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A Review of Common and Rare Genetic Variants in Schizophrenia

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

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List of Abbreviations

MZ: monozygotic

DZ: dizygotic

CDCV: common disease - common variant

CDRV: common disease – rare variant

OR: odds ratio

DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision

DSM-V: Diagnostic and Statistical Manual of Mental Disorders, fifth edition

SNP: single nucleotide polymorphism

GWAS: genome-wide association study

PCR: polymerase chain reaction

LD: linkage disequilibrium

TDT: transmission disequilibrium test

CATIE: Clinical Antipsychotic Trials of Intervention Effectiveness

ISC: International Schizophrenia Consortium

MGS: Molecular Genetics of Schizophrenia

SGENE: Schizophrenia Genetics Consortium

MHC: major histocompatibility

CNV: copy number variant

BAC: bacterial artificial chromosome

CGH: comparative genomic hybridization

WGTP: Whole Genome TilePath

LOD: logarithm of odds

LTP: long-term potentiation

STEP-BD: Systematic Treatment Enhancement Program for Bipolar Disorder

WTCCC: Wellcome Trust Case Control Consortium

Abstract

A REVIEW OF COMMON AND RARE GENETIC VARIANTS IN SCHIZOPHRENIA

By Jonathan Craig Luedders

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2011

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Genetic epidemiology has shown a large role for genetic influences on schizophrenia. However, the nature of the variants involved is debated. The common disease-common variant (CDCV) hypothesis suggests that schizophrenia is caused by common alleles with small effect sizes. According to the common disease-rare variant (CDRV) hypothesis, schizophrenia is caused by rare variants with large effect sizes.

In recent years, evidence has been found for both common and rare variants in schizophrenia. Several SNPs have been associated with schizophrenia through genome-wide association studies (GWAS), supporting the CDCV hypothesis. In support of the CDRV hypothesis, individuals with schizophrenia have been found to have a higher burden of rare copy-number variants (CNVs). Also, several specific rare CNVs have been associated with schizophrenia. The exact mechanisms of these variants are unknown, but common and rare variants appear to affect many of the same pathways in the etiology of schizophrenia.

Chapter 1: Genetic Influence on Schizophrenia

Genetic factors have been implicated as an important part of the etiology of schizophrenia. Family studies have shown aggregation of the disorder in families, and twin and adoption studies show that this aggregation is due largely to genetic factors. Several lines of evidence suggest that many variants are involved in schizophrenia. However, the genetic architecture of the disorder is debated. One hypothesis is that common genetic variants with small effect sizes play an important role in the disorder, while another states that schizophrenia is caused mainly by rare variants with larger effect sizes.

Evidence of Genetic Influence

Family, twin, and adoption studies have shown that there is a strong genetic component in the development of schizophrenia. The aim of family studies is to determine whether a disorder aggregates in families by comparing the frequency of the disorder in relatives of cases and controls. The first major and methodologically sound family studies of schizophrenia were conducted in the 1980s. Kendler and Diehl (1993) reviewed seven family studies of schizophrenia from between 1985 and 1993. The percentage of first-degree relatives of individuals with schizophrenia that had the disorder ranged from 1.4 to 6.5. The percentage of relatives of controls with schizophrenia was consistently lower, from 0 to 1.1. In all but one of these studies, the difference in aggregation was significant. Kendler and Diehl combined the

samples from these studies and found that 4.8% of relatives of schizophrenics in this combined sample had schizophrenia, resulting in a 9.7 times greater risk of schizophrenia in a relative of an individual with schizophrenia compared to a relative of a control.

Table 1 shows the average risk of schizophrenia for various relatives of people diagnosed with the disorder. These risks, from Gottesman (1991) are based on family and twin studies from 1920 to 1987. The major trend in this table is a consistent increase in risk as the degree of relationship becomes closer. For example, second-degree relatives of individuals with schizophrenia have on average just over a 4% risk of developing schizophrenia, and first-degree relatives have on average just over an 11% risk of the disorder.

There are some other interesting observations that can be made from this table. One of these observations is that the risk for children of individuals with schizophrenia is twice as high as the risk for parents. Since parents and children of individuals with schizophrenia would both share half of their genetic material with the proband, this observation is somewhat unusual. One likely reason for this is the reduced fertility associated with schizophrenia, which will be discussed in the next chapter. The lower fecundity of individuals with schizophrenia would make it less likely for a child to have a parent affected with the disorder. Another factor potentially contributing to this phenomenon is the role of de novo variants in schizophrenia, which would result in schizophrenic children being born to unaffected parents.

Another interesting observation is the higher risk in dizygotic twins of probands compared to siblings. Dizygotic twins and siblings both share on average half of their genetic material, so again more similar risks would be expected. The increased risk in dizygotic twins is likely due to the prenatal environment shared by twins but not regular siblings. Prenatal environment could

plausibly play a role in the development of schizophrenia through effects on brain development, and there is some evidence, which will be discussed in the next chapter, that it does.

Since families share environmental exposures as well as genes, other methods besides assessment of relative risks are needed to separate the effects of these two sets of factors. Twin studies compare the correlation for a trait within monozygotic (MZ) and dizygotic (DZ) twin pairs. Monozygotic twins are formed when a single egg is fertilized and splits into two zygotes. Since they are formed from the same egg and sperm, MZ twins are genetically identical. Dizygotic twins are formed by the fertilization of two different eggs by two different sperm. Therefore they only share on average half of their genes, like regular siblings. Since both types of pairs supposedly share the same prenatal, familial, and cultural environmental influences and MZ twins share twice as much genetic information as DZ twins, any significant increase in correlation from DZ to MZ pairs is attributed to genetic influences.

Twin studies have consistently found a larger correlation in schizophrenia diagnosis for MZ twins compared to DZ twins. Table 1 shows the risk of schizophrenia to be on average 48% for MZ twins and 17% for DZ twins. However, correlations have varied widely between studies. An early study by Kallman (1946) found correlations of .86 in MZ twins and only .15 in DZ twins. Fischer et al. (1969) calculated correlations of .56 for MZ twins and .26 for DZ twins. Onstad et al. (1991) found correlations of .48 in MZ twins and .04 in DZ twins. Cannon et al. (1998) calculated correlations of .84 and .34 for MZ and DZ twins respectively. Even though the exact percentages differ, all of these studies show a large genetic influence on schizophrenia. The most commonly accepted heritability estimate, which is the proportion of variance in a trait in a population due to genetic variance, of schizophrenia is

Table 1: Risk of Schizophrenia for Relatives of Probands

Relationship	Lifetime Risk of Schizophrenia
First cousin	2%
Uncle/Aunt	2%
Nephew/Niece	4%
Grandchild	5%
Half sibling	6%
Child	13%
Sibling	9%
DZ twin	17%
Parent	6%
MZ twin	48%

around 80%, which has been found in meta-analyses of twin studies (Cardno & Gottesman, 2000; Sullivan et al., 2003). Since these meta-analyses have larger sample sizes and do not have the methodological issues of earlier studies, this is viewed as a robust heritability estimate for the disorder.

Adoption studies have also been used to separate the effects of genes and environment on the etiology of schizophrenia. There are two major categories of adoption studies. The first type, high-risk adoptee studies, examines the rates of schizophrenia in children adopted away from schizophrenic mothers. Heston et al. (1966) studied 47 children of schizophrenic mothers and 50 children of mothers without schizophrenia in foster homes. Five of the 47 children of schizophrenic mothers developed schizophrenia compared to none of the children of control mothers. Rosenthal et al. (1968) also found higher rates of schizophrenia in the adopted away children of mothers who developed schizophrenia after the adoption. Since the biological parents in this study did not have mental illness at the time of adoption, it is not likely that the increased schizophrenia rates in their children were due to being raised by a schizophrenic parent. These studies support the hypothesis that the increased risk of schizophrenia in offspring of people with schizophrenia is due to genetic rather than postnatal environmental factors. However, prenatal factors may also be partially responsible for these results.

The second type of adoption studies, adoptees' family studies, find adopted children that have developed schizophrenia and study rates of schizophrenia in their biological relatives. For example, the Danish-American adoption studies compared schizophrenic adoptees to control adoptees without schizophrenia and found higher rates of schizophrenia in the biological relatives of schizophrenic adoptees versus the biological relatives of control adoptees (5.0% compared to 0.4%) (Ingraham & Kety, 2000). These results show that genetic relation to an

individual with schizophrenia increases risk of developing the disorder even in the absence of shared environmental factors.

Evidence of Complex Inheritance

Since a large role for genetic factors in the etiology of schizophrenia has been shown, one of the goals of schizophrenia research is to find the genes involved in the disorder. Finding the genes involved in schizophrenia will help predict risk of developing the disorder for individuals and allow for the determination of the mechanisms responsible for the disorder, possibly providing targets for drug therapy treatment. An important part of this process is determining the genetic architecture, which includes the number of genes involved, their frequencies and effect sizes, and how they interact.

Two major categories of genetic disorders are single-gene and complex disorders. Single-gene disorders are, as the name implies, caused by mutations on one gene. Several different patterns of inheritance are found for these disorders, depending on whether one or two copies of the mutant allele are needed and whether the gene is located on an autosomal chromosome, a sex chromosome, or within mitochondrial DNA. Complex disorders result from variants in multiple genes, and environmental factors can be involved as well. They do aggregate in families but do not show the clear patterns of inheritance seen in single-gene disorders.

Several lines of evidence suggest that schizophrenia is a complex disorder. For one, the results of family studies are inconsistent with a single-gene model. According to James (1971), risk for a single-gene disorder will drop 50% for every degree of relationship away from a proband. For example, if a first-degree relative of a proband has a 50% chance of developing a single-gene disorder, a second-degree relative will have a 25% risk. In schizophrenia, the drop in risk

between first and second-degree relatives is from about 11% to about 4% (Gottesman, 1991), which is greater than a 50% drop and suggestive of multi-gene inheritance. This dramatic decrease in risk is reflected in the lack of large pedigrees with many cases, which are common for single-gene disorders. In fact, it is likely that at least 60% of schizophrenia cases are sporadic, meaning no first or second-degree relatives are affected (Gottesman & Erlenmeyer-Kimling, 2001).

The relatively high prevalence of schizophrenia is also inconsistent with a single-gene model. As will be discussed in the next chapter, schizophrenia has negative effects on longevity and fertility. If a single gene were responsible for schizophrenia, that gene would be expected to face strong selective pressure. This would lower the frequency of the disorder to levels typical of severe single-gene disorders. For example, Huntington's disease occurs in only 5-7 out of 100,000 individuals (Walker, 2007), a much lower frequency than about 0.30-0.66% of individuals diagnosed with schizophrenia (McGrath et al., 2008).

The results of linkage studies also imply a complex pattern of inheritance for schizophrenia. In a linkage study, markers throughout the genome are used to detect chromosomal regions that segregate with a particular trait in a family. These studies work particularly well for single-gene disorders, because pedigrees with multiple affected individuals can be found, and also because the same region will segregate with the disorder in most families. The exception would be locus heterogeneity, where there are multiple risk loci for a disorder, but only one locus is responsible for much of the risk for the disorder in any given patient or family. Linkage studies led to the successful identification of the genes involved in Huntington's disease (Gusella et al., 1983), polycystic kidney disease (Reeders et al., 1985), and cystic fibrosis (Beaudet et al., 1986), among others. Several significant results have been found from linkage studies for schizophrenia.

However, there has been little replication of results between studies. This lack of consistency is reflected in the observation that in 31 schizophrenia linkage studies conducted before 2008, no regions have been significantly linked in more than 4 studies, and 58% of the genome has been significantly linked once (Sullivan et al., 2008). A meta-analysis of 32 genome-wide linkage studies, which included 3,255 pedigrees, found nominally, but not genome-wide, significant evidence for regions of chromosomes 1, 2q, 3q, 4q, 5q, 8p, and 10q (Ng et al., 2009). This meta-analysis had sufficient power to detect a small number of genes with large effects. Therefore, it appears from the lack of genome-wide significant results that one or a few highly penetrant genes are not responsible for schizophrenia.

Debate over Genetic Architecture

The evidence above has reinforced the fact that schizophrenia is a complex disorder, with many genetic and environmental factors, which will be discussed in Chapter 2, involved in its etiology. However, determining ideal methods for finding causal genetic variants requires knowledge of the frequencies and effect sizes of the variants. This has led to a debate over the genetic architecture of the disorder. The two primary hypotheses in this debate are the common disease-common variant (CDCV) and common disease-rare variant (CDRV) hypotheses. This debate is not unique to schizophrenia, and is commonly discussed in the context of other complex disorders.

The CDCV hypothesis suggests that complex diseases are caused mainly by common variants. Although the general definition of a common variant is simply a frequency greater than 1%, these variants are believed to be found in most populations. They are also believed to have small effect sizes, usually with odds ratios (OR) less than 2.An odds ratio is a ratio of the odds of an

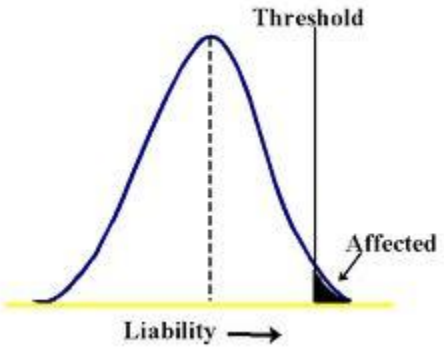
event occurring in two separate groups. For example, if a disease occurs in 90% of people with allele *A* of a gene and in 80% of people with allele *a* of the same gene, the odds ratio for allele *A* is:

$$(0.9/0.1)/(0.8/0.2)=2.25$$

The CDCV hypothesis also posits that the genes involved in a complex trait can act in either an additive or multiplicative manner. An additive model results in a linear increase in risk for every additional risk allele without interaction between risk alleles, whereas a multiplicative model leads to a non-linear increase in risk with increasing alleles and assumes interaction. A purely additive model of polygenic inheritance would suggest a liability threshold model for schizophrenia. This model states that risk for a disorder is a normally distributed variable consisting of many genetic and environmental factors and that the disorder develops when an individual's risk is over a particular threshold. Figure 1 illustrates this model. However, a study by McGue et al. (1985) tested the fit of this model with a goodness-of-fit test to family data sets and found only marginal evidence of the model fitting the data ($p = 0.066$), suggesting that while not significantly different from the data, the threshold liability model does not fit it well.

One early paper supporting the CDCV hypothesis was by Lander (1996), who gave some early examples of common variants involved in disease, including APOE4 in Alzheimer's, ACE in heart disease, and CKR-5 in HIV resistance. Lander also discussed the prospect of using a collection of common variants in association studies for diseases. These studies, which search for risk alleles by comparing allele frequencies between cases and controls, will be discussed in depth in Chapter 3. Reich and Lander (2001) concluded from mathematical models that if

Figure 1: Liability Threshold Model



(from www.uic.edu)

This is an illustration of the liability threshold model. The horizontal axis represents liability for a disorder, which is assumed to be normally distributed. This liability includes genetic and environmental risk factors. The threshold is the point at which liability becomes high enough to result in affection with the disorder.

selection for a disease susceptibility allele is low, which would be the case for variants with small effect sizes, the frequency of the allele should decrease very slowly.

This would allow relatively high frequencies to be maintained over a long time. Some researchers, including Pritchard (2001), hypothesize that evolutionary processes are unlikely to lead to common risk variants, and instead propose the CDRV hypothesis. This hypothesis states that complex diseases are caused by rare variants, often family or individual specific, with moderate effect sizes, generally with ORs between 5 and 20. It also suggests that there is substantial allelic heterogeneity, meaning there are multiple alleles at a single locus that can cause a disease. Some examples of rare variants that have been found to increase risk for complex disorders include variants in the APC gene that increase risk for colorectal cancer (Azzopardi et al., 2008) and variants in ABCA1 that cause low levels of HDL cholesterol (Cohen et al., 2004).

One early paper supporting the CDRV hypothesis (Pritchard, 2001) showed that the mutation rate at many loci for complex diseases would be higher than the selection rate for these loci. This would result in several disease alleles forming at a single locus, with none of them having a high frequency. However, the models in this paper assumed that the allele frequencies have reached equilibrium in the population, meaning the frequencies are relatively stable.

Additional evidence supporting of the CDRV hypothesis is the observation that association studies for common variants have only been able to explain a small portion of the heritability for most complex traits. For example, the genes that were associated with height in three large GWASs only accounted for about 5% of the heritability of height, which is 90%, leaving about

85% of the heritability unexplained (Maher, 2008). These small percentages leave a lot of room for rare genetic variants that cannot be detected through association studies.

The importance of this debate lies in the fact that common and rare variants are best detected by different methods and are important in different ways. Association studies work well for detecting common variants, since they can be detected in samples with a few thousand cases, depending on the effect sizes of the variants. The fact that association studies should work well for common variants with odds ratios above 1.5 contributed to the popularity of the CDCV hypothesis and GWAS methods. Also, common variants contribute to risk in large numbers of people since they have high frequencies. Since genotyping arrays are not well designed for detecting rare variants, association studies are not a very suitable method for identifying risk-increasing rare variants. Instead, sequencing of cases is considered the best method for finding rare variants. Since they are uncommon, rare variants cannot be used to identify large numbers of people at risk for the disorder. However, genes that contain a rare risk variant for a disorder may contain additional risk variants and be good candidate genes for future studies. Also, a detected rare variant is more likely to be the actual variant responsible for the disorder, since rare variants are more likely to be functional (Gorlov et al., 2008).

These two hypotheses also differ in how they explain the relatively high prevalence of schizophrenia. As will be discussed in the next chapter, schizophrenia results in significant impairments in survival and reproduction. Due to these impairments, it is puzzling that natural selection has not reduced the frequency of the disorder to lower levels. Proponents of the CDRV hypothesis argue that this is due to new risk-increasing mutations occurring often, leading to many variants that individually increase risk in a small number of people (Pritchard, 2001). Supporters of the CDCV hypothesis believe that schizophrenia remains common because the

variants involved in schizophrenia face weak natural selection due to their small effect sizes. This would lead to their frequencies, and the prevalence of schizophrenia, being reduced very slowly. This argument is supported by the results of Reich and Lander (2001) mentioned above.

Another possible reason for the high frequencies of some SNPs that increase risk for schizophrenia is balancing selection, meaning that an allele that increases risk may also be beneficial in some instances. The classic example of this concept is the allele causing sickle cell anemia. Individuals with one copy of the risk allele do not develop the disorder but do have increased resistance to malaria. This has resulted in increased frequencies of sickle cell anemia in areas where malaria is common.

A few different examples of advantages in biological relatives of individuals with schizophrenia have been proposed. One of these was an increase in fertility in relatives. Carter & Watts (1971) compared 64 relatives of schizophrenia patients to 88 relatives of individuals without schizophrenia. They observed an average of 2.88 children in relatives of schizophrenia patients compared to 2.11 children in relatives of controls ($p = 0.0032$). However, this pattern has not been replicated in most other studies and is not generally supported (Haukka et al., 2003; Svensson et al., 2007; Bundy et al., 2011).

Carter & Watts (1971) also observed a lower incidence of viral infections in relatives of schizophrenia patients. After correcting for age, since children are more likely to acquire viral infections, they found 93 viral infections in 77 relatives of schizophrenia patients. The expected incidence in this group based on the rate in relatives of controls (not given) was 125 viral infections. This difference was statistically significant ($p = 0.0047$).

Another possible advantage in relatives of schizophrenia patients may be creativity or giftedness. Karlsson (1970) compared relatives of schizophrenia patients to members of three kindred groups in Iceland. He observed that relatives of schizophrenia patients were almost twice as likely to be listed in the book Who Is Who in Iceland, a listing of individuals that have made important contributions to Icelandic culture and society. In his study of children adopted away from mothers with schizophrenia, Heston (1966) found that several of the children who did not develop schizophrenia were exceptionally creative. However, these findings were anecdotal and not statistically tested. A recent study by Kyaga et al. (2011) found that siblings and parents of schizophrenia patients were more likely to have creative occupations.

One important fact to note is that the CDCV and CDRV hypotheses are not mutually exclusive. It is possible that both of these types of variants are involved in the etiology of the same trait, and there is some empirical evidence for this. For example, Johansen et al. (2010) sequenced genes with common variants that were associated with plasma lipid concentrations and found many rare variants as well. It is possible that a similar pattern of observations could be found in schizophrenia.

In conclusion, genetic epidemiology studies have shown that genes play an important role in the etiology of schizophrenia and that it is a complex genetic disorder. However, there is debate over whether the variants involved are common with small effect sizes or rare with larger effect sizes.

Chapter 2: Overview of Schizophrenia

Schizophrenia is a severe psychotic disorder that affects almost 1% of the population. It impairs functioning and productivity and increases mortality in those affected. Schizophrenia is also a complex disorder, with multiple genetic and environmental factors involved in its etiology. Improving treatment for schizophrenia will require increased understanding of these causal factors.

Symptoms

Schizophrenia includes several symptoms, both positive and negative. Positive symptoms are symptoms present in individuals with schizophrenia, but not in most people. Negative symptoms are deficits in normal functions, such as communication and motivation. The negative symptoms in particular impair the functionality of people with schizophrenia. Also, negative symptoms are more resistant to treatment.

Positive symptoms of schizophrenia include delusions, hallucinations, and thought disorder. Hallucinations are sensory perceptions of stimuli that do not exist. The most common hallucination in schizophrenia is hearing voices. Delusions are fixed false beliefs that are strongly held despite a lack of basis in reality and evidence to the contrary. For example, an individual with schizophrenia may believe there is a code hidden within a book. Often, the delusions of individuals with schizophrenia are bizarre, meaning they are completely implausible

for example, believing one's brain has been taken. Thought disorder consists of disorganized thinking expressed through incoherent speech patterns, such as nonsensical combinations of words. An example of this would be "Colorless ideas sleep furiously."

Negative symptoms, traits which are absent or deficient in people with schizophrenia, include blunted affect, alogia, anhedonia, avolition, and asociality. Blunted affect consists of a lack of emotional expression through verbal or non-verbal means. Alogia is a lack of unprompted speech in conversation. Anhedonia is the inability to find pleasure in normally enjoyable activities. Avolition consists of a lack of motivation to achieve goals. This results in the individual being disengaged from work or sitting still for a long time. Asociality is a lack of desire or ability to form close relationships with others.

Diagnosis

According to the DSM-IV-TR, three criteria are needed for a diagnosis of schizophrenia. First, the individual must have two or more of the following symptoms: hallucinations, delusions, thought disorder, and any of the negative symptoms listed above. Exceptions to this requirement include presence of bizarre delusions or auditory hallucinations where a voice is narrating the individual's actions or two or more voices are conversing. In these cases, a second symptom is not required.

The second criterion for diagnosis of schizophrenia is evidence is dysfunction. To meet this, the individual must show significant impairment in work, interpersonal relationships, and/or self-care. The third consideration is duration. The dysfunction must last at least six months, with at least a month of symptoms being present. Cases lasting less than a month are considered brief

psychotic disorder, and cases lasting between one and six months are classified as schizophreniform disorder.

There are also five subtypes of schizophrenia recognized in the DSM-IV-TR. Paranoid schizophrenia consists of hallucinations and delusions without thought disorder and blunt affect. Individuals with disorganized schizophrenia have both thought disorder and blunt affect. In catatonic schizophrenia, the individual may be immobile or may have random, agitated movements. In undifferentiated schizophrenia, delusions and hallucinations are present, but the three previous subtypes do not fit. Residual schizophrenia consists of low levels of the positive symptoms. However, some experts are suggesting that these subtypes should not be included in the DSM-V (Pierre, 2008).

Overlap with Other Psychiatric Disorders

There are several other disorders besides schizophrenia that include symptoms of psychosis. Schizophreniform disorder and brief psychotic disorder, which have the same symptoms as schizophrenia but for a shorter duration, are mentioned above. Other diagnoses of psychotic disorders include schizoaffective disorder and delusional disorder.

Individuals with schizoaffective disorder experience mood episodes as well as the psychotic and negative symptoms of schizophrenia. These mood episodes can be either manic or depressive. During a manic episode, the individual feels overly happy and in some cases agitated. He may also be easily distracted and engage in impulsive actions, such as a spending spree or an unplanned remodeling. Individuals in depressive episodes feel empty and do not enjoy activities they once did. Also, they have trouble concentrating and remembering and may think about or attempt suicide.

Delusional disorder is characterized by non-bizarre delusions, such as believing someone famous is in love with the person or someone is spying on the person. However, there are not visual or auditory hallucinations. Also, individuals with delusional disorder do not have the negative symptoms of schizophrenia and are often high-functioning.

Psychotic symptoms have also been associated with mood disorders. Bipolar disorder includes episodes of mania and depression. During these episodes, individuals may experience psychotic symptoms. The affective and psychotic symptoms are similar to schizoaffective disorder. However, individuals with bipolar disorder do not have the negative symptoms found in schizophrenia and schizoaffective disorder. Also, diagnosis of schizoaffective disorder requires the presence of psychosis outside of mood episodes, which does not occur in bipolar disorder. In rare and severe cases, individuals with major depressive disorder may also experience psychosis.

In addition to the common symptom of psychosis, schizophrenia and bipolar disorder are also similar in other aspects. Although bipolar disorder is slightly more common than schizophrenia with a lifetime prevalence of 3.9% (http://www.nimh.nih.gov/statistics/1BIPOLAR_ADULT.shtml), both disorders have typical onset in early adulthood. Also, both disorders are associated with increased risk of suicide.

This symptomatic overlap, in particular the overlap between schizophrenia, schizoaffective disorder, and bipolar disorder, presents a challenge for research of these disorders. It is not uncommon for an individual with one of these disorders to be misdiagnosed with another. For example, in a study by Werry et al. (1991) that examined early-onset cases of these disorders, 55% of subjects who were diagnosed with schizophrenia before the study were diagnosed with bipolar disorder during the study. There is also some genetic overlap, which will be discussed

later, that has led some to believe that the diagnostic boundaries of these disorders may not be valid (Craddock & Owen, 2005; Lichtenstein et al., 2009).

Another disorder that has shown a degree of overlap with schizophrenia is autism. A study by Konstantareas and Hewitt (2001) interviewed 14 schizophrenia patients and 14 autism patients to determine if they also met criteria for the other disorder. They found that seven of the individuals with autism met criteria for schizophrenia, in particular for the disorganized subtype. Five of these patients displayed positive symptoms of schizophrenia, including bizarre behavior and thought disorder. Six of the autism cases showed negative symptoms of schizophrenia, including blunted affect, alogia, attention difficulties, and asociality. Spek & Wouters (2010) also observed negative symptoms in both a sample of 21 individuals with schizophrenia and 21 individuals with autism. However, it should be noted that these studies have very small sample sizes.

Prognosis and Lifestyle

The prognosis of schizophrenia is highly variable. A meta-analysis of schizophrenia outcome studies found that 40% of people with schizophrenia achieved social recovery, meaning they were financially independent, lived on their own, and had minimal social disruption. Also, 20% of people with schizophrenia had complete recovery, which means loss of psychotic symptoms and normal level of functioning (Warner, 2009).

Hospitalization is required in a portion of schizophrenia cases. Whitehorn et al. (2004) examined rates of hospitalization at time of diagnosis and within the first year after diagnosis. They found that 54% of patients were in the hospital when they were initially diagnosed, and 17% of patients required hospitalization within a year after diagnosis. These percentages are similar to other studies (Sipos et al., 2001; Hickling et al., 2001; Malla et al., 2002). Individuals who presented

manic or negative symptoms or have had untreated schizophrenia for a longer period of time were more likely to be admitted (Sipos et al., 2001).

Individuals with schizophrenia also tend to have lower marital and fecundity rates, especially males (Nimgaonkar, 1998; Hutchinson et al., 1999; Bundy et al., 2011). This is likely due to the effects of schizophrenia on behavior, which would make establishing a relationship more difficult. The stronger effect on males could be explained by the earlier onset of schizophrenia in males. This would make the disorder more likely to occur before the opportunity for marriage and reproduction arises.

Schizophrenia also results in increased mortality, with people with schizophrenia dying 12-15 years younger than the general population (Saha et al., 2007). There are several factors involved in this increased mortality. Individuals with schizophrenia generally have unhealthier diets, with more saturated fat and fewer fruits and vegetables (McCreadie et al., 2003; Ryan et al., 2003). This diet, along with the side effects of anti-psychotic medications, can result in increased rates of obesity and diabetes. People with schizophrenia also have higher rates of smoking, which also raises mortality through cardiovascular and respiratory problems. Suicide is also a contributor to this increased mortality, as 10% of people with schizophrenia commit suicide, and estimates of attempted suicide range from 18-55% (Siris, 2001). Another factor in the increased mortality appears to be homicide. Hiroeh et al. (2001) found that schizophrenic individuals were more likely to be murdered than people in the general population.

Epidemiology

The prevalence of schizophrenia is 0.30-0.66%, and men are more likely to be affected with a male: female ratio of 1.4:1 (McGrath et al., 2008). Schizophrenia also occurs earlier in males,

with peak risk for males between ages 20 and 28 and peak risk for females between 26 and 32 (Castle et al., 1998). According to Loranger et al. (1984), about 91% of men and 66% of women have onset before 30, and 17% of women have onset after 35 compared to 2% of men. Roy et al. (2001) found that men are also 1.75 more likely to develop deficit schizophrenia, which is defined as schizophrenia with at least two negative symptoms lasting six months.

There are some cases of schizophrenia with onset before or after the age range of highest risk. The prevalence of schizophrenia with onset before 15 is estimated to be about 0.14 in 1,000 (Eggers & Bunk, 1997). Childhood onset schizophrenia is similar to adult onset schizophrenia, and similar diagnostic criteria can be reliably used (Kumra et al., 2002). However, individuals with childhood onset schizophrenia tend to have greater social impairment (Eggers & Bunk, 1997). However not all evidence is consistent, including one study that estimated that 23.5% of individuals with schizophrenia had late-onset schizophrenia, defined by onset after age 40 (Howard et al., 2000).

Treatment

There are two main classes of medication for schizophrenia, typical and atypical antipsychotics. Both of these classes of medication primarily act by blocking dopamine receptors in the brain. A meta-analysis by Leucht et al. (2009) found that some atypical antipsychotics, including amisulpride, clozapine, olanzapine, and risperidone, were more effective than typical antipsychotics in treatment of symptoms, but for other atypical antipsychotics there was not a difference. Some of the side effects of these medications include motor control disabilities, particularly with haloperidol, weight gain, and sedation.

There is a substantial portion of people with schizophrenia, about 30%, that do not respond to antipsychotics (Meltzer, 1997). These patients tend to have earlier onset of the disorder and poorer function before onset. Also, some patients do not require medication for recovery (Fenton & McGlashan, 1987).

Various forms of therapy have also been used in the treatment of schizophrenia with some success. Cognitive behavioral therapy has been shown to have some success in reducing psychotic symptoms (Zimmerman et al., 2005; Wykes et al., 2008). Cognitive remediation therapy is used to reduce the cognitive deficits found in schizophrenia. It can lead to improvements in memory and executive function (Wykes et al., 2002). Family therapy, which aims to help the individual and their family cope with the condition, has been shown to reduce symptoms and improve functioning (Giron et al., 2010).

Environmental Risk Factors

Despite the large genetic influence on schizophrenia discussed in Chapter 1, environmental factors have also been shown to play a role in the etiology of the disorder. Prenatal and perinatal factors appear to be involved in some cases of schizophrenia. Cannon et al. (2002) performed a meta-analysis of population-based studies on obstetric complications. They found that complications during pregnancy, such as bleeding, preeclampsia, diabetes, and rhesus incompatibility, were associated with schizophrenia. Delivery complications, including asphyxia, emergency Caesarian section and uterine atony, and problems with fetal growth, including low birth weight, congenital deformities, and small head circumference, were also found more often in individuals with schizophrenia than controls (Cannon et al., 2002). The odds ratios for these risk factors are shown in Table 2. Khashan et al. (2008) found that increased psychological stress

Table 2: Odds ratios of Prenatal and Perinatal Factors from Cannon et al. (2002)

Complication	Odds Ratio
Maternal diabetes	7.76
Birth weight < 2,000 grams	3.89
Emergency Caesarean section	3.24
Congenital deformities	2.45
Uterine atony	2.29
Rhesus incompatibility	2.00
Asphyxia	1.74
Bleeding during pregnancy	1.69
Head circumference < 32 cm	1.38
Preeclampsia	1.36

to the mother during the first trimester was associated with an increased risk for schizophrenia (risk ratio = 1.67). According to Brown (2006), several infectious agents, including rubella (10 to 20-fold increase), influenza (3-fold increase), and toxoplasmosis (2.5-fold increase), can increase risk for schizophrenia if the mother is infected during pregnancy. Brown and Susser (2008) examined schizophrenia rates in individuals born during two major famines and found that rates increased two-fold, suggesting a role for prenatal nutrition. These findings of the involvement of prenatal and perinatal factors support the idea that neurodevelopment plays a role in schizophrenia, despite the usual adult onset of the disorder. These findings of prenatal influence may also explain the higher risk of schizophrenia in DZ twins of probands than in siblings, as mentioned before.

Other environmental factors have also been associated with schizophrenia. Krabbendam and van Os (2005) found that rates of schizophrenia are twice as high in urban areas compared to rural areas. It is not known what factors are responsible for this association, but some believe it may be due to a lower level of trust and bonding between people in urban areas. Another possible explanation is that individuals with schizophrenia are more likely to move to cities, where they can live anonymously. First and second generation immigrants are also more likely to develop schizophrenia, with an odds ratio of 2.9 (Cantor-Graae & Selton, 2005). This odds ratio was higher (4.8) for dark-skinned individuals migrating to largely Caucasian countries, suggesting that social issues such as discrimination may play a role.

In conclusion, schizophrenia has several symptoms, including hallucinations, delusions, and deficits in emotional expression, sociality, and speech. Functioning is often significantly impaired, resulting in reduced fertility and increased mortality. Medication and therapy have had

some success in treatment of the disorder, but determining the genetic variants involved may help in understanding the pathology of schizophrenia and improving treatment.

Chapter 3: Evidence for Common Variants

The most used method for finding common variants is association studies, which compare the frequencies of genetic variants between cases and controls. At first limited to candidate genes, these studies can now be performed on a genome-wide scale. This ability to systematically search for common variants has led to several associations of genes with schizophrenia.

Methods for Finding Common Variants

Since by definition common variants have relatively high frequencies in the population (greater than 1%), association studies are a useful method for their detection. In these studies, SNP frequencies are compared between cases and controls, which can come from either population or family samples. A SNP is a base pair in the DNA sequence where the nucleotide present differs between individuals. For example, some people may have an adenine nucleotide at a particular site and other individuals may have a guanine nucleotide. A SNP in an exon can change the protein that is produced by the gene by changing an amino acid or prematurely stopping transcription, particularly if it is in the first or second position of the nucleotide triplet. SNPs in the third position of the triplet are often synonymous and do not change the amino acid produced. SNPs that are not within exons, but in regulatory regions, can also affect phenotypes, mainly by affecting gene expression. The mutation rate for SNPs is about 2.0×10^{-8} per base pair per generation, which when extrapolated to the entire genome results in every individual having approximately 128 de novo SNPs (Nachman & Crowell, 2000; Kondrashov, 2003).

In many cases, the associated SNP is not actually responsible for the disorder, but is in linkage disequilibrium with the causal variant, meaning these alleles are found together more often in the population than expected by chance. Due to recombination, linkage disequilibrium only extends over short distances, about 50-60 kb in European populations and 5 kb in African populations (Reich et al., 2001). Since linkage disequilibrium extends over short distances, hundreds of thousands of markers are needed to account for the majority of variation due to SNPs in the genome. For example, 250,000 SNPs capture 85% of the variation in European populations, with more needed for African populations because of lower LD (Barrett & Cardon, 2006). Until about 2005, genotyping technology did not allow very large numbers of SNPs to be genotyped feasibly, so the first association studies focused on candidate genes for a disorder. These candidate genes were selected either because their function suggested involvement in the disorder or because they were located in regions that had been implicated in linkage studies. Then association tests would be performed for selected SNPs within those genes.

There are several methods that can be used for selecting functional candidate genes. If the biological pathways involved in a disease are known, genes involved in those pathways are often strong candidates. Gene expression studies can be used to find genes that are differentially expressed in the disorder and may be candidate genes. If certain environmental agents, such as chemicals, increase risk for a disorder, genes involved in the processing of these chemicals can be examined. The same logic applies for medications that interfere with the pathology of a disorder.

Another challenge of candidate gene studies is selecting the SNPs within a gene to test for association. In most association studies before GWAS, the most highly prioritized SNPs are the ones located in exons that either change the amino acid produced or stop transcription. SNPs that

are located in regulatory regions are also selected, since they may change expression of the gene. Linkage disequilibrium is also considered when selecting SNPs for testing. If a group of SNPs is in linkage disequilibrium, only one of the SNPs is selected since the genotypes of the other SNPs can be estimated from the genotype of one.

With the improvement of genotyping technology and the systematic characterization of common variation and linkage disequilibrium across the genome by the HapMap project, genome-wide association studies (GWAS) became feasible. These studies use genotyping arrays that contain probes for hundreds of thousands of SNPs. Instead of being limited to testing genes that were hypothesized to be involved in the trait of interest, researchers could now test SNPs throughout the genome for association without a priori hypotheses.

Some GWAS were performed in the early 2000's for traits including myocardial infarction, diabetic nephropathy, and osteoarthritis. However, these studies are not considered true GWAS studies because they genotyped fewer than 100,000 SNPs, and therefore failed to test most of the common variation across the genome. Also, these studies used PCR for SNP genotyping instead of genotyping arrays and only used SNPs located within genes.

The first GWAS generally recognized as such was performed in 2005 by Klein et al. This study included 96 cases with age-related macular degeneration and 50 controls and genotyped 116,204 SNPs. They found an association between age-related macular degeneration and complement factor H.

Phase I of the International HapMap Project was also completed in that year. This project examined the linkage disequilibrium patterns around SNPs identified by the Human Genome

Project. Identifying these LD patterns allowed genotyping arrays to more efficiently cover the genome since many SNPs can be indirectly genotyped due to LD with other SNPs.

One landmark GWAS (Burton et al., 2007) was performed by the Wellcome Trust Case Control Consortium to study several complex diseases, including bipolar disorder, coronary artery disease, Crohn's disease, hypertension, rheumatoid arthritis, type 1 diabetes, and type 2 diabetes. This study used 2,000 cases for each disease and a set of 3,000 shared controls and found association for 24 different loci. Burton et al. also addressed several issues related to GWAS studies, including screening controls, using shared controls for multiple analyses, and sample size. These early successes were an encouraging sign that GWAS may help identify genes involved in psychiatric disorders, including schizophrenia.

Despite these successful findings, there are several issues that need to be considered in association studies. One issue is that large samples are needed for power to detect associated SNPs that have small effect sizes. To reliably detect a common SNP with an OR between 1.5 and 2, 1,000 to 2,000 cases are needed, and reliable detection of SNPs with ORs between 1 and 1.5 requires 8,000 to 20,000 cases (Psychiatric GWAS Consortium, 2009). One feasible way to obtain sample sizes that large is through combining multiple samples in a meta-analysis. In a meta-analysis, the effect sizes of multiple studies are combined through a regression model. This provides one effect size with a larger sample size, increasing power. Also, in GWAS specifically, since hundreds of thousands of tests are being performed, a more stringent probability threshold is needed to maintain the same genome-wide level of significance. A Bonferroni corrected p-value of approximately $5x10^{-7}$ is considered appropriate for GWAS studies (Burton et al., 2007).

Another issue that association studies must deal with is population stratification. Population stratification arises when there is a difference in allele frequencies and a difference in disease prevalence between populations. For example, frequencies of the C allele at a locus in TCF7L2 range from 0.013 to 0.488 in 11 different HapMap populations (Adeyemo & Rotimi, 2010). To avoid issues with major differences in ancestry, association studies commonly use just one racial group, such as only Caucasians or only African-Americans. More subtle differences can be corrected through statistical methods. One method, genomic control, compares the frequencies between cases and controls of loci that are not connected to the trait. An overall genomic inflation factor is calculated from these frequencies and adjusts the results by this factor. However, the genomic inflation factor used may not be accurate for all SNPs since population differences in allele frequencies are not uniform between SNPs. Another common method for correcting population stratification is the Eigenstrat method. This method uses principle components analysis to determine axes of genetic variation and adjusts genotypes accordingly.

One statistical method of association testing that is robust to population stratification is the transmission disequilibrium test (TDT). This is a family-based design that requires trios of affected probands and their parents. The transmission of alleles from the parents to the offspring is measured. If a particular allele is transmitted more or less often than expected by chance, it is considered to be associated with the disorder. Since this is a within-family measure, population stratification is effectively controlled. However, family samples are harder to obtain than case-control samples, so obtaining sufficient power, particularly for GWAS, is more difficult.

Common Variants Found

Functional Candidate Gene Association Studies

As can be seen from the SzGene database (Allen et al., 2008), many genes were tested for association with schizophrenia through functional candidate gene studies. A large portion of these candidate genes were selected on the basis of the dopamine hypothesis of schizophrenia, which suggests the symptoms of schizophrenia are caused by overtransmission of dopamine in the brain. This hypothesis is popular because many of the drugs used to treat schizophrenia act by blocking dopamine receptors. Serotonin receptor genes have also been considered candidates because atypical antipsychotics show an affinity for these receptors. Some genes that have been tested on the basis of these hypotheses include DRD2, DRD3, and 5HTR2A. Neurotrophin genes, including BDNF, CNTF, and NT-3, have also been studied because of the role of neurodevelopment in schizophrenia. However, none of these functional candidates has been consistently replicated (Virgos et al., 2001: Allen et al., 2008). There were several issues with these studies that likely contributed to their lack of success. Since the proposed mechanisms for schizophrenia are highly speculative, it is possible that the candidate genes studied were poor choices. Also, in many of these studies only a few SNPs within each gene were tested, so a large portion of the variation in these genes may not have been tested. These studies often also had small samples, so they were underpowered for detecting associations with small effect sizes.

Positional Candidate Gene Association Studies

Other association studies have focused on positional candidate genes, which are selected because they are located in regions that have been implicated in linkage studies. These studies have had more success than functional candidate studies, with a two genes receiving fairly strong support.

Straub et al. (2002) found an association for the gene DTNBP1, within the 6p linkage peak. Other studies also found association with DTNBP1, but the associated markers differed between studies (Schwab et al., 2003; Williams et al., 2004; Kirov et al., 2004). Also, some studies have not found this association (Van Den Bogaert, 2003; Li & He, 2007; Sanders et al., 2008)

The association of neuregulin 1 (NRG1) with schizophrenia, which is within the 8p linkage peak and plays a role in neuronal development, was first reported by Stefansson et al. (2002). This association was replicated in some later studies (Stefansson et al., 2003; Corvin et al., 2004; Li et al., 2006). However, some other studies have not found this association (Iwata et al., 2004; Munafo et al., 2006; Sanders et al., 2008).

As will be seen in the following sections, DTNBP1 and NRG1 also have not been supported strongly in GWAS studies. One possible reason for this lack of support is that the multiple testing correction for GWAS is too high for the effects of these genes to be detected. Also, there may be issues with heterogeneity between study samples.

Pooled GWAS Studies

Several of the first GWAS used pooled genotype data, meaning a mixture of DNA from different cases was compared to a mixture of DNA from different controls. While cheaper, pooling samples also reduces power. Mah et al. (2006) used a discovery sample with 320 cases and 325 controls, as well as a replication sample with 200 cases and 230 controls. They found suggestive association with the gene PLXNA2 in the discovery (OR = 1.49, p = 0.06) and the replication (OR = 1.38, p = 0.07) samples. However, attempts to replicate this association have largely been unsuccessful (Sullivan et al., 2008; Budel et al., 2008). Kirov et al. (2008), in a study using 574 family trios, found 40 SNPs that had significant transmission to probands, using a threshold of p

< 0.05. The most significant p-value, $p = 1.2x10^{-6}$, was for a SNP in the gene CCDC60. Shifman et al. (2008) used a pooled sample of 660 cases and 2,771 controls for their initial GWAS and an individually genotyped sample of 745 cases and 759 controls to analyze their best results. They found an association with a marker in the gene RELN (OR = 2.1, $p = 9.8x10^{-5}$), but only in women. Liu et al. (2010) replicated this association in a sample of 721 cases and 1,455 controls, but other studies have not replicated this association.

Individual Genotyping GWAS

Lencz et al. (2007) performed the first schizophrenia GWAS with individual genotyping. This study used a sample of 178 cases and 144 controls. They found association with a marker near the gene CSF2RA (OR = 3.23, $p = 3.7x10^{-7}$). Another GWAS with individual genotyping, the CATIE study by Sullivan et al. (2008), which used a sample of 738 cases and 733 controls, did not find any genome-wide significant associations. However, it must be noted that these two studies, as well as the three pooled genotype studies mentioned above, were greatly underpowered for current estimates of effect sizes, with fewer than 1,000 cases.

Another GWAS with individual genotyping, by O'Donovan et al. (2008) also had a relatively small discovery sample, with 479 cases and 2,937 controls. However, they followed up their top results in two larger replication samples, with a total of 5,807 cases and 10,056 controls. They found an association with a marker in the gene ZNF804A, which fell just short of significance in the replication sample (OR = 1.09, $p = 9.25x10^{-5}$). However, this marker was found to be significant when the two replication samples were combined with the discovery sample (OR = 1.12, $p = 1.61x10^{-7}$). This finding was replicated in several later studies (Riley et al., 2010; Williams et al., 2010; Zhang et al., 2011; Xiao et al., 2011) and is considered the first

consistently successful finding of schizophrenia GWAS. The function of ZNF804A is not known, but as will be mentioned in Chapter 5, some studies have implicated the schizophrenia-associated SNP with brain functioning.

GWAS Meta-analyses

Three larger GWAS, with discovery samples of over 2,000 cases each, were published in 2009. Purcell et al. (2009) had 3,322 cases and 3,587 controls, and their strongest associations included a SNP in the gene MYO18B ($p = 3.4 \times 10^{-7}$) and many SNPs in the major histocompatibility (MHC) region, with the most significant having a p-value of 4.79×10^{-8}. Shi et al. (2009), with a sample of 2,681 cases and 2,653 controls, found suggestive evidence of association for the genes CENTG2 ($p = 4.59 \times 10^{-7}$), NTRK3 ($p = 8.10 \times 10^{-7}$), and EML5 ($p = 9.49 \times 10^{-7}$). Stefansson et al. (2009) used a discovery sample of 2,663 cases and 13,498 controls and a follow-up sample of 4,999 cases and 15,555 controls. They found suggestive evidence of association for three markers in the MHC region, with p-values between 3.1×10^{-6} and 4.9×10^{-7}.

These three groups then combined their samples to perform meta-analyses of their most significant results. This combined sample had a total of 8,008 cases and 19,077 controls. Each group achieved significant association for markers in the MHC region, although the specific markers that were associated varied between groups. The most significant SNP from Purcell et al. (2009) was located near a histone gene cluster in the MHC region and had a p-value of 9.5×10^{-9}. Shi et al. (2009) also obtained their most significant result with the same SNP ($p = 9.54 \times 10^{-9}$). The most significantly associated SNP in Stefansson et al. (2009) was located near the gene PRSS16 ($p = 1.4 \times 10^{-12}$), also in the MHC region. The odds ratios for these associated SNPs were small, generally in the range of 1-1.3. Stefansson et al. (2009), after adding extra

cases and controls for a sample of 12,945 cases and 34,591 controls, also found significant association for SNPs in the genes NRGN ($p = 2.4 \times 10^{-9}$) and TCF4 ($p = 4.1 \times 10^{-9}$). These odds ratios for the associated SNPs in these genes were also small, with an OR of 1.15 for NRGN and an OR of 1.23 for TCF4.

Another meta-analysis, by Chen et al. (2010), used 17,198 cases and 11,380 controls from 25 different population and family samples. The first stage of this study used two of these samples, totaling 1,658 cases and 1,655 controls, to identify markers of interest through GWAS. The 1,128 SNPs that had unadjusted p-values ≤ 0.05 in both samples were then ranked through a bioinformatic approach. SNPs that changed the resulting amino acid or were located within a schizophrenia gene were awarded 2 points. SNPs found within an evolutionary conserved region or an untranslated region, or at a transcription factor binding site received 1 point. Any SNP within 2 kb of a gene received 0.5 points. The most highly ranked SNPs were then tested for replication in other samples. The strongest associations found in this study were for two markers in the gene CMYA5. These two markers had ORs of 1.11 and 1.07 and p-values of 0.00082 and 0.00030. Another SNP within CMYA5 was found to be associated by Li et al. (2011) in a sample of 2,797 cases and 2,808 controls.

Evidence for Polygenic Inheritance

There is also strong evidence for a general pattern of polygenic inheritance for schizophrenia. Some of this evidence comes from the GWAS paper by Purcell et al. (2009) mentioned above. They used a discovery sample to select alleles that met increasingly liberal probability thresholds in association tests, such as alleles with an association p-value under 0.1 or 0.5. Risk scores were then calculated for individuals in a target sample based on the number of risk alleles they have at

each probability threshold, weighted by log odds ratio. The mean scores of cases and controls were then compared to see if cases had higher average risk scores.

The initial discovery sample included 2,176 cases and 1,642 controls, all male, and the initial target sample consisted of 1,146 cases and 1,945 controls, all female. As more liberal thresholds were used and more SNPs included, the predictive value of the risk score increased, showing that even SNPs that were only weakly associated with schizophrenia play a role in the etiology of the disorder. When all SNPs that had $p < 0.5$ in the discovery sample were included, this risk score accounted for about 3% of the variance in the target sample ($p = 9x10^{-19}$) (Purcell et al., 2009). To rule out the possibility of stratification, this test was performed between discovery and target samples from different countries, and similar results were found.

Purcell et al. then used simulations of a variety of genetic models to estimate the portion of variance due to markers tagging causal alleles (V_M), based on the variance explained by aggregate risk scores across probability thresholds in the study. The models that fit the data estimated V_M to be around 34% (Purcell et al., 2009). These results show that at least a third of the variability for schizophrenia can be explained by common variants. The actual figure is likely higher due to imperfect tagging of the actual causal alleles.

Another study that supported the overall involvement of common variants in schizophrenia was performed by Moskvina et al. (2009). In this study, only SNPs within genes were used. After GWAS, genes were then tested for association in two ways, by examining the smallest p-value for a SNP in each gene and through threshold-truncated products of the p-values of the SNPs in each gene. The primary aim of this study was not to identify specific genes associated with schizophrenia but to determine if more genes were associated with schizophrenia than expected

from permutations. For both methods of testing, significantly more genes were found to be associated with schizophrenia than expected at multiple probability thresholds, suggesting that there are many common variants for schizophrenia.

In summary, several genes, including DTNBP1, NRG1, ZNF804A, NRGN, TCF4, and the MHC region, have been implicated in positional candidate association studies and GWAS. There is also evidence for an overall influence of common variants in the etiology of schizophrenia. However, as can be seen from Purcell et al. (2009), common variants likely do not explain all of the variance in schizophrenia. The next chapter will discuss other genetic variants, with lower frequencies and larger effect sizes, which are also involved in the etiology of schizophrenia.

Chapter 4: Evidence for Rare Variants

In addition to common SNPs, rare variants with moderate effect sizes are also involved in the etiology of schizophrenia. GWAS arrays are designed primarily for detection of common SNPs and CNVs, and are not very suitable for detection of rare SNPs. Since sequencing is still a fairly expensive technique, especially for large samples, there have not been many risk-increasing rare SNPs detected. However, there has been some success in finding rare variants involved in schizophrenia, in particular rare CNVs.

Methods for Finding Rare Variants

There are two major types of rare variants that affect complex traits, rare SNPs and rare copy number variants (CNVs). A CNV is a segment of DNA, ranging in size from a few to millions of base pairs, that differs in number of copies between individuals. An individual may be missing a copy, which would be a deletion, or have an extra copy, a duplication. A CNV may change the structure of a protein if it duplicates or deletes part of the gene coding for the protein, or the quantity of a protein if it duplicates or deletes the entire gene or if it disrupts a regulatory region of the gene. Deletions often result in a larger change in protein quantity because a decrease in gene copy number from two copies to one results in a two-fold change in gene dosage, compared to a 1.5-fold change when gene copy number is increased from two to three (Baross et al., 2007). A CNV may also unmask a recessive allele located at the same locus on the other chromosome

of the pair. The rate of mutation for CNVs is estimated to be between $1.7x10^{-6}$ and $1.0x10^{-4}$ per locus per generation (Zhang et al., 2009). Based on an extrapolation to the entire genome of the frequencies of deletions and duplications in a gene that causes muscular dystrophy, Van Ommen (2005) estimated that a de novo deletion occurs in about 1 in 8 newborns and a de novo duplication occurs in about 1 in 50 newborns.

Two methods have been commonly used to detect CNVs in schizophrenia cases. In the first method, array comparative genomic hybridization (CGH), case and control DNA are differentially labeled with fluorescent tags and both are hybridized to an array spotted with DNA clones. Various types of clones, including BACs, fosmid clones, or oligonucleotides, may be used. Then the fluorescence intensity for each clone is compared between the case and control DNA to detect regions where copy numbers differ. The resolution of array CGH depends on the type and number of clones used. For example, Neill et al. (2010) compared the detection ability of a BAC array with about 4,600 clones and an oligonucleotide array with 105,000 clones, and found that the oligonucleotide array detected several CNVs that the BAC array did not detect. However, oligonucleotide clones have a poor signal to noise ratio, meaning a higher portion of hybridizations are non-specific. This leads to greater variation between arrays in the signaling ratio of case and control DNA. To compensate for this, several arrays have to be averaged.

Genotyping arrays provide a second method for CNV detection. With this method, one strand of DNA is hybridized to the array, and signal intensities of the probes on the array are used to detect CNVs. Since the probes used on these arrays are smaller than the clones used in array CGH, genotyping arrays provide better resolution and can detect smaller CNVs. For example, a study by Redon et al. (2006) that compared the Affymetrix 500K Mapping Array to the Whole Genome TilePath array (WGTP) found that the average length of CNVs found by the 500K set

was 206 kb. In contrast, the average CNV length detected by the WGTP array was 341 kb. Early genotyping arrays only included probes for SNPs, so CNV detection in areas of the genome that had low SNP density was poor. More recent genotyping arrays, however, have added non-polymorphic probes for these areas of the genome to aid in CNV detection.

For rare SNPs, the best method for detection is sequencing of candidate genes in cases in the population. If any rare SNPs are found within these genes, controls are then sequenced to determine if the frequency of the SNP differs between cases and controls. Also, various methods, such as analysis of the protein structure and functional assays, are used to try to determine the effect of the rare SNP on the gene product.

There are some issues that make this method difficult to implement. Selection of candidate genes for sequencing requires knowledge of the biology of a disorder, which is limited for psychiatric disorders. Also, sequencing the large samples of individuals that would be needed can be expensive compared to genotyping. For example, as of August 2011, sequencing one genome costs about $5,000, but genotyping one individual by array costs only about $250. Additionally, to reliably detect a SNP that only occurs in .1% of the population, at least 1,000 cases would need to be sequenced, which would cost approximately $5,000,000. Also, after sequencing, it may be difficult to determine the effects of any rare variants that are found. Due to the high cost of sequencing, this method has only been recently implemented in schizophrenia research, but will certainly become more common as sequencing technology improves and associated costs are reduced.

Table 3: Results of CNV Burden Studies

Study	Sample	Significant Results
Walsh et al. (2008)	150 cases and 268 controls	Rare gene-altering CNVs (OR = 3.37, p = 0.0008) In cases with onset before 18 (OR = 4.82, p = 0.0001)
Kirov et al. (2009)	471 cases and 2,792 controls	CNVs over 1 Mb (OR = 2.26, p = 0.00027) Deletions over 1 Mb (OR = 4.53, p = 0.00013) Duplications over 1 Mb (OR = 1.68, p = 0.04)
Xu et al. (2008)	152 sporadic cases and 159 controls	Rare de novo CNVs 8 times more common in sporadic cases than controls (p = 0.00078)
Xu et al. (2009)	48 familial cases and 159 controls	Rare inherited CNVs 2 times more common in familial cases than controls (p = 0.01)
Stone et al. (2008)	3,391 cases and 3,181 controls	Cases had a 1.15-fold increase in rare CNVs ($p = 3 * 10^{-5}$) and a 1.41-fold increase in genes affected by rare CNVs ($p = 2 * 10^{-6}$)

CNV Burden in Schizophrenia

Several studies have found an increased frequency of rare CNVs in individuals with schizophrenia, and the major results of these studies are shown in Table 3. Stone et al. (2008), using the Affymetrix 5.0 and 6.0 genotyping arrays, and Walsh et al. (2008), using oligonucleotide array CGH, both found that individuals with schizophrenia had more rare CNVs than controls. Stone et al. also found that cases had more genes disrupted by CNVs. Kirov et al. (2009) did not find a significant difference in overall CNV burden, but did find that individuals with schizophrenia had significantly more CNVs of 1000 kb or larger. The lack of an overall effect from Kirov et al. may be due to the small sample size of the study. Also, Kirov et al. used the Affymetrix 500K Mapping Array, which has been shown to be poor at detecting CNVs (Redon et al., 2006). Of these three studies, only Walsh et al. provided percentages of individuals that had rare CNVs. They found that 15% of cases carried rare CNVs, compared to only 5% of controls. The effect sizes from Stone et al. (2008) are likely the most accurate estimates, since the sample size in this study was much larger than the others.

As can be seen from the table, Walsh et al. (2008) found an especially large increase in rare CNV burden in a sample of individuals with onset of schizophrenia before age 18. In these cases, the early age of onset may reflect a more severe form of the disorder. If this form impairs function more than typical schizophrenia, it is possible that the variants responsible would have large effect sizes and low frequencies.

Two studies by Xu et al. (2008, 2009), which used the Affymetrix 5.0 genotyping array, compared rates of de novo and inherited rare CNVs in sporadic cases and familial cases of schizophrenia. A case is considered sporadic if no first or second-degree relatives are affected.

They found that while about 40% of both types of cases carried rare CNVs, in familial cases all of the rare CNVs were inherited whereas 10% of the sporadic cases carried de novo CNVs. In the control cohort, about 1% and about 20% of individuals carried de novo and inherited rare CNVs respectively. These studies suggest that familial cases of schizophrenia are often associated with inherited CNVs, while sporadic cases are generally associated with de novo CNVs.

Rare Variants Found

In addition to individuals with schizophrenia having a greater overall CNV burden, several specific rare CNVs have also been implicated in schizophrenia. In one study searching for rare CNVs, Kirov et al. (2008) used array CGH with BAC clones in a sample of 93 cases and their families. Array CGH was also performed on 372 individuals with other disorders for comparison. Of the 13 rare CNVs found in schizophrenia cases, two appeared to be potentially pathogenic. One was a duplication at 15q13.1 found in a proband. This duplication included three genes: APBA2, NDNL2, and TJP1. The other CNV of interest was a deletion at 2p16.3, which occurred in one of the probands, the unaffected mother, and an affected sibling. The only gene affected by this deletion was NRXN1, which is believed to play a role in neurotransmitter release.

A few studies have had sample sizes large enough to test specific rare CNVs for association with schizophrenia. Stefansson et al. (2008) performed a two stage study to test de novo CNVs for association. To find de novo CNVs, they analyzed 9,878 parent-offspring transmissions. The sample for the first stage of association testing included 1,433 cases and 33,250 controls. The second stage sample contained 3,285 cases and 7,951 controls. CNVs were detected using the

HumanHap 300, HumanHap 550, and Affymetrix 6.0 genotyping arrays. Three deletions achieved significant association in the second sample. A deletion at 1q21.1 (OR = 14.83, p = 2.9 x 10^{-5}) covers several genes, including GJA8, which has been associated with schizophrenia (Ni et al., 2007). The second deletion, at 15q11.2 (OR = 2.73, p = 6.0 x 10^{-4}), includes the gene CYFIP1, which is involved in some of the behavioral features found in Angelman and Prader-Willi syndromes. The third deletion is located at 15q13.3 (OR = 11.54, p = 5.3 x 10^{-4}). One of the genes in this deletion is CHRNA7, which has been associated with schizophrenia in some studies (Freedman et al., 1997; Xu et al., 2001).

Stone et al. (2008) also searched for CNVs that were associated with schizophrenia, using the same sample that they used to calculate overall CNV burden in individuals with schizophrenia. They found three deletions that were significantly associated. Two of these deletions, 15q13.3 (OR = 17.9, p = 0.046) and 1q21.1 (OR = 6.6, p = 0.046), were also implicated in the Stefansson et al. (2008) study mentioned above. The other significantly associated deletion was at 22q11.2 (OR = 21.6, p = 0.0046). Deletion of this region is responsible for velo-cardio-facial syndrome and DiGeorge syndrome. Previous studies have found that about 30% of individuals with this deletion develop psychosis (Murphy et al., 1999), so the significant association of this deletion was expected. None of the genes within this deletion have been established as responsible for this increased risk, although COMT has had inconsistent support in association studies (Li et al., 1996; Shifman et al., 2002).

Some CNV association studies have focused on specific genes or regions instead of searching through the whole genome. Rujescu et al. (2009) performed a study to test the association of NRXN1 CNVs with schizophrenia, because of the deletions found within this gene in previous studies. This study included 2,977 cases and 33,746 controls. Suggestive evidence was found for

association of CNVs that disrupted any part of NRXN1 (OR = 1.73, p = 0.13). Since CNVs that disrupt exons are more likely to be pathogenic, Rujescu et al. also tested only CNVs that disrupted NRXN1 exons, leaving out CNVs that only disrupted non-coding regions of the gene, and found significant association (OR = 8.97, p = 0.0027).

Another gene that has been targeted in the search for rare variants is ZNF804A. The interest in this gene is largely due to the fact that it has achieved significance in association studies for schizophrenia, as mentioned previously. Steinberg et al. (2010) examined 4,235 patients with either schizophrenia or bipolar disorder and 39,481 controls for CNVs in ZNF804A. They found CNVs in two cases but no controls, resulting in a significant association with psychosis (p = 0.013). However, the genome-wide studies mentioned above have not found significantly associated CNVs within ZNF804A.

McCarthy et al. (2008) performed a study focusing on the 16p11.2 region, due to previous findings of CNVs in this region that were associated with autism and mental retardation. This study found a microduplication in this region that was associated with schizophrenia in both an initial sample of 1,906 cases and 3,971 controls (OR = 25.8, $p = 1.2 \times 10^{-5}$) and a replication sample of 2,645 cases and 2,420 controls (OR = 8.3, p = 0.022). Of the 28 genes located within this duplication, 17 are expressed in the brain, so there are several potential candidates for explaining the increased risk of schizophrenia.

Another rare structural variant that has been connected to schizophrenia is a translocation between chromosomes 1 and 11. This translocation has been shown to segregate with mental illness, including schizophrenia, in a Scottish family, with an LOD of 6.0 (Millar et al., 2000). This translocation disrupts two genes on chromosome 1, DISC1 and DISC2. DISC2 is not

thought to be translated, so DISC1 has been considered the likely causal gene. Other linkage studies have found evidence for linkage in this region, suggesting that there may be multiple rare variants of DISC1 involved in schizophrenia (Ekelund et al., 2001; Hovatta et al., 1999). Some association studies for DISC1 have also achieved significant results (Hodgkinson et al., 2004; Callicot et al., 2005; Cannon et al., 2005; Schumacher et al., 2009).

Due to the high cost of sequencing there have yet to be any studies that have sequenced the whole genome in a large number of cases. However, smaller sequencing studies have been conducted and a few rare SNPs have been implicated in schizophrenia. Piton et al. (2010) sequenced synaptic genes located on the X chromosome, because of the involvement of synapses in schizophrenia and the earlier onset of the disorder in males. They found SNPs in the gene MAOB in 5 out of 190 cases that were not detected in 190 controls. Gauthier et al. (2010) sequenced another synaptic gene, SHANK3, in 185 cases and 285 controls. A nonsense mutation was found in three affected brothers and a missense mutation was found in an affected woman, but neither of these mutations was found in controls. However, it should be noted that both of these studies had very small sample sizes, so the lack of the rare SNPs in controls may be due to chance. As sequencing costs decrease, larger and more statistically valid sequencing studies will become more feasible.

One common trend of these studies, with a few exceptions, is that the rare variants involved contain several genes. For some of these CNVs, there is fairly strong evidence pointing to a particular gene as the gene responsible for increasing risk. However, more research will be needed to definitively establish the causal genes in these CNVs.

Another important observation is that some genes, such as ZNF804A, GJA8, DISC1, and CHRNA7, have been implicated in both association studies and rare variant searches. This pattern suggests that many genes may have both common and rare variants that increase schizophrenia risk. Therefore, genes that have been implicated in association studies may be strong candidates for future sequencing studies for rare variants.

Also, while rare CNVs do appear to play a role in schizophrenia, it must be noted from the studies above that the majority of individuals with schizophrenia did not carry rare CNVs. For example, in the Walsh et al. (2008) study, only 15% of cases had rare CNVs. It is likely that there are many CNVs that have not been detected, due to incomplete coverage and lack of resolution for smaller CNVs. However, even a reasonable hypothetical correction for this would still not account for all of the cases, meaning there are many cases in which common variants are primarily responsible for the etiology of schizophrenia. One of the major interests of research now is to determine how these variants increase risk for the disorder. Some of this research will be reviewed in the next chapter.

Chapter 5: Biological Mechanisms of Risk Variants

Identifying genetic variants that increase risk for schizophrenia may have some value for prediction of risk in individuals. However, it is not well understood how these variants increase risk for schizophrenia. Examination of how the functions of the affected genes relate to the neurological characteristics of schizophrenia will help in exploring this question. Exploring the effects of the various types of risk variants, as well as their involvement in other disorders may provide some insight as well.

Functions of Mutated Genes

Since schizophrenia is a mental disorder, it would be expected that the brain is the organ responsible for its development, and molecular genetic studies have been consistent with this expectation. For example, Walsh et al. (2008) used two classification systems to determine whether any particular functional pathways were overrepresented among the genes that were partially or entirely located within CNVs in people with schizophrenia. They found that several pathways involved in brain development were overrepresented. No pathways were overrepresented among genes partially or entirely located within CNVs in controls, showing that the CNVs in brain development pathways are deemed responsible for the increased schizophrenia risk. The following paragraphs form a discussion of the functions of some of the genes that have been implicated in schizophrenia and how these functions relate to the biology of

the disorder. First, genes containing risk-increasing common variants, including DTNBP1, NRG1, ZNF804A, the MHC region, TCF4, and NRGN are discussed. Then, the functions of NRXN1 and DISC1, which contain rare variants implicated in schizophrenia, are explored.

DTNBP1

Several studies have suggested that DTNBP1, which produces the protein dysbindin, is involved in neurotransmission, the passage of signaling molecules between neurons. Numakawa et al. (2004) found that overexpression of DTNBP1 in neuronal cultures increased expression of SNAP25 and synapsin I, two proteins that are involved in synaptic vesicle function. Synaptic vesicles are small sacks that store neurotransmitters and release them into the synapses between neurons. Based on the increased expression of SNAP25 and synapsin I, Numakawa et al. expected increased release of glutamate, a common neurotransmitter, and indeed found this result. Also, down-regulation of DTNBP1 resulted in lower expression of SNAP25 and synapsin I and decreased glutamate release. Murotani et al. (2007) discovered reduced dopamine levels in the hippocampus and cortex in mice that did not express dysbindin. Chen et al. (2008), also using mice without dysbindin, found that lack of dysbindin resulted in slower vesicle release, lower probability of release, and fewer vesicles that are ready for release.

These findings of altered neurotransmission fit in with the hypothesis that dysfunctional neurotransmission is involved in the development of schizophrenia. This hypothesis is partially based on findings of altered levels of neurotransmitters, including dopamine and glutamate, in individuals with schizophrenia (Frankle et al., 2003). In particular, there is strong evidence for the involvement of NMDA receptors, a type of glutatmate receptor that regulate passage of calcium, potassium, and sodium ions, in schizophrenia (reviewed by Marsman et al., 2011).

NRG1

NRG1 is involved in several aspects of the nervous system. Rio et al. (1997) showed that blocking NRG1 impairs the migration of neurons from their place of origin to other regions of the brain. Stefansson et al. (2002) found that mice with an NRG1 knockout had fewer NMDA receptors. Vartanian et al. (1999) found that lack of neuregulin impaired the development of oligodendrocytes, cells that insulate the axons of neurons, in mice.

These functions of NRG1 have been found to be impaired in individuals with schizophrenia. Akbarian et al. (1996) and Rioux et al. (2003) found altered distribution of neurons in schizophrenia patients, which could be due to abnormal neuronal migration. Mohn et al. (1999) observed schizophrenia-like motor and social behaviors in mice with reduced expression of NMDA receptors. Gao et al. (2000) observed lower levels of mRNA for NMDA subunits in the postmortem hippocampi tissue of individuals with schizophrenia. Low density of oligodendrocytes has also been found in schizophrenia (Uranova et al., 2001; Hof et al., 2002).

ZNF804A

The function of ZNF804A has not been definitively determined. Riley et al. (2010) suggested on the basis of bioinformatic analysis that the gene may contain binding sites for transcription factors. Chung et al. (2010) found the gene to be a target of the gene Hoxc8. Since Hox genes are involved mainly in development, this finding suggests that ZNF804A may play a role in neurodevelopment.

Even though the function of ZNF804A has not been determined, some studies have tested the schizophrenia-associated SNP within this gene for association with schizophrenia-related phenotypes. Esslinger et al. (2009), in a study using individuals without schizophrenia, observed

decreased connectivity, a measure of correlation between fMRI time series, within the dorsolateral prefrontal cortex in individuals that carried the risk allele during a cognitive task but increased connectivity between this region and the hippocampus. They also noticed increased connectivity from the amygdala to the hippocampus and the cortex. Walter et al. (2010), also studying individuals without schizophrenia, observed reduced activation in several areas of the cortex during a social cognition task, as well as altered connectivity between regions. These patterns are consistent with studies of brain activity in individuals with schizophrenia (Meyer-Lindenberg et al., 2001; Brunet et al., 2003).

Major Histocompatibility Complex

The MHC is mostly known for its role in immune function. This complex codes for proteins found on the surface of the cell. These proteins let the immune system know if foreign material is present in the cell so an immune response can be initiated. However, the MHC has also been shown to be involved in the nervous system (reviewed by Garay & McAllister, 2010). Dysfunctional neural plasticity has been observed in individuals with schizophrenia (Daskalakis et al., 2008). Also, the involvement of the MHC in both immune response and neurodevelopment may provide a mechanism for explaining the increased rates of schizophrenia in cases of maternal infection mentioned in Chapter 2.

NRGN

NRGN is a target of NMDA receptors, which are affected in schizophrenia as mentioned above, and binds to calmodulin, a calcium-binding protein. When glutamate binds to NMDA receptors, calcium ions flow into the cell and oxidate NRGN. This oxidation causes NRGN to release calmodulin. In turn, calmodulin activates calmodulin-dependent kinase II. Through this pathway,

NRGN plays a role in the induction of long-term potentiation (LTP) (Bliss & Collingridge, 1993), which enhances communication between neurons. The role of NRGN in LTP provides a mechanism for this gene to cause connective abnormalities found in schizophrenia. Also, LTP is believed to be involved in the process of memory, which has been shown to be impaired in schizophrenia (Goldman-Rakic, 1994; Aleman et al., 1999).

TCF4

TCF4 codes for a transcription factor, which is a protein that binds to DNA and regulates its transcription to RNA. Although mechanisms are not known, several studies have shown that this gene is important in brain development. Flora et al. (2007) observed that mice lacking the Tcf4 protein failed to develop pontine neurons. Also, variants in TCF4 have been shown to cause Pitt-Hopkins syndrome, a disorder with symptoms including mental retardation, hyperventilation, and distinctive facial features (Amiel et al., 2007; Brockschmidt et al., 2007), further supporting its role in neural development. Brzozka et al. (2010) observed that mice overexpressing TCF4 were impaired in fear conditioning, the ability to associate a neutral event with a traumatic one when exposed to both simultaneously, and sensorimotor gating, the ability to filter out and ignore unimportant sensory information. Sensorimotor gating in particular is known to be affected in schizophrenia (Braff & Geyer, 1990).

NRXN1

NRXN1 is one the genes that codes for neurexin proteins. NRXN1 gene has two separate promoters, allowing it to code for α-neurexins and β-neurexins. The neurexin proteins are located mainly in presynaptic terminals and form trans-synaptic complexes with neuroligins located in the post-synaptic terminals (Sudhof, 2008). Missler et al. (2003) examined mice with knock-outs

of one or more types of α-neurexins and observed impaired neurotransmitter release, due to reduced function of calcium ion channels. Li et al. (2007) observed similar results in Drosophila, as well as a reduction in the number of presynaptic terminals as neurons, suggesting that neurexins may be involved in synapse formation. These results suggest that NRXN1 may be another contributor to the dysfunctional neurotransmission found in schizophrenia.

DISC1

DISC1 has been associated with abnormal neurodevelopment in a few studies. Ozeki et al. (2003) observed impaired outgrowth of axons and dendrites from neurons in DISC1 mutant mice. Studies that knocked down DISC1 expression also found this effect (Kamiya et al., 2005). Kamiya et al. also observed impaired neuron migration in mice with reduced DISC1 expression, as well as misorientation of neurons. These abnormalities in neurodevelopment are similar to those seen in schizophrenia (Akbarian et al., 1993; Harrison, 1997). A common SNP is DISC1 has been associated with smaller volume in the hippocampus, a brain region that is strongly affected in schizophrenia, and abnormal patterns of activation in this region during memory tasks (Callicott et al., 2005).

In summary, two processes in particular, neurodevelopment and communication between neurons, are mentioned often in this discussion of mechanisms of candidate genes. This pattern is not surprising considering the structural and functional brain abnormalities in schizophrenia, including enlarged ventricles, reduction of areas of the cortex, and abnormal activation patterns (Ross et al., 2006). In turn, these abnormalities likely lead to the perceptual and cognitive dysfunction found in schizophrenia.

Effects of SNPs and CNVs Compared

SNPs and CNVs can both affect phenotypes by changing the quality or quantity of proteins produced. However, these two types of variants act through different mechanisms, possibly contributing to the larger effect sizes of CNVs. As mentioned in Chapter 3, SNPs are single base pair changes that can change the amino acid composition of a protein if they occur in an exon. The degree to which this change affects the structure and function of the protein depends on the amount of difference between the ancestral and altered amino acids. For example, if the SNP results in one positively charged amino acid being replaced with another, there will not be much change in protein function. However, a SNP that replaces a positively charged amino acid with a negatively charged one will likely have a large effect on protein function. Similarly, some nucleotide changes in regulatory regions have a larger effect on transcription factor binding and change the level of gene expression more.

A study by Richards et al. (2011) shows that many of the common variants involved in schizophrenia act through changes in gene expression. In this study, SNPs that were at least weakly associated with schizophrenia in the ISC and MGS schizophrenia GWAS ($p < 0.5$) were tested for association with expression levels of nearby transcripts. The SNPs were then sorted into groups based on the strength of their association with expression levels, and the groups were tested in both cases and controls for differences in their polygenic scores, similar to Purcell et al. (2009). A greater difference was found between cases and controls for the SNPs associated with expression levels, supporting the idea that common variants involved in regulation of gene expression play a role in schizophrenia.

The epidemiology of schizophrenia suggests that epistasis between SNPs, in which the effects of a gene are modified by other genes, is also involved in the etiology of schizophrenia. Dempster & Lerner (1950) stated that disorders with high heritability and low prevalence, which are characteristics of schizophrenia, are most likely to feature epistasis. Risch (1990) concluded that the dramatic decrease in risk to relatives, discussed in Chapter 1, is most consistent with a model that includes epistatic effects.

The larger effect sizes of CNVs could possibly be explained by their larger size. Instead of changing one amino acid in a protein, CNVs can delete or duplicate several amino acids in a protein, greatly changing its structure and function. Also, the changes in expression level that result from CNVs are usually larger than the changes in expression level caused by SNPs. Another factor that likely contributes to the larger effect sizes of CNVs is the fact that they often span multiple genes. This can result in a CNV having a larger effect on a single pathway or may even lead to multiple pathways being affected.

Involvement of Variants in Other Disorders

Some of the variants that have been implicated in schizophrenia have turned out to also increase risk for other mental disorders, in particular bipolar disorder, autism, and mental retardation. As mentioned in Chapter 2, there is some symptomatic overlap between schizophrenia and bipolar disorder, with psychosis occurring in both disorders. Several studies have also found these two disorders to have significant genetic overlap as well. Using the aggregate risk score method described in Chapter 3, Purcell et al. (2009) found that the schizophrenia risk alleles with p-values less than 0.5 in the ISC sample achieved higher mean scores in cases than controls in two bipolar samples. In the STEP-BD sample, these SNPs explained 1.9% of the variance for bipolar

disorder with a p-value of $7x10^{-9}$. The associated SNPs explained 1.4% of the variance in the WTCCC sample with a p-value of $1x10^{-12}$. Moskvina et al. (2009) performed GWAS on a schizophrenia sample and a bipolar sample. They counted the number of genes that were significant in both samples at significance thresholds of 0.01 and 0.05 and compared their observations to the expected number of genes shared based on permutations. At both of these thresholds, a higher number of genes than expected were found to be significantly associated with both disorders (p-values = 0.007 and 0.014 respectively).

In addition to this general genetic overlap, specific variants have been found to be associated with both disorders as well. In their GWAS that implicated ZNF804A in schizophrenia, O'Donovan et al. (2008) added 2,865 bipolar cases to their replication sample and achieved a more significant association for ZNF804A than found in the schizophrenia samples alone ($9.96x10^{-9}$ compared to $1.61x10^{-7}$). A meta-analysis by Williams et al. (2011) also achieved a similar increase in significance ($2.54x10^{-11}$ without bipolar samples, $4.1x10^{-13}$ with bipolar samples).

Another gene that has been implicated in both schizophrenia and bipolar disorder is CACNA1C. This gene was first associated with bipolar disorder in a GWAS meta-analysis with a p-value of $7.0x10^{-8}$ (Ferriera et al., 2008). Green et al. (2010) tested the associated marker for association with schizophrenia in a sample of 479 cases and 2,938 controls and observed a significant association (OR = 1.15, p = 0.034). Association was also found when the control sample was expanded to 11,361 (OR = 1.13, p = 0.043).

In contrast to the SNPs that are associated with both schizophrenia and bipolar disorder, the genetic variants associated with schizophrenia, mental retardation, and autism are mainly CNVs.

Guilmatre et al. (2009) examined 236 cases with schizophrenia, 260 cases with autism, and 247 cases with mental retardation for 28 CNVs that have been found in at least one of these disorders in previous studies. They observed several cases of CNVs involved in multiple disorders, including 22q11 and NRXN1 deletions and 15q13 and 16p11 duplications. Another interesting note is that cases of childhood-onset schizophrenia, which often features developmental delays similar to autism, are often enriched for rare CNVs. In a sample of 83 childhood-onset schizophrenia cases, Walsh et al. (2008) observed that 28% of these cases carried rare CNVs, making them especially enriched for rare CNVs.

There are a few conclusions that can be drawn from these examples of genetic overlap between schizophrenia and other disorders. First, the genetic overlap between schizophrenia and bipolar disorder, along with the presence of psychosis in both and similarity in course and epidemiology, suggests that sharply differentiating these two disorders is not accurate. Also, Lichtenstein et al. (2009) found that relatives of bipolar patients were more likely to develop schizophrenia. These findings have led some researchers have proposed that schizophrenia and bipolar disorder be placed on a spectrum (Craddock & Owen, 2005; Lichtenstein et al., 2009).

Second, genetic overlap implies similar mechanisms are involved in disorders. In the case of schizophrenia and bipolar disorder, neuron communication appears to be an important mechanism in both disorders. CACNA1C is involved in the transport of calcium ions across the membrane, which can affect the function of synapses. While the function of ZNF804A is unknown, the risk allele for schizophrenia in this gene has been shown to affect connectivity in the brain, as mentioned above. The CNVs implicated in schizophrenia, autism, and mental retardation are mainly involved in brain development and neurotransmission. These CNVs have

not been implicated in bipolar disorder, suggesting that brain development does not play a major role in its etiology.

Third, the fact that different variants overlap between schizophrenia and other disorders may help in associating variants with particular symptoms. For example, the influence of CACNA1C and ZNF804A in schizophrenia and bipolar disorder may hint at a role for these genes in the development of psychosis. On the other hand, rare CNVs may play a greater role in the negative symptoms of schizophrenia, based on their role in autism and mental retardation as well. Studies testing particular risk variants for association with symptoms could be useful in determining these relationships.

Fourth, since these variants can lead to multiple disorders, there are likely other factors, genetic and environmental, that modify the effects of these variants and determine the specific phenotype of the individual. An example of a genetic modifier was found by Fanous et al. (2004), who observed an association between HT2A and affective symptoms in a sample of schizophrenia patients. Since the increased presence of affective symptoms partially differentiates bipolar disorder from schizophrenia, it would be plausible that this gene could play a role in determining whether someone susceptible to psychosis develops schizophrenia or bipolar disorder. Similarly the variants associated with psychotic disorders could be involved in determining the phenotype of individuals with rare CNVs. Establishing effects such as these would require diagnostic examination of individuals genotyped at multiple loci. For example, individuals with NRXN1 deletions could be genotyped for the risk alleles in ZNF804A and CACNA1C to determine if these genotypes differ based on diagnosis.

In conclusion, many of the genes mutated in schizophrenia are involved in brain development and neurotransmission, two processes that seem to be disrupted in schizophrenia. Common SNPs and rare CNVs both appear to affect similar pathways. However, due to their larger size, CNVs likely disrupt these pathways to a greater degree, explaining their larger effect sizes. The variants implicated in schizophrenia also appear to be involved in other psychiatric disorders. In particular, some SNPs increase risk for both schizophrenia and bipolar disorder, and several rare CNVs have been implicated in autism and mental retardation as well as schizophrenia.

Chapter 6: Conclusions

Even though much effort has been invested in studying schizophrenia, the causes of the disorder are still poorly understood. Genetic epidemiology studies of the disorder provide evidence for a substantial role for genetic influences in the etiology. These studies also underscore that this genetic influence has a complex nature, with many genes being involved.

However, the search to clarify the genetic predisposition to schizophrenia will depend on determining the genetic architecture of the disorder, which is the subject of the CDCV-CDRV debate. Some researchers have maintained that the majority of risk is due to common SNPs with small effect sizes, which can be detected through association studies. Other researchers support the hypothesis that schizophrenia is caused mainly by rare variants with large effect sizes. These variants cannot be feasibly detected through association studies so other methods are required.

Two genes, DTNBP1 and NRG1, have received fairly strong support from positional candidate association studies. Genome-wide association studies have also had some success, with ZNF804A, the MHC region, NRGN, and TCF4 being some of the major genes implicated in these studies. Evidence for an overall effect of common variants on schizophrenia risk has also been found by measuring the aggregate predictive ability of associated SNPs.

However, rare variants with larger effect sizes have also been implicated in schizophrenia. Several studies have found that individuals with schizophrenia have a larger burden of rare

CNVs. Also, several particular rare structural variants have been implicated in the disorder, including deletions at 22q11.2, 15q13.3, 15q11.2, 1q21.1, and NRXN1, duplications at 16p11.2, and a translocation involving the gene DISC1. Early sequencing studies have also discovered some rare SNPs in schizophrenia cases, including markers in MAOB and SHANK3.

As expected, the genes that have been implicated in schizophrenia are involved in brain development and function. Some of the pathways and functions affected by these genes include neuron development and migration, oligodendrocyte formation, synaptic plasticity, and neurotransmission. There do not seem to be any differences in the types of pathways affected by SNPs and CNVs. However, due to their larger size, CNVs are likely to have larger effects on these pathways and more likely to affect multiple pathways. This could explain the larger effect sizes and lower frequencies of CNVs.

Some of the variants implicated in schizophrenia are also involved in other psychiatric disorders. SNPs in the genes ZNF804A and CACNA1C have been associated with both bipolar disorder and schizophrenia. A general overlap of common variants has also been found between these disorders. These findings, along with other similarities, have led some to argue that the diagnostic boundary between these disorders is invalid. Genetic overlap has also been shown between schizophrenia, autism, and mental retardation, with several CNVs being implicated in all of these disorders. Based on these findings of genetic overlap, it appears that at least some of the variants involved in schizophrenia are not specific to that disorder but have general effects on brain functioning. The exact phenotype that results from these variants is likely determined by other genetic variants as well as environmental factors.

In conclusion, it appears that both the CDCV and CDRV hypotheses are valid for schizophrenia, with evidence being found for both types of variants. These two classes of variants act through the same pathways, but rare variants likely disturb these pathways to a greater degree. The best model for the underlying risk in schizophrenia is likely similar to the threshold liability model, but allows for gene-gene and gene-environment interactions. This model would allow for the involvement of both common and rare variants, as well as environmental factors. A model with normally distributed risk is also supported by findings of neurological abnormalities and cognitive deficits in siblings of schizophrenia patients (Callicot et al., 1998; Egan et al., 2001; Callicot et al., 2003). These findings show that individuals with an increased, but not sufficient, liability for schizophrenia still display phenotypes relatively similar to those found in the disorder. The relatively high prevalence of schizophrenia is likely maintained by several factors, including weak selection against common SNPs, de novo mutations, and possibly balancing selection involving SNPs.

Due to the findings of both common and rare variants, it is important to continue efforts to search for more of both types of variants. GWAS meta-analyses appear to be a promising route in the search for common SNPs involved in schizophrenia. A meta-analysis from the Psychiatric Genetic Consortium is expected to be published soon. This meta-analysis will include 17,836 cases, more than any schizophrenia GWAS to date. Also, there is plenty of room for improvement for algorithms used for the detection of CNVs through genotyping arrays (Zhang et al., 2011). The development of more accurate algorithms will likely lead to the discovery of more rare CNVs associated with the disorder. As mentioned before, sequencing studies for rare SNPs involved in schizophrenia have only recently started and will become more common as sequencing becomes more affordable.

As well as searching for more variants, it is also important to study the mechanisms of known risk variants. The method used by Esslinger et al. (2009), in which neurological phenotypes associated with schizophrenia are measured in carriers and non-carriers of the risk allele, would be a useful method for common variants. However, this method may not be as feasible for studying rare variants, since sufficient sample sizes would be more difficult to obtain. Biochemical assays could be a useful method for determining the effect of a variant on the protein produced. Animal models have also been shown to be useful in studying the effects of genes on brain function, although some of the behavioral aspects of schizophrenia cannot be easily replicated in animals.

It is also necessary to study the intermediate phenotypes that link genetic variants to the phenotype of schizophrenia. These include RNA and protein expression levels, which can be measured with microarrays. Neurological phenotypes, such as abnormalities in the volumes of brain regions and dysfunctional connectivity in the brain, can also be useful in elucidating the mechanisms of schizophrenia. MRI and other brain scanning techniques are useful methods of study in this area.

The search for genes involved in schizophrenia is just starting to bear fruit, with both common and rare variants being implicated. The continued use of GWAS meta-analyses should allow more common SNPs to be discovered. The discovery of rare variants should be aided through the development of better CNV detection algorithms as well as the increased use of sequencing.

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