cells in vessel walls and may, therefore, be involved in migraine headaches that have a vascular origin. As noted earlier, there is evidence that ion channels are likely very important to the pathophysiology of BD. Together, these features of *SLC24A3* seem to make it a somewhat better BD gene candidate than *RIN2*. A more definitive assessment of the role of *SLC24A3* will require further evaluation of the genetic variation at this locus and the relationship of that variation to the occurrence of BD.

This study has yielded initial support for the hypothesis that some of the effects of the genetic variation underlying BD are modified by gender. The approach described here is novel to the study of BD and has proven worthwhile in its ability to detect a potential disease locus that has been implicated by earlier linkage and association studies and that is biologically plausible. The plausibility of there being a locus predisposing to BD in the vicinity of rs6046396 is supported most strongly by the independent discovery of two nearby, highly significant SNPs with moderately strong linkage to rs6046396. Their detection and independence from rs6046396 greatly lowers the probability that the detection of rs6046396 is spurious. The presence of two reasonable gene candidates flanking rs6046396, either of which could be mechanistically

associated with BD, provides additional support for the validity of the detection of rs6046396 as an important marker for susceptibility to BD.

While these results regarding rs6046396 are potentially significant, it should be borne in mind that this marker has only been detected, both in this study and two earlier ones (Smith et al., 2009; Wang et al., 2010), in subjects taken from the same data set. The data set that was analyzed in the current study, as is the case with many genetic studies of mental illness, is complex and represents the contributions of materials and information from many institutions over a period of nearly 2 decades. Consequently, this data set may contain errors and/or biases that might lead to erroneous conclusions. Therefore, in order to reach a higher level of confidence regarding the true importance of the locus detected by rs6046396, this result will need to be replicated in other studies that evaluate a different group of subjects.

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