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ADDRESSING ISSUES IN THE DETECTION OF GENE-ENVIRONMENT INTERACTION THROUGH THE STUDY OF CONDUCT DISORDER

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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List of Abbreviations

3'UTR	3' Untranslated Region
5-HT	Serotonin
5HTTLPR	Serotonin Transporter Promoter Polymorphism
ADHD	Attention Deficit Hyperactivity Disorder
AIC	Akaike's Information Criteria
ASE	Asymmetric Standard Error
ASP	Antisocial Personality Disorder
CAPA	Child and Adolescent Psychiatric Assessment
CD	Conduct Disorder
cDNA	Complementary Deoxyribonucleic Acid
DAT1	Dopamine Transporter 3'UTR Polymorphism
DIC	Deviance Information Criteria
DNA	Deoxyribonucleic Acid
DSM-III R	Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, revised
DZ	Dizygotic
DZF	Dizygotic Females
DZM	Dizygotic Males
GxE	Genotype-environment interaction

IRT	Item Response Theory
MAOA	Monoamine Oxidase A (enzyme)
MAOA	Monoamine Oxidase A (gene)
mRNA	Messenger Ribonucleic Acid
MZ	Monozygotic
MZF	Monozygotic Females
MZM	Monozygotic Males
OFC	Orbitofrontal Cortex
rGE	Genotype-Environment Correlation
SERT	Serotonin Transporter (protein)
SLC6A3	Dopamine Transporter (gene)
SLC6A4	Serotonin Transporter (gene)
SNP	Single Nucelotide Polymorphism
VNTR	Variable Number of Tandem Repeats
VTA	Ventral Tegmental Area
VTSABD	Virginia Twin Study of Adolescent and Behavioral Development

Abstract

ADDRESSING ISSUES IN THE DETECTION OF GENE-ENVIRONMENT INTERACTION THROUGH THE STUDY OF CONDUCT DISORDER

By Elizabeth Chin Prom, Ph.D.

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2007

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This work addresses issues in the study of gene-environment interaction (GxE) through research of conduct disorder (CD) among adolescents and extends the recent report of significant GxE and subsequent replication studies. A sub-sample of 1,299 individual participants/649 twin pairs and their parents from the Virginia Twin Study of Adolescent and Behavioral Development was used for whom Monoamine Oxidase A (*MAOA)* genotype, diagnosis of CD, maternal antisocial personality symptoms, and household neglect were obtained.

This dissertation (1) tested for GxE by gender using *MAOA* and childhood adversity using multiple approaches to CD measurement and model assessment, (2) determined whether other mechanisms would explain differences in GxE by gender and (3) identified and assessed other genes and environments related to the interaction *MAOA* and childhood adversity.

Using a multiple regression approach, a main effect of the low/low *MAOA* genotype remained after controlling other risk factors in females. However, the effects of GxE were modest and were removed by transforming the environmental measures. In contrast, there was no significant effect of the low activity *MAOA* allele in males although significant GxE was detected and remained after transformation. The sign of the interaction for males was opposite from females, indicating genetic sensitivity to childhood adversity may differ by gender. Upon further investigation, gender differences in GxE were due to genotype-sex interaction and may involve *MAOA*.

A Markov Chain Monte Carlo approach including a genetic Item Response Theory modeled CD as a trait with continuous liability, since false detection of GxE may result from measurement. In males and females, the inclusion of GxE while controlling for the other covariates was appropriate, but was little improvement in model fit and effect sizes of GxE were small.

Other candidate genes functioning in the serotonin and dopamine neurotransmitter systems were tested for interaction with *MAOA* to affect risk for CD. Main genetic effects of dopamine transporter genotype and *MAOA* in the presence of comorbidity were detected. No epistatic effects were detected.

The use of random forests systematically assessed the environment and produced several interesting environments that will require more thoughtful consideration before incorporation into a model testing GxE.

CHAPTER 1 Introduction

Recent reports of measured genotype-environment interaction (GxE) in explaining risk for conduct disorder (CD) have provoked a great deal of excitement regarding the applicability of GxE to assess disease susceptibility. The detection of GxE, interpretation of significant results and expansion of GxE research to identify and test a variety of measured genotypes and environments to advance understanding of disease etiology has encouraged a great deal of discussion (Eaves, 2006; Moffitt, 2005; Moffitt, Caspi, & Rutter, 2005; Rutter, Dunn, Plomin et al., 1997; Rutter, 2005; Rutter, Moffitt, & Caspi, 2006). Some issues affecting the detection and interpretation of GxE include the measurement of CD, the assessment of alternate biologically relevant processes, and the inclusion of alternate genetic and environmental risk factors. This work identifies and addresses these issues in the study of GxE through research of CD among adolescents and serves to extend the recent report of significant GxE and subsequent replication studies.

A Summary of Study Goals

In order to advance the study of CD and GxE in general, it is necessary to identify and address issues which might hinder the feasibility of GxE research and ultimately our understanding of GxE. This dissertation addresses the previously addressed concerns by (1) Testing for the effects of GxE by gender (Chapter 2), (2) Determining whether other biologically relevant mechanisms such as X-linkage may yield results similar to those of GxE by gender (Chapter 3), (3) Assessing whether GxE

is robust between alternate approaches to outcome measurement and model assessment (Chapter 4), and (4) The identification and assessment of other genes (Chapter 5) and environments (Chapter 6) that are related to *MAOA* and childhood adversity in defining risk for CD.

An Overview of Conduct Disorder

Conduct Disorder is defined by the Diagnostic and Statistical Manual, 4th edition as a repetitive and persistent pattern of behavior that violates the basic rights of others and/or major age-appropriate social norms. A psychiatric diagnosis of CD results from the presence of any three behaviors categorized into the 4 diagnosis groups of Conduct Disorder for a 12-month period, with at least one behavior persisting during the 6-months prior to diagnosis (American Psychiatric Association, 1994). The diagnosis groups of Conduct Disorder include (1) aggressive conduct, causing or threatening physical harm to people or animals (ie: bullying, initiation of physical fights, use of a weapon that can harm others, physical cruelty to people and/or animals, stealing while confronting a victim, or forcing sexual activity), (2) non-aggressive conduct causing property damage or loss (ie: deliberate fire setting, deliberate vandalism of property), (3) deceitfulness or theft (ie: breaking into someone else's property, lying to obtain goods/favors or to avoid obligations, or stealing valuable items without victim confrontation) and (4) serious violations of rules/laws (ie: staying out of the house at night past parental restrictions, running away from home at least twice while living with a parent or other caretaker, or frequent truancy from school beginning at age 13 years).

The prevalence of Conduct Disorder ranges from 6%-16% in males under 18 years and 2%-9% in females (American Psychiatric Association, 1994). The costs incurred to public agencies is 10 times greater for children and adolescents with a CD diagnosis and 3.5 times greater for those with a borderline CD diagnosis compared to those without (Foster, Jones, & Group, 2005). In addition, childhood CD is a risk factor for future adult antisocial personality disorder, conviction for violent crimes in adolescence and adulthood, depression, conduct disorder, antisocial behavior, substance abuse, peer rejection, poor school performance, poor performance in the workplace, and school dropout (van Lier & Crijnen, 2005).

Environmental Risk Associated with Conduct Disorder

Several risk factors have been separately associated with CD and can be differentiated as proximal or distal. Proximal risk factors are individual-specific and include biological processes as well family-level risk factors. These risk factors include parental antisocial personality disorder, poor parenting, physical/sexual abuse, parental neglect, decreased frontal lobe functioning, low serotonin levels, low salivary cortisol, underarousal of the autonomic nervous system, maternal prenatal smoking, birth complications, lead exposure, negative temperaments (ie: negative emotionality, intense/reactive responding and inflexibility), attachment problems in early childhood, reading problems, behavioral impulsivity, lack of social cognition, and poor timing of puberty. Distal risk factors operate outside the individual and the immediate family environment and include peer rejection, association with deviant peers, community

disorganization, community unemployment, neighborhood violence, and community availability of drugs. Further, distal risk factors are particularly important in addressing CD as public health problem through public policy and community prevention programs (Bassarath, 2001; Burke, Loeber, & Birmaher, 2002; Simonoff, 2001).

Poor Family Environment as an Environmental Risk Factor for Conduct Disorder. Family-level risk factors such as inter-parental violence, inconsistent parenting and parental neglect define environmental risk in this study and are important in the development of CD. Exposure to inter-parental violence is hypothesized to present a model of aggression in the household as a normative part of family relationships that can be used to control others and many times can go unpunished (Osofsky, 1995). Therefore, household aggressive behavior may be imitated, giving rise to difficulty in social adjustment outside the home (Dodge, 1986; Fergusson & Horwood, 1998). Inconsistent parenting is hypothesized to contribute to CD risk through a failure to restrain a child's impulse towards deviance and antisocial behaviors (Gottfredson & Hirschi, 1990). Finally, parental neglect, defined by Burgess and Conger (1978) as "the harming of a child through lack of care or supervision" (Burgess & Conger, 1978) has been most commonly observed as a risk factor for CD (Bassarath, 2001). It is hypothesized to manifest a lack of parental control over a child's exposure to social risk factors outside of the home, such as deviant peers (Scaramella, Conger, Spoth, & Simons, 2002).

Biological Risk Factors Associated with Conduct Disorder

The serotonin and dopamine neurotransmitter systems have been associated with CD. The serotonin system plays an important role in the regulation of mood and affective regulation, cognition, satiety, and various autonomic functions when responding to stress. The dopamine system may help to explain the observed comorbidity between Attention Deficit Hyperactivity Disorder (ADHD) and CD (Thapar, Harrington, & McGuffin, 2001) as well as associations observed between externalizing problems in children and the gene regulating dopamine transport (Young, Smolen, Corley et al., 2002).

Measures of the functioning of the hypothalamic-pituitary-adrenal (HPA) axis such as salivary cortisol levels may serve as a possible endophenotype for CD, measuring the response to stressful environments. Exposure to stress results in the release of corticotrophin releasing hormone from the hypothalamus, which triggers the release of ACTH from the anterior pituitary gland, followed by the release of glucocorticoid from the adrenal cortex (reviewed in Barr, Newman, Schwandt et al., 2004). Therefore, an individual's reaction on both biological and behavioral levels to stressful or abusive environments may be better understood through the study of the HPA axis and the genes associated with neurotransmitters related to its function including monoamine oxidase-A, serotnin transporter and dopamine transporter by attempting to understand the role of these genes in the development of CD (Barr, Newman, Schwandt et al., 2004).

Monoamine Oxidase-A as a Candidate Gene in the Study of Conduct Disorder.

Monoamine oxidase-A (MAOA, EC 1.4.3.4) is responsible for the degradation of biogenic amines including the neurotransmitters epinephrine, norepinephrine, dopamine, and serotonin via deamination. *MAOA* is localized to Xp11.4-Xp11.3. A nonsense mutation in exon 8 (Gln296Stop) causes the truncation of the protein at codon 296, resulting in the loss of MAOA activity (Brunner, Nelen, Breakefield, Ropers, & van Oost, 1993). Males with the exon 8 mutation have engaged in impulsive/aggressive behaviors including rape, arson, and assault (Brunner et al., 1993). A mutation in transgenic mice results in the deletion of exons 2 and 3, yielding a non-functioning enzyme that is associated with increased aggressiveness and injury among male mice and their cage-mates (Cases, Seif, Grimsby et al., 1995).

The *MAOA* promoter region contains a variable number tandem repeat (VNTR) polymorphism with suggested effects on transcription level. Studies have reported low transcription activity for the 3- and 5-repeat elements while the 3.5- and 4- repeats had high transcription activity (Denney, Koch, & Craig, 1999; Sabol, Hu, & Hamer, 1998). However, studies of brain tissue, fibroblasts, neuroblastoma, and choriocarcinoma cell lines do not converge on similar conclusions. For example, studies using neuroblastoma cell lines do not agree on the transcriptional activity of the 3.5-repeat allele as either low or high (Deckert, Catalano, Syagailo et al., 1999; Sabol et al., 1998). There has also been difficulty evaluating the transcription and enzyme activities of the rare 2- and 5- repeat alleles (Balciuniene, Emilsson, Oreland, Pettersson, & Jazin, 2002; Denney et al., 1999). Finally, in a study of human post-mortem brain samples,

Balciuniene and colleagues (2002) reported that there were no significant differences in enzyme activity for alleles of the *MAOA* promoter region. They also noted that the differences in transcriptional regulation in brain samples and the cell transfection studies may be attributable to differences in the regulation of *MAOA* in the different cells types. The inconsistent results of transcription and enzyme activity associated with alleles of the *MAOA* promoter therefore make the assessment of association studies of this locus difficult to interpret. This study defines *MAOA* transcription activity for each allele in the manner reported by Sabol, Hu and Hamer (1998), as low activity for the 3- and 5- repeat alleles and high activity for the 3.5- and 4- repeat alleles. This definition of transcription activity was used because the reported transcription efficiencies were consistent across 2 different cell types (neuroblastoma and placental choriocarcinoma) and because these results were replicated in a later study of skin fibroblasts (Denney et al., 1999).

Inclusion of Females Heterozygous for *MAOA* is Complicated by X-Inactivation. X-inactivation is the mechanism by which X chromosome dosage (2 in females and 1 in males) is compensated between males and females (Lyon, 1963). X-inactivation is caused by methylation of the X-inactivation center on either one of the X chromosomes in each female cell and silences genes on that chromosome. X-inactivation is thought to occur randomly, with paternally and maternally derived X chromosomes equally as likely to be inactivated, resulting in functionally mosaic cell populations consisting of X chromosomes from both parents. Additionally, once an X

chromosome is inactivated, it remains inactivated for the life of the cell and all the resulting daughter cells will also have the same inactive X (Heard & Disteche, 2006; Nussbaum, McInnes, Willard, & Boerkoel, 2001).

In general, X-inactivation in females is expected to be random, such that 50% of active X chromosomes are paternal and 50% maternal. Departure from this expectation is referred to as skewed X-inactivation (Heard et al., 2006; Nussbaum et al., 2001). Highly skewed inactivation patterns can result, for example, from an X-chromosome abnormality. Cells with an active damaged chromosome may have a significant survival disadvantage and so be underrepresented in the adult carrier (Amos-Landgraf, Cottle, Plenge et al., 2006). This is a passive process, which occurs after inactivation itself, and may affect all daughter cells or only those in certain tissues.

The study of allele-specific MAOA transcription in heterozygous females is potentially difficult. First, because of X-inactivation, assessment of both inactivation and expression must be made at the level of individual cells. Second, skewed inactivation will introduce error in the allele-specific expression values. One report suggested that MAOA escapes inactivation, which would simplify these assessments since heterozygotes would express both alleles (Carrel & Willard, 2005), but this has not been supported (Benjamin, Van Bakel, & Craig, 2000; Nordquist & Oreland, 2006; Pinsonneault, Papp, & Sadee, 2006). Further, a recent study showed no evidence for skewed inactivation patterns due to MAOA polymorphism alleles (Pinsonneault et al., 2006). Since there is no evidence for skewing of inactivation on the basis of MAOA

promoter polymorphism alleles and limited evidence for escape from X-inactivation, cells of heterozygous females are equally expected to express either allele.

Genotype, Environment and their Combined Effects on Conduct Disorder

The distinction made between genetic and environmental risk has been helpful in identifying and describing risk factors of CD, though their separation ignores processes that may be important in its etiology. Three descriptions of combined genotype and environment effects are likely sources of variation and important in understanding how genes and environments common in families might operate to produce CD. These factors are genotype-environment interaction, genotype-environment correlation and assortative mating.

Genotype-Environment Interaction. Genotype-environment interaction is defined as a genotype-specific sensitivity to environmental exposure of an organism (Fisher, Immer, & Tedin, 1932) and has also been reported to moderate risk of environmental exposure in adoption, twin and singleton samples. Careful studies of GxE in plant and animal models have described three specific characteristics on the nature of the interaction. First, genes contribute to the sensitivity of an organism to specific environments. Perkins and Jinks (1971) who reported that the average performance and sensitivity among 82 inbred lines of *Nicotiana rustica* was due to genetic control specific to each line. Second, genes with sensitivity to a particular environment may function differently from other genes. Mather and Jinks (1982)

demonstrated this characteristic when estimating the average number of loculi per fruit in several crosses of different tomato species, observing that the additive and dominance properties of the Danmark X Johannisfeuer cross differed from that of similar crosses. Third, various genes are responsible for sensitivity to different environments as observed in the work of Mather and Caligari (1976) who observed among *Drosophila melanogaster*, genes responsible for average sternopleural chaeta number resided on chromosome 3, while temperature sensitive genes resided on chromosome 2 (Eaves, 1984; Mather & Caligari, 1976; Mather & Jinks, 1982; Perkins & Jinks, 1971) .

General characterizations, such as those of scalar and non-scalar GxE, are used to describe how genotypes and environmental exposures influence liability for a phenotype. Scalar GxE refers to an increase in the probability of illness by genotype group across increasing environmental exposure, without any change in the ranking of genotype groups across levels of exposure. In comparison, non-scalar GxE interaction refers to a change in the probability ranking of illness across increasing environmental exposure for each genotype (Figure 1.1) (Eaves, Chen, Maes, Neale, & Silberg, 2005; Kendler & Eaves, 1986; Mather et al., 1982).

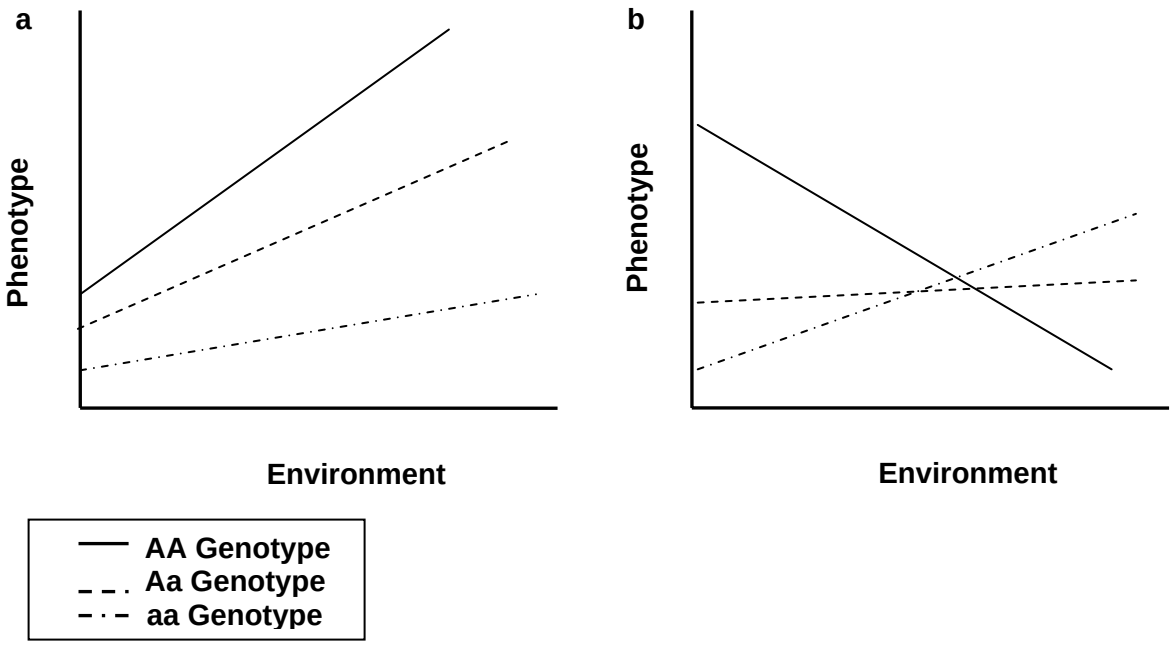


Figure 1.1 Examples of (a) Scalar and (b) Non-Scalar Gene-Environment Interaction (Taken from Psychiatric Genetics, 2005)

Human studies have observed GxE in several different phenotypes including Alzheimer's disease, ischaemic heart disease, response to infections, response to medication, alcohol sensitivity, antisocial personality, substance abuse, anxiety, depression, as well as cognitive scores (Rutter & Silberg, 2002).

Human behavioral genetic studies have reported genotype-environment interaction (GxE) in the development of CD and antisocial behavior. Antisocial behavior refers to those latent traits leading to a CD diagnosis, or those behaviors that are physically violent or non-violent, with acts involving property. Adoption studies have been the first to highlight the importance of GxE and have reported that genetic

risk, identified as alcoholic or antisocial behavior in the biological parents, in the presence of an adverse adoptive environment increased risk for child antisocial behavior (eg: Cadoret, Yates, Troughton, Woodworth, and Stewart, 1995). Cadoret reported no gender difference in a model of GxE for aggression and CD (Cadoret, Yates, Troughton, Woodworth, & Stewart, 1995), while other studies have reported gender differences in those genetic and environmental factors important to CD (Cadoret & Cain, 1974; Goldstein, Prescott, & Kendler, 2001). Studies of aggression in non-human primates have observed increased aggression resulting from interactions between male rhesus monkeys with the low activity *MAOA* allele and exposure to aggression either from maternal-only or peer-raised contexts, suggesting a sensitivity to specific social environments (Newman, Syagailo, Barr et al., 2005).

Twin studies have implicated interaction between genetic liability to CD and exposure to maltreatment (Jaffee, Caspi, Moffitt et al., 2005). Among a community sample of singletons, Caspi and colleagues (2002) reported a measured genotype-environment interaction for males with a low-activity MAOA allele and household maltreatment, defined as maternal rejection, inconsistent presence and identification of any particular primary caregiver, harsh discipline, physical abuse, and sexual abuse among males. The finding in males has been replicated (Foley, Eaves, Wormley et al., 2004; Kim-Cohen, Caspi, Taylor et al., 2006; Nilsson, Sjöberg, Damberg et al., 2005), although non-replication has also been reported (Haberstick, Lessem, Hopfer et al., 2005; Young, Smolen, Hewitt et al., 2006). This interaction was also reported in females, encouraging further investigation (Caspi, McClay, Moffitt et al., 2002).

Genotype-Environment Correlation. Gene-environment interaction will be detected at the statistical level when, at the functional level, genetic differences are observed in sensitivity to an environment (Mather et al., 1982). However, a statistically significant interaction can be due to either gene-environment correlation (rGE), GxE or their combination. Gene-environment correlation (rGE) occurs because parents and offspring share their genes and home environments and confounds the detection of GxE. rGE is defined as a genetic control of exposure to the environment (Jinks & Fulker, 1970) and can be further distinguished by three different taxonomies. The first, as described by Cattell (1963) identified within-family relationships such as parent-child interaction and sibling relationships as well as between-family genotype-environment correlations such as neighborhoods and schools that contribute to variation in behavior (Catell, 1963).

Plomin described another taxonomy to rGE by describing passive, active and evocative forms of rGE (Plomin, DeFries, & Loehlin, 1977; Scarr & McCartney, 1983). Passive rGE is defined as children receiving genotypes that are correlated with their family environment. For example, parents with antisocial personality disorder (ASP) would both transmit genes and produce environments that increase risk for CD in their offspring. Evocative rGE refers to a situation where the child's genotype and behavior elicits parental, familial or teacher responses such as neglect. For example, a child of difficult temperament is often punished for their actions with aggressive contact and consequently perpetuates this behavior. Active rGE refers to individuals who seek out

environments that correspond to their genetically influenced traits. For example, children with difficult and aggressive temperaments may be more likely to seek out friends who are also aggressive. While it is difficult to separately estimate active and evocative rGE using the majority of study designs (Falconer & Mackay, 1996), direct measurement of specific environments over time in an adoption design is anticipated to adequately assess the direction of effects between the child's genotype and their environmental exposure (Cadoret et al., 1995).

A third taxonomy of rGE addresses the conceptualization of rGE by Cattell and the difficulty in its estimation noted by Jinks and Fulker (1970) by defining gene-environment covariance (CovGE) as resulting from environments selected by genotypes, sibling effects or cultural transmission (Eaves, Last, Martin, & Jinks, 1977). The phrase "environments that are selected by genotypes" refers to individuals with genotypes that are associated with a particular phenotype seeking or creating environments that reinforce the behavior. This is similar to the aforementioned active or evocative types of rGE, though it doesn't attempt to distinguish the two. Individuals with susceptibility genes for CD might create environments through their interactions with the environment outside of the home, such as associating with low-achieving or behaviorally deviant peers and provoking negative responses from teachers. Sibling interaction occurs when siblings modify their personal behaviors with respect to the behavior of the other sibling. For example, one sibling may develop aggressive behaviors in response to the aggressive nature of their sibling (imitation effects) or a sibling may develop passive behaviors in contrast to the aggressive behaviors of the

other sibling as a result of the observation of severe punishment for such behavior (contrast effects) (Eaves, 1976). Cultural transmission is understood as a phenotype of the parent influencing the phenotype of the child through the environment. This is similar to passive rGE, where parents with ASP transmit susceptibility genes for CD and a high-risk home environment.

Gene-environment correlation for antisocial behavior, particularly evocative rGE, has been reported in adoption studies of CD. O'Conner et al reported that adoptees with higher genetic risk were also more likely to receive negative parenting (O'Connor, Deater-Deckard, Fulker, Rutter, & Plomin, 1998a). Ge and colleagues reported similar results of evocative rGE, where biological parents with ASP-related psychiatric disorders were significantly associated with the adopted children's antisocial/hostile behaviors (Ge, Conger, Cadoret et al., 1996). Gene-environment correlation has also been observed in other phenotypes such as depression and alcohol abuse (Rutter et al., 2002). Twin studies partition the total variance to include variance due to rGE in the absence of a specified genotype. Meyer and colleagues observed both strong additive genetic and familial effects (as measured by family adaptability), indicating the role of passive rGE in twin CD using an extended twin design (Meyer, Rutter, Silberg et al., 2000). Gene-environment correlation may play a role in CD development since (1) diagnosis of parental ASP is associated with neglect of children in the household (American Psychiatric Association, 1994), (2) ASP is a heritable disorder (Lyons, True, Eisen et al., 1995), and (3) passive (Ge et al., 1996; Meyer et al., 2000) and evocative (O'Connor, Deater-Deckard, Fulker, Rutter, & Plomin, 1998b;

Riggins-Caspers, Cadoret, Knutson, & Langbehn, 2003) rGE have been identified in the etiology of CD.

Assortative Mating. The non-random mating of individuals resulting from factors other than those of biological relatedness is known as assortative mating. The classical treatment of assortative mating assumes that mates choose one another for the phenotype of interest and is often indicated by a significant phenotypic correlation between mates. This phenotype may be influenced by both genetic and environmental factors. In turn, when partners select one another by phenotype, they also indirectly select mates who are also similar genetically and culturally. Therefore, assortative mating for a particular phenotype may affect the transmission, magnitude and correlation of genetic and environmental effects on that outcome (Neale & Maes, 2002).

The importance of assortative mating in the etiology of CD has been consistently reported (Krueger, Moffitt, Caspi, Bleske, & Silva, 1998). Additionally, the quality of the home environment is a reflection of parental behavior. Therefore, any association between parental ASP and child CD reflects the genetic correlation between parents and children as well as the parent-influenced environment (Meyer et al., 2000; Moffitt, Caspi, Rutter, & Silva, 2001a).

Genotype-environment interaction, rGE and assortative mating highlight the complexity in understanding the etiology of CD in families. Assortative mating increases the genetic and environmental correlations between relatives. In the study of CD, it indexes the extent to which antisocial parents shape the risk environments around

themselves and their children (Meyer et al., 2000). Additionally, the omission of rGE and GxE also results in a potential bias of estimated genetic and environmental effects (Eaves et al., 1977) since the exclusion of rGE and GxE treats environmental exposure as a random rather than a systematic variable. In order to understand the relationship between genes and environments as risk factors of CD, it is important to include gene-environment correlation along with GxE in order to disentangle and assess their individual roles (Eaves, Silberg, & Erkanli, 2003b). For example, Foley et al (2004) reported a significant GxE between low *MAOA* genotype in the presence of childhood adversity on risk for CD after testing for the effects of evocative rGE and controlling for the effects passive rGE using parental ASP in males. Thus, the sensitivity of males with the low activity *MAOA* allele to household maltreatment was not confounded by the passive genotypic control of environmental exposure.

Issues in the Detection and Interpretation of GxE in Addressing Risk for CD

Several issues hinder our ability to utilize GxE as a tool for public health prevention of CD. First, the functional significance of polymorphisms of candidate genes is unclear, which presents difficulties in determining the role of candidate genes in CD. For example, it is difficult to draw a conclusion regarding the function of the MAOA promoter polymorphism as a result of conflicting reports of transcription and enzyme activities (Balciuniene et al., 2002; Deckert et al., 1999; Denney et al., 1999; Sabol et al., 1998). Additionally, most studies have avoided inclusion of females with heterozygous genotypes due to the uncertainty in inactivation of MAOA and of whether

X-inactivation is skewed as a result of a specific MAOA allele. Second, measures of the environment with reference to behavior are often problematic and may either diminish our ability to detect or overestimate the effects of GxE. It is important to clearly distinguish the environment as one with known risk for a disorder of interest (Moffitt, 2005; Moffitt et al., 2005). However, measurements of the environment and those leading to diagnosis vary from study to study as well as between respondents (ie: parents versus children). In addition, the timing of measurement is important, since mental health disorders often develop during different times of the life span. Conduct disorder has the highest prevalence during middle to late adolescence (Lahey, Schawb-Stone, Goodman et al., 2000). Therefore, risk factors and their interactions may change over time as genetic sensitivity may change throughout periods of development (Moffitt et al., 2005). Also, the definition of "environment" with respect to GxE studies of CD have focused on proximal, or familial environment, mainly parental neglect, abuse and ASP. However, the study of environmental risk and its application to GxE suffers from a degree of haphazard choice in environment by using the "best" proximal environment based on previous, focused studies of specific risk factors at the expense of ignoring other potential environments. No known studies have systematically assessed proximal and distal environments to determine which environments may be risk factors either alone or via interaction with each other or with any gene of interest.

Third, our ability to detect GxE and adequately measure its effects is hindered by our treatment of the data as well as current methodologies to detect and quantify GxE. In the absence of specified genetic markers, twin and adoption studies are left to

partition the variance associated with GxE from their estimates of genetic and environmental effects according to the strengths of their specific study designs (Eaves et al., 1977; Heath, Kendler, Eaves, & Markell, 1985; Jinks et al., 1970). Recently, several advances have been made by incorporating more innovative ways at partitioning the total variance to reflect the variance contributed by GxE (Eaves & Erkanli, 2003a; Heath et al., 1985; Purcell, 2002). However, as more functional genetic variants are used, it becomes important and feasible to model specific genetic effects. Therefore, GxE might be better understood as an alteration of risk as a function of a specific genotype rather than alterations of the estimates of anonymous additive genetic effects (Eaves et al., 1977).

In the presence of specific genes and environment, epidemiological samples of unrelated individuals have often utilized a case-control approach and compared the relative risks and odds ratios for individuals with specific genotypes stratified by environmental exposure. However, it is difficult to collect data and match environmental exposures across members of both groups to ensure that members of each group only differ by exposure level. A multiple or logistic regression approach can also estimate the contributions of genetic and environmental, although GxE is not considered to be a source of large variation and may be difficult to detect (Eaves et al., 2003a). It is often commented that any statistical interaction should take place in the presence of individual main effects. However, if genes and environments work in a synergistic manner, it is possible that main effects are not always detected because of the need for genetic and environmental risks to be assessed simultaneously. In addition,

when studying disorders like CD that have a low prevalence in the general population, the power to detect main genetic effects and GxE is low. Also, studies of GxE often fail to address the inherent issue of gene-environment correlation, which is known to function with GxE (Eaves et al., 2003b). Finally, the discrepancy between a statistical interaction and its meaning with regard to biological interaction makes it difficult for GxE to be informative to public health.

The Virginia Twin Study of Adolescent Behavioral Development: A Description of the Study Population and Methodologies

Data for all analyses were obtained from the Virginia Twin Study of Adolescent Behavioral Development (VTSABD), which was designed as a longitudinal study of psychiatric symptoms and disorders of adolescent twins to (1) determine the rates of child/adolescent psychiatric disorders in a community sample, (2) assess the roles of genetic and environmental variation leading to the susceptibility to childhood psychopathology, (3) describe the development of psychopathology from childhood to adulthood, (4) study the mechanisms underlying the comorbidity of psychopathology, and (5) clarify issues of measurement, conceptualization and definition of childhood psychiatric disorders (Hewitt, Silberg, Rutter et al., 1997).

The VTSABD is part of a twin population of 5,413 twin pairs born between 1974 and 1983 and residing in Virginia (Meyer, Silberg, Simonoff, Kendler, & Hewitt, 1996). Specifically, the VTSABD is a sequential cohort consisting of 1,412 twin pairs (2,824 children) and their parents followed prospectively at approximately 15-month intervals over four waves of data collection. The ascertainment and data collection of this sample has been described in detail elsewhere (Hewitt et al., 1997) (Meyer et al., 1996). Briefly, twins and their parents were ascertained through the Virginia public and private school systems in 1987 and 1988. The first wave of data collection took place between March 1990 and March 1992 and twins in this cohort ranged in age from 8-17 years old. As the study progressed, twins turning 8 years old were included and those over the age of 17 were considered too old for inclusion and aged out of the sample. A

second group of twins was ascertained between 1992 and 1993 to include a "high risk" population for conduct disorder and attention deficit hyperactivity disorder. After a telephone screen, 48 twin pairs and their parents participated. These families were administered the same protocol as the other VTSABD families and were followed over two waves.

Informed consent was obtained from both parents and twins at each wave of data collection. Only anonymous family and twin identification numbers identify each observation from this dataset. All other identifying variables were stripped from this dataset prior to receipt by staff of the Mid Atlantic Twin Registry.

Demographic Profile and Representation of the VTSABD to the General Population

The community demographic profiles of the families in the VTSABD were assessed and summarized using 1990 United States census data. These analyses were performed on the block-group level, defined as a geographic space containing on average 400 housing units. The range of participant per capita income (mean income for all individuals in a block-group) is similar to that of the general Virginia population as assessed by non-parametric analyses of variance (Meyer et al., 1996). However, participating families had a significantly higher median per capita income ($15,531) than those who were not interviewed ($14,260, $p < 0.01$). Families from urban neighborhoods and lower per capita incomes are underrepresented in this sample. However, the effects of such sampling bias on the prevalence rates of adult

psychopathology were small (Meyer et al., 1996). Additionally, while this sample is representative of white rural and urban communities throughout Virginia, it was not meant to reflect other racial groups. Due to the low sample size of black and other racial group respondents (approximately 17%), their data did not provide adequate power for meaningful analyses and were not included.

Assessment Procedures

Field interviewers performed assessments in family homes. Field interviewers received 3 weeks of standardized training from the study primary investigators prior to administering these measures. These training sessions consisted of practiced interviews, both in the training site and in the field and were monitored by trained staff. Interviewers had often obtained a Masters degree in Social Work or equivalent graduate training, or had extensive experience in administering psychiatric interviews. During the course of the study, a pair of interviewers was randomly assigned to a household and further assigned one parent and one twin to assess. Measurements were conducted in a standardized order. First, twins were interviewed simultaneously in different locations to avoid potential biases in data collection resulting from twin similarities/differences or discussion between the twins. Parent interviews followed the twins, where parents were administered measures about one specific twin. Once all measures about the first twin were completed, the process was repeated for the second twin. This procedure was implemented in an attempt to avoid comparisons between the twins for each measure. In order to ensure proper comprehension of items, twins under

the age of 11 or having less than a 6th grade reading ability were read questionnaire material by the interviewer. Additionally, for self-report questionnaires, interviewers were available to twins and parents to answer content-related questions. Regular meetings of field interviewers and primary investigators were also conducted for protocol review and to ensure the maintenance of interview standardization (Hewitt et al., 1997).

Several measurements were administered at each assessment, or wave, over the course of the study to determine child psychopathology, intelligence, reading ability, individual risk factors, environmental exposures, and parental psychopathology. Physical measures such as height and weight were taken and DNA was also collected. A teacher assessment of psychopathology was sent to those teachers identified to know a child well. DNA was collected from parents and twins during the third wave and administered by the interviewers. Interviewers instructed parents and children to rinse their mouths with water first and were each given 2 buccal swabs to scrape the inside lining of the cheek for 45 seconds. Further instructions asked that participants not touch the brushes with their fingers nor brush their teeth or gums in order to avoid contamination. Once DNA collection was completed, samples were packaged, labeled by family and twin identification numbers and sent to the Virginia Institute for Psychiatric and Behavioral Genetics molecular lab for storage. Tables 1.1 and 1.2 detail the instruments used during the home interview to measure each dimension of interest by informant (parent or child) for each wave of administration.

Table 1.1 Instruments and Dimensions Measured in the VTSABD by Wave- Twin Assessment

Wave	Instrument	Dimensions Measured
1-4		Height, Weight
1-4	Slosson Oral Reading Test	Vocabulary/Reading Level
1-4	Child and Adolescent Psychiatric Assessment (CAPA-C)(Angold & Costello, 2000)	Family Structure and Function Peer Relationships School Performance and Behavior Truancy/School Attendance Separation Anxiety Worry and Anxious Affect Depression/Depressed Affect Suicide and Self-Injurious Behavior Food-Related Behavior Sleep Problems Pubertal Stage Oppositional Disorder Conduct Disorder Alcohol Use Drug Use Incapacity- Effect of symptoms on daily life
1-4	FSSC	Specific Fears
1-4	Mood and Feelings Questionnaire	Depressed Feelings and Mood
1-4	What I Think and Feel	Manifest Anxiety
1-4	Behavior and Activities Checklist	Delinquent and Aggressive Behavior
1-4	Life Events Checklist	Shared and Individual Life Events
1-4	EASI Temperament Scales	Temperament
1-4 1-4	Sibling Inventory and Differential Experiences (SIDE)	Twin Similarities and Differences
1-4	Family Adaptability and Togetherness	Family Interaction
3	Standard Ravens Progressive Matrices	General/Non-Verbal Intelligence
3	Buccal Swab	DNA Collection
3	Section R	Peer Relations
3	Section E	Parent-Child Interaction/Household Environment

Table 1.2 Instruments and Dimensions Measured in the VTSABD by Wave- Parent Assessment

Wave	Instrument	Dimensions Measured
1-4	Child and Adolescent Psychiatric Assessment (CAPA-P)	Hyperactivity/ADD Food-Related Behavior Sleep Problems Pubertal Stage Family Structure and Function Peer Relationships School Performance and Behavior Truancy/School Attendance Separation Anxiety Worry and Anxious Affect Depression/Depressed Affect Suicide and Self-Injurious Behavior Oppositional Disorder Conduct Disorder Tobacco Use Alcohol Use Drug Use Incapacity- Effect of symptoms on daily life
1-4	FSSC	Specific Fears
1-4	Mood and Feelings Questionnaire	Depressed Feelings and Mood
1-4	What I Think and Feel	Manifest Anxiety
1-4	Behavior and Activities Checklist	Delinquent and Aggressive Behavior
1-4	Life Events Checklist	Shared and Individual Life Events
1-4	EASI Temperament Scales	Temperament
1-4	Sibling Inventory and Differential	
1-4	Experiences (SIDE)	Twin Similarities and Differences
1-4	Family Adaptability and Togetherness	Family Interaction
1-4	Rutter-A2	
1-4	Dyadic Adjustment Scale	Marital Relations
1	Section A	Prenatal and Perinatal Development
1	Sections L and M	Lifetime and Recent Parental Psychiatric History
1	Section S	Parental Antisocial Personality
2	Section P	Parental Report of Socioeconomic Status
2	Section E	Parent-Child Interaction
3	Section AA	Child Medical Problems
3	Buccal Swab	DNA Collection
3	Section CL	Religious Affiliation

Zygosity Determination

Zygosity for the like-sex twin pairs was evaluated using either blood group typing or DNA polymorphisms when such information was available for 231 or 21% of like-sex pairs. Zygosity was established for the remaining pairs using survey data and photographs and applying an algorithm previously validated against genotyped twins. Survey questions consisted of (1) the frequency the twins were mistaken for one another by strangers (frequently, occasionally or never), (2) how alike the twins were ("alike as two peas in a pod" or "of only family likeness") and (3) the parental assessment of zygosity ("definitely identical, "probably identical", "probably fraternal", or "definitely fraternal"). Maternal reports were preferred over paternal reports because they have been shown to have greater validity over paternal reports. In the absence of maternal reports, paternal reports were then used. Polaroid photographs of individual twins were taken and two independent raters were asked to score the twins as "definitely monozygotic", "probably monozygotic", "probably dizygotic", "definitely dizygotic" or "indeterminate" (Eaves, Silberg, Meyer et al., 1997).

A Summary of the Sub-Sample Used for Study

Study Population

With the exception of a single study (Chapter 3, "Detection of GxE in Twin Pairs"), all analyses are based on a sub-sample of 1299 individual participants/ 649 twin pairs and their parents from the VTSABD whom twin *MAOA* genotype, diagnosis of conduct disorder, and maternal antisocial personality symptoms and household neglect

information were obtained. The range of eligible participants upon entry into the study was 8 -17 years (mean ± SD, males- 11.12 ± 2.28 years, females- 11.24 ± 2.54 years). Sample sizes vary across studies and reflect different inclusion criteria by variables used for study. Table 1.3 summarizes the samples used in each chapter and the criteria used to produce them.

Table 1.3 Summary of Sample Sizes Used by Chapter and Sample Inclusion Criteria

Chapter	Sample Size	Inclusion Criteria
2	1299 individuals	*MAOA* genotype, childhood adversity, CD diagnosis, and maternal antisocial personality symptoms
3	1124 twin pairs	Wave 1 CD symptoms and childhood adversity
4	540 twin pairs	*MAOA* genotype, wave 3 CD symptoms and childhood adversity
5	1218 individuals	*MAOA* genotype and either *5HTTLPR* or *DAT1* genotypes, CD diagnosis and ADHD diagnosis
6	1299 individuals	*MAOA* genotype, childhood adversity, CD diagnosis, and maternal antisocial personality symptoms

Sample Representativeness

Individuals included in the sub-sample were younger than those who were not ($p<0.0001$) as expected due to older participants "aging-out" of the study after age 18 before data collection, including DNA collection was completed over all waves. The rates of CD were comparable between sub-sample members (3.9%) and those not included (5.4%, $p = 0.07$). The average number of maternal ASP symptoms upon entry into the study between sub-sample members (0.76 ± 0.90) and those not included (0.94

± 1.03) was significantly different (p = <0.0001), as a result of a significant difference between participants and non-participants for maternal ASP symptoms among males. Individuals included in the sub-sample were similar for measured census-based indicators of socioeconomic status such as median family income (p = 0.07), rural vs. urban residence (p = 0.36), and the proportion of individuals over age 18 having received college-level education (p = 0.24).

Assessments

Measures of CD, childhood adversity and parental ASP as well as *MAOA* genotype were used throughout this study and the protocols for their assessments are summarized below.

Diagnosis of Conduct Disorder. All samples consisted of data on individual twins registered in the VTSABD on previous 3-month history of CD at any of the 4 waves as assessed with the Child and Adolescent Psychiatric Assessment (CAPA), which is based on the Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, revised (DSM III-R) criteria (Angold et al., 2000). Symptoms were reported by maternal, paternal or child self-report and a CD diagnosis was defined using a symptom-or rule from any wave of data collection. Under the symptom-or rule, a symptom was rated as being present when either parent or child endorsed the item. This algorithm is particularly helpful by using responses from multiple informants rather than relying on a single respondent (Simonoff, Pickles, Meyer et al., 1997). CD

diagnosis was assigned to those twins having three or more symptoms under the symptom-or algorithm.

For some studies (Chapters 3 and 4), CD was measured by the number of symptoms reported by each rater at a specific wave. Responses to binary items were summed and used as a scale ranging from 0 to 7.

Measurement of Childhood Adversity. Three measures of negative family environment associated with CD indexed childhood adversity, specifically parental neglect, exposure to inter-parental violence and inconsistent parental discipline. Parental neglect was assessed using parent report and utilized three items to determine a lack of care severe enough to be recognized by individuals outside the home, including notification from others on the lack of general care for the children, illness due to insufficient parental care and failure to seek medical attention for the children when such care was necessary. Exposure to inter-parental violence was measured by child report and utilized two items to determine whether parents make physical contact (ie: pushing, shoving or hitting) with one another during disagreements. Inconsistent parental discipline was obtained by child report to determine whether each parent maintained consistent responses to child rule breaking. Responses to the binary items were summed and used as a scale ranging from 0 to 7.

Parental Antisocial Personality Symptoms. Maternal and paternal ASP symptoms were measured separately as the sum of the following seven binary items:

inconsistent work behavior, failure to conform to social norms and laws, irritability/aggression or involvement in fighting or assault, failure to honor financial obligations, impulsivity, recklessness in the safety of self or others, and no long-term (>1 year) monogamous romantic relationships. Responses to the binary items were summed to form a measure of ASP, having a scale ranging from 0 to 7.

DNA Extraction and *MAOA* Genotyping. DNA was obtained from buccal cells using cytology brush for collection. DNA was isolated using the InstaGene Matrix kit protocol for cell lysis absorption (Bio-Rad Laboratories, Hercules, CA).

Genotyping of the *MAOA* promoter polymorphism used samples with a working concentration of 5-20 ng/µl. Primer sequences previously described were used (Sabol et al., 1998), specifically MAO APT1 labeled with the FAM-6 fluorophore (5'ACAGCCTGACCGTGGAGAAG3') and MAO APB1 (5'GAACGGACGCTCCATTCGGA3'). Polymerase chain reaction amplification of the *MAOA* promoter region VNTR was performed in 96-well microtitre plates, using a 10 µl volume containing 50-200 ng of genomic DNA, 10X PCR Buffer (Invitrogen), 0.3 mM 2' deoxynuceloside 5' triphosphate (Invitrogen), 50 mM Magnesium Chloride, 0.3 µmol each of forward and reverse primer, and 0.5 U Platinum Taq DNA Polymerase (Invitrogen). Cycling reactions were performed on a PTC-225 DNA engine (MJ Research Inc., Waltham, MA) with 3 a minute initial denaturation at 95ºC, followed by 35 cycles at 95ºC for 3 minutes, 62ºC for 1 minute, 72ºC for 1.5 minutes, and concluding with a final extension at 72ºC for 8 minutes. Products were analyzed using

an SCE-9610 capillary sequencer (Spectrumedix, State College, PA), ROX-labelled GX-500 size standard and Genospectrum v 2.6 DNA fragment analysis software (SpectruMedix). Classification of *MAOA* activity (high or low) was assigned to each allele resulting from previous work in the efficiency of transcription activity of the *MAOA* gene promoter (Sabol et al., 1998).

CHAPTER 2 Gender Differences in the Interaction of Monoamine Oxidase-A and Childhood Adversity as Risk Factors for Conduct Disorder

Abstract

Recent studies among males have reported a genotype-environment interaction (GxE) in which low activity alleles at the Monoamine Oxidase-A (*MAOA*) locus confer greater sensitivity to the increasing effects of childhood adversity on conduct disorder, although not all studies have replicated this finding. So far, few attempts have been made to generalize these findings to females and compare how GxE differs by gender. The current study tests for interaction between *MAOA* genotype and exposure to childhood adversity as predictors for antisocial behavior in females and compares the findings with males.

A longitudinal study of adolescent twins from the Virginia Twin Study of Adolescent Behavioral Development (VTSABD) assessed risk for Conduct Disorder (CD) using *MAOA* genotype, exposure to childhood adversity and parental antisocial personality disorder (ASPD) as risk factors. Mixed models were fitted that specified fixed additive (homozygous) and dominance (heterozygous) effects on CD of genotypes at the *MAOA* locus together with the main effect of adversity and the interaction of additive and dominance effects with adversity. Secondary analysis including male data assessed the role of gender as an additional risk factor.

The analysis revealed significant main effects of increasing maternal, but not paternal ASPD. A significant main effect of genotype was detected, where alleles classified as low activity imparted the greatest risk to CD in girls. Marginally significant GxE was detected, suggesting higher sensitivity to childhood adversity in the presence of low activity *MAOA* genotype among females.

Introduction

Although gender differences are consistently reported with respect to the prevalence of Conduct Disorder (CD) (Eley, Lichtenstein, & Stevenson, 1999; Farrington & Loeber, 2000; Jacobson, Prescott, & Kendler, 2002a; Moffitt et al., 2001a; Simonoff et al., 1997), the development of such differences is not well understood. The male to female ratio for CD prevalence varies from 2:1 to 5:1 (AACAP official action, 1997; Loeber, Burke, Lahey, Winters, & Zera, 2000; Maughan, Rowe, Messer, Goodman, & Meltzer, 2004) despite similarities in the factors of risk, comorbidity patterns and age of onset (Herrera & McCloskey, 2001a; Ilomaki, Viilo, Hakko et al., 2006; Moffitt et al., 2001a). Further, though males have higher rates of physical aggression and violence, males and females engage in partner violence to a similar degree. Male and females with CD also share similar outcomes following adolescence including a poor transition into adulthood, drug and alcohol related offenses and the likelihood of engaging in intimate relationships with other adults having attitudes that encourage antisocial behavior (Moffitt et al., 2001a). Less clear is the how the development of CD differs between gender and the role of genetic risk, specifically polymorphisms of monoamine oxidase-A (*MAOA*), in the presence of environmental risk defined here as family-level risk factors.

Environmental Risk Factors of Conduct Disorder

Family-level risk factors such as inter-parental violence, inconsistent parenting and parental neglect are important to understanding the development of CD and have been the subject of a few studies addressing gender differences in CD prevalence. Males and females have been reported to be equally exposed to inter-parental violence (Moffitt, Caspi, Rutter, & Silva, 2001b), though it is unclear whether this risk factor functions similarly in the development of CD in both groups (Becker & McCloskey, 2002; Kinsfogel & Grych, 2004; Herrera & McCloskey, 2001b). Becker and McCloskey (2002) reported an association between witnessing family violence and conduct problems in females only. In contrast, witnessing marital violence predicted offending behaviors, such as those characterizing CD in both males and females. Further, females witnessing parental violence and who were physically abused were most likely to engage in offending behavior (Herrera et al., 2001b). However, after controlling for physical abuse, risk of offending behavior from witnessing family violence was not significant (Herrera & McCloskey, 2003).

One study has reported non-significant gender differences for exposure to inconsistent parenting as a risk factor for CD (Moffitt et al., 2001b). Few studies have tested the gender differences in exposure to inconsistent parenting and risk for CD, highlighting the need for further research in this area. Additionally, the lack of studies on gender differences in exposure to parental neglect and risk for CD makes it difficult to determine whether a gender difference in parental neglect accounts for the gender

difference in CD. Since CD is a risk factor for criminal arrests and criminal offenders are likely to commit serious acts of antisocial behavior, samples of incarcerated individuals are sometimes used to study risk factors for CD (Giodarno, Cernkovich, & Lowery, 2004). Among juvenile delinquents, females were more likely to have experienced physical neglect than boys, though both were equally as likely to be exposed to emotional neglect (McCabe, Lansing, Garland, & Hough, 2002). Like exposure to inconsistent parenting, more work is required to address whether gender differences in exposure to parental neglect also accounts for gender differences in CD.

Genetic Risk Factors of Conduct Disorder

Behavioral genetic studies of gender differences have provided limited insight into the genetic and environmental contributions in the development of CD. In general, CD is considered a heritable trait. Reports of gender differences in the magnitude of genetic and environmental influences using twin studies have been mixed. Several studies report significant gender differences in the heritability of CD and antisocial behavior in general. For example, within an unselected sample, Graham and Stevenson (1985) found stronger evidence for genetic effects in males compared to females for behavioral deviance (Graham & Stevenson, 1985). Similarly, gender differences in the heritability estimates of antisocial behaviors were reported, with a greater additive genetic effect occurring in females compared to males for non-aggressive delinquent behavior. However, an absence of gender differences was also reported for the heritability estimates of aggressive delinquent behavior (Eley et al., 1999). Another

study reported a substantial additive genetic effect in females and a high shared environment effect in males for childhood antisocial behaviors, while no gender differences were reported for additive and shared environmental effects in adolescent antisocial behavior (Jacobson et al., 2002a). Hudziak et al. also report a weak gender difference in heritability estimates for childhood aggression (Hudziak, van Beijsterveldt, Bartels et al., 2003).

In contrast, Eaves et al (1997) reported no gender differences in additive genetic and shared environment effects for CD using self-report questionnaires. Likewise, studies of adult antisocial behaviors show no gender differences in heritability (Rhee & Waldman, 2002; Slutske, Heath, Dinwiddie et al., 1997). Further, specific items used in the diagnosis of CD such as stealing without confrontation, use of weapons and fighting show a similar degree of moderate heritability in males and females after accounting for differences in prevalence by gender (Gelhorn, Stallings, Young et al., 2005). Adoption studies are also varied with respect to gender differences of genetic and environmental effects. Some studies report that the same genetic factors are responsible for antisocial behaviors in both males and females (Cadoret & Cain, 1980), while others suggest greater genetic effects in female CD compared with males (Langbehn, Cadoret, Yates, Troughton, & Stewart, 1998).

A Biometrical Genetic Approach to Detect Main Genetic Effects and Genotype-Environment Interaction Using a Measured Genotype

In the absence of experimental data to determine the levels of *MAOA* expression of homozygotes and heterozygotes, it remains possible to specify their phenotypic differences by genotype. Fisher, Immer and Tedin (1932) define the phenotype mid-way between the homozygous phenotypes as *m*. The value *h* is used to identify the phenotypic departure of the heterozygote from *m*. $+d$ and $-d$ are the phenotypic differences of the homozygotes from the mid-point. Thus, *d* refers to the fixable or additive genetic variation, while *h* reflects the unfixable heritable variation or dominance properties. Here, *additive genetic variation* refers to the sum of the average effects of the individual alleles on a phenotype. *Dominance* refers to Mendel's classical experiments where the progeny phenotype of a cross between two pure-breeding lines would favor one parent more than the other (Neale et al., 2002). The heterozygous progeny did not have a phenotype exactly midway between that of the parents and *h* can have a positive or negative value. Thus, dominance only reflects the effect of an allele on shifting the heterozygote phenotype away from the mid-point. Adapting the continuous phenotype framework to that of a dichotomous phenotype (ie: affected or unaffected), the contribution associated with one homozygous genotype (AA) to the phenotype can be denoted as 1, while the contribution of the other homozygous genotype (aa) is defined as –1 and the heterozygote as 0 (Figure 2.1) (Fisher et al., 1932).

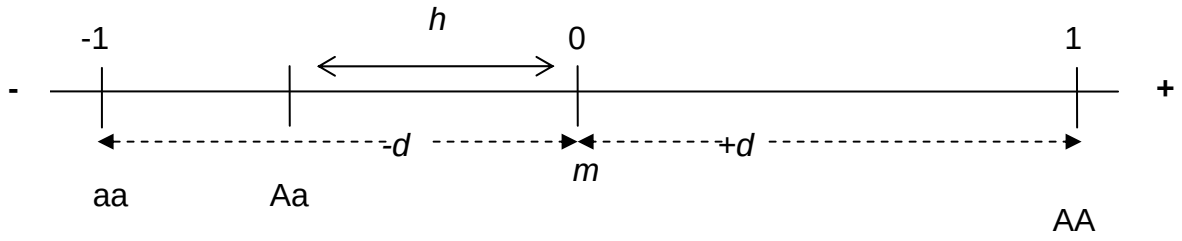


Figure 2.1 Genotypic Representation of a Continuous Trait
(Adapted from Mather and Jinks, 1982)

Three possible models can be specified for the effects of the *MAOA* locus in females using the classical biometrical-genetic model of Mather (Mather et al., 1982)) following the original conception of Fisher (Fisher, 1918): 1) An "additive" model, or one with no dominance where the phenotypic mean of the heterozygotes is between the means of the two homozygotes; 2) A model of "complete dominance" in which the heterozygote mean is the same as either of the two homozygotes; 3) A model of "incomplete dominance" in which the mean phenotype of heterozygotes resides between the two homozygotes, while differing from the mid-point. Both additive and dominance effects may interact with the environment if genotypes differ in their sensitivity to the environment.

Among males and females, Caspi et al reported the significant effect of GxE for the low activity *MAOA* polymorphism and increasing household adversity for CD and a non-significant effect of the low activity *MAOA* allele. However, heterozygous females, which comprised 46% of the sample, were not included because of concerns

surrounding the perceived inability to estimate genotypic effects as a result of X-inactivation. Further, this study of GxE did not include the effects of rGE. In this chapter, we test for the presence of the main effects of gender, *MAOA* and childhood adversity as well as any effect associated with GxE in the presence of passive rGE, defined by parental ASP as risk factors for CD, utilizing females of homozygous and heterozygous *MAOA* genotypes. Additionally, differences in the risk for CD by gender groups are highlighted.

Methods

Study Population

This study is based on a sub-sample of 578 male and 721 female individual participants and their parents from the Virginia Twin Study of Adolescent and Behavioral Development (VTSABD). The current sub-sample consists of those individuals and their parents for whom *MAOA* genotype, maternal ASP and household neglect information were obtained.

Assessments

Diagnosis of Conduct Disorder. Previous 3-month history of CD as assessed with the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al., 2000) and reported by maternal, paternal or child self-report using a symptom-or rule at any wave of the four waves of data collection (Simonoff et al., 1997).

Measurement of Childhood Adversity. Three measures of negative family environment associated with CD indexed childhood adversity, specifically parental neglect, exposure to inter-parental violence and inconsistent parental discipline. Responses to the binary items were summed and used as a scale ranging from 0 to 7.

Parental Antisocial Personality Symptoms. Maternal and paternal ASP symptoms were measured separately as the sum of the following seven binary items. Responses to the binary items were summed to form a measure of ASP, having a scale ranging from 0 to 7.

MAOA Genotyping

Primer sequences previously described were used and classification of *MAOA* activity (high or low) was assigned to each allele resulting from previous work in the efficiency of transcription activity of the *MAOA* gene promoter (Sabol et al., 1998).

Data Analysis

Tests of Gender Differences in Environmental Exposure. Gender differences in CD diagnosis, prevalence of childhood adversity, prevalence of maternal ASP symptoms, prevalence of paternal ASP symptoms, and associations of risk factors with CD diagnosis were assessed using the χ^2- test for association. As a result of low cell frequencies at the highest levels of exposure, measures of childhood adversity and

parental ASP symptoms were collapsed from a continuous measure utilizing the full range of responses to ordinal measures of 0, 1, 2, and 3 or more exposures.

Test of Hardy-Weinberg Equilibrium. Female allele frequencies were determined using a randomly selected allele from each *MAOA* genotype. Transcription activity (high or low) was assigned to each *MAOA* allele. Since human males are not diploid on the X-chromosome, Hardy-Weinberg equilibrium (HWE) was tested in the female genotypes. Male and female allele frequencies were then tested for significant differences in distribution as a population-level evaluation of HWE. If a population is determined to be in HWE, it is not subject to assortative mating, population bottleneck, mutation, or population admixture due to inmigration.

Assessing Appropriate Measures of Environmental Exposures. Alternate scales of childhood adversity measured as 0/1+, 0/1/2+ or 0/1/2/3+ exposures were considered in males and females separately. Since there were no a priori expectations of the most appropriate scale for measuring environmental exposure and none of these measures offered significant improvement in the prediction of CD over scales utilizing the full range of measure (0-5 exposures), each scale could be considered equally informative. Further, a scale consisting of 0/1/2/3+ exposures increases the cell size of those exposed at the highest levels and minimizes loss of information that results from collapsing the scale. Therefore, the ordinal measure of childhood adversity using a scale of 0/1/2/3+ exposures was used. This measure was treated as a continuous variable to maintain

model interpretability between gender groups, while attempting to address the issue of low frequency at the highest levels of exposure. Maternal ASP (measured as 0-5 symptoms) was treated in an identical manner and a scale of 0/1/2/3+ symptoms was used. The assessment of environmental exposure was assessed separately for males and females.

Testing for Additive and Dominance Effects of MAOA Genotype on Conduct Disorder in Females. A preliminary sequence of logistic regression models was tested to evaluate the relationship between *MAOA* and CD in the absence of environmental exposure separately for males and females. The most general genetic model (incomplete dominance) specified free parameters for both homozygous (additive) and heterozygous (dominance) differences, where the mean phenotype of heterozygotes resides between the two homozygotes, while differing significantly from their mid-point. The effects of three non-nested constraints on the heterozygous effect were compared as an additive genetic model, where the heterozygous mean corresponds to the mean of the two homozygotes in the absence of dominance; complete dominance for low activity, and complete dominance for high activity, both of which reflect the heterozygous mean as being the same as either of the two homozygote means. Each of the four genetic models was compared with a null model in which only the intercept was included.

Testing for Main and Interaction Effects of MAOA Activity and Environmental Risks to Conduct Disorder. Models were tested using a sample for which data for maternal ASP, childhood adversity, gender, and *MAOA* genotype was available (N= 1299). Observations from both genders were included for which data for maternal ASP, paternal ASP, childhood adversity, and *MAOA* genotype were available. Paternal ASP symptoms was not found to be a significant predictor of CD nor improved model fit as either a main or interaction term and was later removed.

Models of decreasing complexity were fitted involving the four predictors (childhood adversity, maternal ASP, gender, and *MAOA* genotype) that were expected to affect liability to CD in males and females separately. First, the model of simple regression of all main effects and all combinations of two- and three-way interactions was fitted. Subsequent models removed non-significant parameters as measured by p-values greater than 0.05, until no significant increase in deviance resulted. Models were compared using goodness-of-fit and parsimony and were assessed as deviance and Akaike's Information Criterion (AIC) respectively. Akaike's Information Criterion (AIC) measuring model parsimony (Akaike, 1974) is calculated as:

$$AIC = deviance + 2p$$

where p = Number of parameters in the model. Deviance is similar to the often used -2ln likelihood and is estimated as the difference between the -2ln likelihood for a null model (intercept only) and a saturated one (one parameter per observation). This measure of deviance follows a chi-square distribution and allows for model comparison.

Lower values of AIC indicate more parsimonious models and higher values of deviance imply improved model fit.

Models best describing risk for CD were determined to have (1) the lowest values of AIC, (2) non-significant differences in deviance from more complex models and (3) significant parameter estimates. Model assessment was performed by logistic regression using PROC GENMOD in SAS (SAS version 9.1.3; SAS Institute, Cary, NC). Random residual effects of twin resemblance and repeated measurement were accommodated by using the Generalized Estimating Equation algorithm incorporated in the SAS GENMOD procedure on the simplifying assumption of constant correlation between measures within monozygotic and dizygotic twin clusters.

Alternate measures of environmental exposure were also assessed to determine whether other truncated representations of measurement scale were more appropriate for inclusion, since each genotype group was present at low frequencies for the highest levels of environmental exposure. These measures were then standardized to reflect a mean of 0 and standard deviation of 1 in order to adequately establish the magnitude of main and interaction effects of final models using PROC STANDARD in SAS. A "main effect" estimated in the final models is defined as the effect of a parameter averaged across all levels of the other parameters in the model, with each parameter in the model having a mean of 0.

Results

MAOA Allele Frequency and Test of Hardy Weinberg Equilibrium

The *MAOA* allele distribution (Table 2.1) is comparable to those in other studies (Caspi et al., 2002), with 3- and 4-repeat alleles having the highest frequencies. There were no significant differences in expected allele frequencies resulting from female genotypes ($p = 0.86$), nor between males and female allele frequencies ($p = 0.10$), and thus no significant departure from HWE.

Table 2.1 *MAOA* Allele Distribution

Allele	Repeat Number	Activity	Males		Females	
			Frequency	Percent	Frequency	Percent
1	3	low	170	28.3	235	32.2
2	3.5	high	12	2.0	12	1.6
3	4	high	415	69.1	468	64.1
4	5	low	2	0.3	12	1.6
5	2	low	2	0.3	3	0.4

Prevalence of Conduct Disorder and Exposure to Childhood Adversity and Symptoms of Parental Antisocial Personality Disorder

There were 121 (9.3%) individuals affected with CD in the entire sample. Prevalence for CD in males was 11.9%, while females had significantly fewer cases (7.8%, $p = 0.006$). Tables 2.2- 2.4 detail the prevalence of environmental exposures as

ordinal measures of childhood adversity and parental ASP, reflecting 0,1,2, and 3+ exposures/symptoms for males and females. Table 2.2 summarizes the distribution of exposure to childhood adversity. Childhood adversity was significantly associated with CD diagnosis in both males ($\chi^2_{DF=3} = 17.98$, $p = 0.0004$) and females ($\chi^2_{DF=3} = 30.61$, $p < 0.0001$). In general, there were no significant gender differences with respect to the specific items leading to the scale of childhood adversity (ie: inconsistent discipline or parental neglect). However, females reported significantly greater exposure to inter-parental violence (6.7%) than males (2.3%, $p = 0.0002$) although there were no significant differences in the aggregate measure of childhood adversity by gender ($\chi^2_{DF=3} = 3.61$, $p = 0.31$). Each item used to define childhood adversity was also significantly associated with CD in males and females.

Tables 2.3 and 2.4 summarize the distributions of parental ASP symptoms. Maternal ASP was significantly associated with CD diagnosis in males and females. Females reported significantly higher exposure to paternal ASP than males ($\chi^2_{DF=3} = 22.28$, $p < 0.0001$), though paternal ASP was not significantly associated for CD in females ($\chi^2_{DF=3} = 4.34$, $p = 0.23$) or males ($\chi^2_{DF=3} = 3.95$, $p = 0.23$).

Table 2.2 Distribution of Childhood Adversity Events

	0			1			2			3 or more		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Frequency	423	495	918	65	98	163	72	98	170	18	30	48
Percent	73.2	68.7	70.7	11.3	13.6	12.6	12.5	13.6	13.1	3.1	4.2	3.7

Table 2.3 Maternal Symptoms of Antisocial Personality Disorder

	0			1			2			3 or more		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Frequency	283	331	614	215	251	466	54	99	153	26	40	66
Percent	49.0	45.9	47.3	37.2	34.8	35.9	9.3	13.7	11.8	4.5	5.6	5.1

Table 2.4 Paternal Symptoms of Antisocial Personality Disorder

	0			1			2			3 or more		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
Frequency	225	235	460	137	139	276	80	109	189	33	90	123
Percent	47.4	41.0	43.9	28.8	24.3	26.3	16.8	19.0	18.0	6.9	15.7	11.7

Table 2.5 summarizes associations between parental ASP symptoms and childhood adversity as an indicator of passive rGE and assortative mating. In both males and females increasing parental ASP is significantly associated with childhood adversity. Additionally, there is a significant association between increasing maternal and paternal ASP symptoms in both males and females.

Table 2.5 Spearman Correlations of Parental Antisocial Personality Symptoms and Childhood Adversity

	Maternal ASP	Paternal ASP	Childhood Adversity
Maternal ASP		0.33**	0.23**
Paternal ASP	0.23**		0.16*
Childhood Adversity	0.16**	0.11*	

Female correlations are above the diagonal, male correlations are below

*p<0.01

**p<0.0001

Risk of CD by *MAOA* Genotype and Exposure to Environmental Risk Factors

Prevalence of CD in males did not significantly differ between low (9.6%) and high activity (12.8%) *MAOA* alleles. Prevalence of CD was greater for females with low/low genotypes (14.6%) than either low/high (6.2%) or high/high genotypes (5.7%) ($\chi^2_{DF=2} = 8.8$, $p = 0.01$). Among females, 41.6% had a high/high *MAOA* genotype, 45.3% had the heterozygous genotype and 13.1% were found to have a low/low genotype.

There were no significant differences in exposure to maternal or paternal ASP symptoms by gender or across genotypes. Both males with the low allele and females with low/low genotype had increased exposure to childhood adversity, though differences were non-significant. In a test of evocative rGE, *MAOA* genotype did not predict exposure to childhood adversity using a linear regression approach in either males ($\beta = 0.06$, $p = 0.26$) or females ($\beta = 1.8$, $p = 0.24$), suggesting that *MAOA* genotype does not impact exposure to childhood adversity.

Testing for Additive and Dominance Effects of *MAOA* Genotype on Conduct Disorder in Females

Table 2.6 details model fitting results for the contribution of *MAOA* genotype to the diagnosis of CD. The most parsimonious model accounting for improved model fit over the null model was obtained when including only additive effects (model 2, deviance = 372.6, AIC = 374.6). *MAOA* was subsequently modeled in an additive fashion (low activity = 1, low/high activity = 0 and high activity = -1) because (1) dominance effects were not significant within the additive/dominance model, (2) improvement in model fit was observed for the more parsimonious additive model compared with the additive/dominance model, and (3) a model of dominance in the direction of high activity (model 4) appeared to oversimplify the effect of the heterozygotes.

Table 2.6 Summary of Model-Fitting Statistics of Genotypic Contribution to Conduct Disorder in Females

					p-values	
Model- *MAOA* Function	DF	Deviance Difference From Null	Deviance	AIC	Additive	Dominance
Null	0		378.55	378.55		
Additive/Dominance	2	7.31	371.24*	375.24	0.01	0.24
Additivity	1	5.94	372.61*	374.61	0.04	
Dominance, Low Activity	1	2.10	376.45	378.45		0.24
Dominance, High Activity	1	7.02	371.53*	373.53		0.01

* Denotes significant difference of deviance at $p \leq 0.05$

Testing for Main and Interaction Effects of *MAOA* Genotype and Environmental Risks to Conduct Disorder

Table 2.7 summarizes the model fitting results to determine the contribution of genotype and environmental exposure to the diagnosis of CD in females, highlighting model 4 as best explaining the data (deviance = 338.57 and AIC = 336.57). This model consisted of (1) *MAOA* considered as a genotype with additive variance in the heterozygous females, (2) Childhood adversity, (3) Maternal ASP symptoms, and (4) The interaction of childhood adversity and *MAOA* genotype.

Table 2.7 Summary of Backwards Elimination Model Fitting in Females to Predict Conduct Disorder

Model Specified		p-value	Deviance	AIC	Parameters
Model 1	1- Childhood Adversity	0.006	337.45	351.45	7
Full Model	2- Maternal ASP Symptoms	0.02			
	3- *MAOA*	0.13			
	4- *MAOA**Maternal ASP	0.74			
	5- *MAOA**Childhood Adversity	0.10			
	6- Maternal ASP*Childhood Adversity	0.55			
	7- Childhood Adversity*Maternal ASP**MAOA*	0.50			
Model 2	1- Childhood Adversity	0.003	337.65	349.65	6
Drop 7	2- Maternal ASP Symptoms	0.03			
	3- *MAOA*	0.12			
	4- *MAOA**Maternal ASP	0.94			
	5- *MAOA**Childhood Adversity	0.05			
	6- Maternal ASP*Childhood Adversity	0.52			
Model 3	1- Childhood Adversity	0.003	337.65	347.65	5
Drop 4	2- Maternal ASP Symptoms	0.02			
	3- *MAOA*	0.03			
	5- *MAOA**Childhood Adversity	0.06			
	6- Maternal ASP*Childhood Adversity	0.50			
Model 4	1- Childhood Adversity	0.0003	338.57	346.57	4
Drop 6	2- Maternal ASP Symptoms	0.006			
	3- *MAOA*	0.02			
	5- *MAOA**Childhood Adversity	0.05			

Table 2.8 summarizes the parameter estimates and odds ratios for model 4 after standardizing each parameter to accurately estimate main and interaction effects. Significant GxE is present while controlling for the main effects of passive rGE (maternal ASP symptoms), *MAOA* genotype and exposure to childhood adversity. The

ordinal definition of *MAOA*, treated as continuous variable defined greatest risk for the low/low genotype (low/low = 1, low/high = 0, high/high = -1). Therefore, the significant effect of *MAOA* reflects the effect of the low/low genotype on risk for CD across all levels of exposure. Likewise, the direction of the interaction effect represents the lower risk associated with the high/high and heterozygous genotypes in the presence of lower levels of childhood adversity where the distribution of exposure is the greatest, highlighting that the ability to detect this interaction is derived from the extremes of the distribution.

Table 2.8 Parameter Estimates and Odds Ratios for Model Used to Estimate CD Risk in Females

Final Model	Estimate	OR	95% CI	p-value
MAOA	0.46	1.59	1.03-2.47	0.04
Childhood Adversity	0.54	1.72	1.32-2.25	<0.0001
Maternal ASP	0.40	1.50	1.12-2.00	0.006
Childhood Adversity * *MAOA*	-0.26	0.77	0.59-0.99	0.05

Table 2.9 summarizes the model fitting results to determine the contribution of genotype and environmental exposure for CD risk in males. Model 3 was identified as the model best explaining the data and consisted of (1) *MAOA* considered as a genotype with additive variance, defining low activity alleles as 1 and high activity alleles as -1, (2) Childhood adversity, (3) Maternal ASP symptoms, (4) The interaction between maternal ASP symptoms and *MAOA*, and (5) The interaction of childhood adversity and

MAOA (deviance= 387.57 and AIC= 397.57). Removing the interaction between *MAOA* and maternal ASP resulted in a model where the interaction between MAOA and childhood adversity was non-significant (model 4a). This model also differs from the model used in Foley et al. (2004), because it contains truncated ordinal measures (0/1/2/3+) of the household environment. When full measures (0-5) were used, the previously reported results were replicated (model 4b), suggesting an inconsistency in the ability to detect significant GxE as a result of how the environment is measured. Therefore, model 3 was chosen as the model that best represented risk for CD in males because the deviance values associated with this model were similar to more complex models while also having the lowest value of AIC and highlighted a robust interaction between *MAOA* and childhood adversity.

Table 2.9 Summary of Backwards Elimination Model Fitting in Males to Predict Conduct Disorder

	Model Specified	p-value	Deviance	AIC	Parameters
Model 1	1- Childhood Adversity	0.02	387.18	401.18	7
Full Model	2- Maternal ASP Symptoms	0.49			
	3- *MAOA*	0.99			
	4- *MAOA**Maternal ASP	0.10			
	5- *MAOA**Childhood Adversity	0.22			
	6- Maternal ASP*Childhood Adversity	0.74			
	7- Childhood Adversity*Maternal ASP**MAOA*	0.55			
Model 2	1- Childhood Adversity	0.01	387.32	399.32	6
Drop 7	2- Maternal ASP Symptoms	0.17			
	3- *MAOA*	0.67			
	4- *MAOA**Maternal ASP	0.11			
	5- *MAOA**Childhood Adversity	0.03			
	6- Maternal ASP*Childhood Adversity	0.44			
Model 3	1- Childhood Adversity	0.006	387.57	397.57	5
Drop 6	2- Maternal ASP Symptoms	0.38			
	3- *MAOA*	0.70			
	4- *MAOA**Maternal ASP	0.07			
	5- *MAOA**Childhood Adversity	0.02			

Table 2.9. Summary of Backwards Elimination Model Fitting in Males to Predict Conduct Disorder (Continued)

	Model Specified	p-value	Deviance	AIC	Parameters
Model 4a	1- Childhood Adversity (0/1/2/3+)	0.01	392.14	400.14	4
Drop 4	2- Maternal ASP Symptoms	0.01			
	3- *MAOA*	0.07			
	5- *MAOA**Childhood Adversity	0.06			
Model 4b	1- Childhood Adversity (0-5)	0.01	391.88	399.88	4
Drop 4	2- Maternal ASP Symptoms	0.01			
	3- *MAOA*	0.07			
	5- *MAOA**Childhood Adversity	0.05			

Table 2.10 summarizes the parameter estimates and odds ratios for model 3 after standardizing each parameter to accurately estimate main and interaction effects. Significant GxE is present while controlling for the main effects of passive rGE (maternal ASP symptoms), *MAOA* genotype and exposure to childhood adversity.

Table 2.10 Parameter Estimates and Odds Ratios for Model Used to Estimate CD Risk in Males

Final Model	Estimate	OR	95% CI	p-value
MAOA	-0.17	0.85	0.64-1.11	0.22
Childhood Adversity	0.34	1.40	1.06-1.86	0.02
Maternal ASP	0.29	1.33	0.97-1.84	0.08
Childhood Adversity**MAOA*	0.27	1.31	1.05-1.64	0.02
Maternal ASP**MAOA*	-0.29	0.75	0.55-1.01	0.06

The inconsistent detection of GxE in males (Table 2.9) demonstrated that the ability to detect the interaction is derived from cases at the extremes in the distribution of environmental exposures. However, very few cases reside at the highest levels of exposure in either gender (Tables 7 and 8), indicating low power to detect significant GxE using this model. In an attempt to address this issue, a modified ridit transformation (Bross, 1958) was performed to adjust the measure of environmental exposure by the sample size at each level of childhood adversity. The modified ridit transformation determines a "ridit" or score for each category, which is defined as the percentile rank of an item in the reference population. This score is calculated as the proportion of individuals within a less severe category plus one-half the proportion of individuals in the category itself. Therefore, each ridit score reflects the category severity of an ordinal scale and sample size for each level and limits the variance of each level to produce a uniform distribution with a range between 0 and 1.

After ridit transformation, a significant main effect of *MAOA* remained (β = 0.46, OR = 1.58; 95% CI, 1.01-2.48; p = 0.05) and GxE was non-significant (β = -0.25, OR = 0.78; 95% CI, 0.57-1.07; p = 0.12) in females. Among males, the weak GxE remained significant (β = 0.25, OR = 1.29, 95% CI, 1.01-1.64; p = 0.04). Thus, there is evidence for a weak main effect of *MAOA* genotype in females and the presence of GxE in the development of CD in males, while controlling for the main effects of passive rGE (maternal ASP symptoms), *MAOA* genotype and exposure to childhood adversity.

Figure 2.2 and 2.3 detail the prevalence of conduct disorder as a function of *MAOA* allele/genotype and exposure to childhood adversity among females and males

respectively. Figure 2.2 visualizes the low/low *MAOA* genotype increasing risk for CD at all levels of exposure in females, although there were no affected individuals with low/low *MAOA* at the highest level of exposure present. Figure 2.3 visualizes the interaction effect of low activity *MAOA* and increasing childhood adversity in males. Additionally, an interaction was observed between childhood adversity and genotype for heterozygous and high activity genotype. Tables 2.11 and 2.12 detail the sample sizes used to produce each figure and demonstrates the small cell sizes at the highest levels of exposure for both males and females.

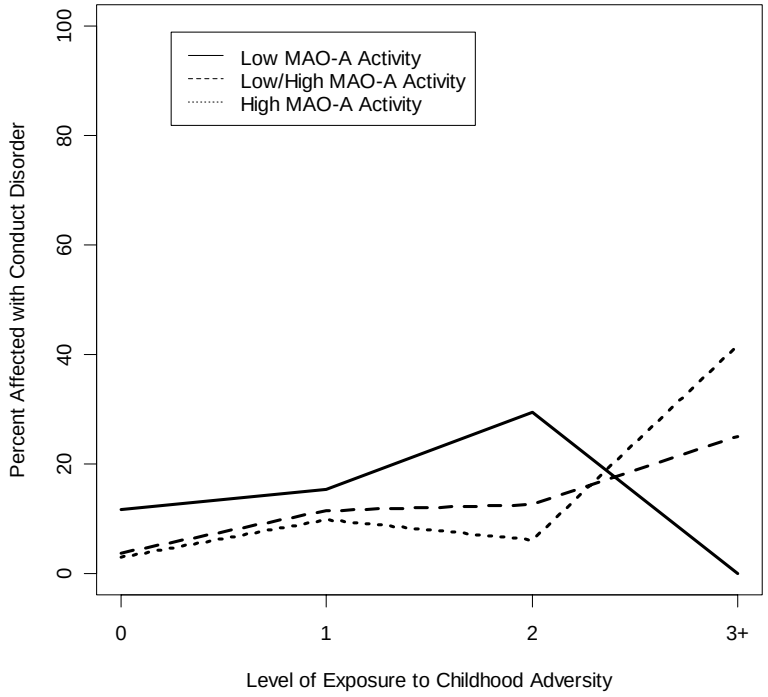


Figure 2.2 Prevalence of Female Conduct Disorder by Childhood Adversity and *MAOA* Genotype

Table 2.11 Prevalence of Female Conduct Disorder by Childhood Adversity and *MAOA* Genotype

Level of Exposure to Childhood Adversity	Low *MAOA*		Low/High *MAOA*		High *MAOA*	
	N/Total	%	N/Total	%	N/Total	%
0	7/60	11.7	8/222	3.6	6/213	2.8
1	2/13	15.4	5/44	11.4	4/41	9.8
2	5/17	29.4	6/48	12.5	2/33	6.1
3 or More	0/6	0	3/12	25.0	5/12	41.7
Total	14/96	14.6	22/326	6.2	17/299	5.7

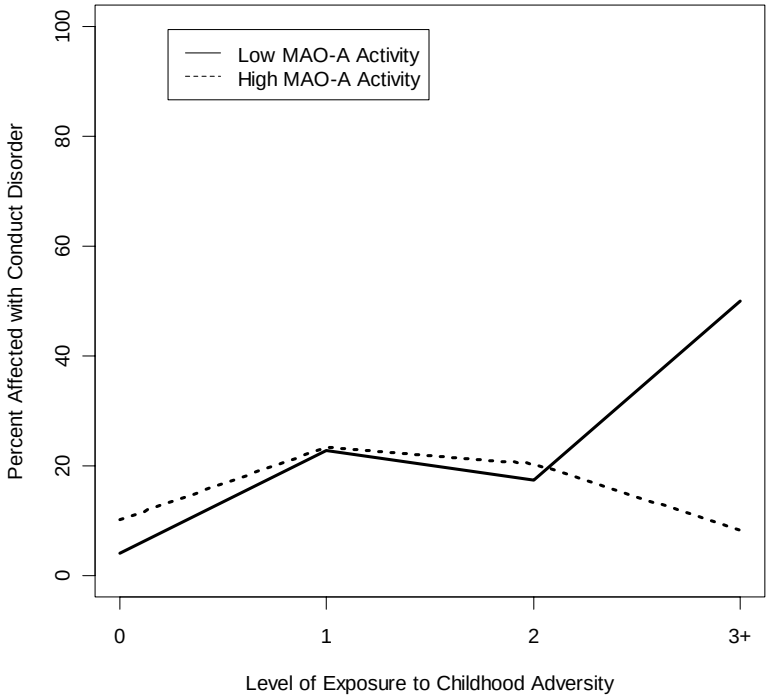


Figure 2.3 Prevalence of Male Conduct Disorder by Childhood Adversity and *MAOA* Genotype

Table 2.12 Prevalence of Male Conduct Disorder by Childhood Adversity and *MAOA* Genotype

Level of Exposure to Childhood Adversity	Low *MAOA*		High *MAOA*	
	N/Total	%	N/Total	%
0	5/127	3.9	30/296	10.1
1	5/22	22.7	10/43	23.3
2	4/23	17.4	10/49	20.4
3 or More	3/6	50.0	1/12	8.3
Total	17/178	9.6	51/400	12.8

Discussion

In order to detect main genotypic effects and GxE in females using an X-chromosome marker, the contribution of *MAOA* genotype to CD diagnosis was modeled. By defining both the homozygous (additive) and heterozygous (dominance) effects of *MAOA*, we have demonstrated that the inclusion of females heterozygous for the low and high activity *MAOA* alleles is reasonable and yields meaningful results in spite of ambiguity around the issue of X-inactivation. Additionally, the risk associated with the heterozygous *MAOA* genotype is between that of the homozygous groups and resembles trajectory of the high activity genotype in risk for CD (Meyer-Lindenberg, Buckholtz, Kolachana et al., 2006).

Initial molecular studies with regard to this locus reported non-skewed patterns of inactivation in genomic DNA obtained from blood samples (Benjamin et al., 2000). A second study of monozygotic female twins described non-skewed inactivation in a majority (85%) of samples (Fraga, Ballestar, Paz et al., 2005), supporting random X-inactivation. Another study of allelic expression of a single nucleotide polymorphism in exon 6 of *MAOA* in human skin fibroblasts also demonstrated random monoallelic expression (Nordquist et al., 2006). A recent study reported that *MAOA* is subject to X-inactivation using a measure of allelic expression imbalance in human brain tissue concluded that there was no evidence for skewing in normal individuals (Pinsonneault et al., 2006). Furthermore, a recent study of functional response of *MAOA* genotype for amygdala and cingulate volume demonstrated the functioning of heterozygous females to be in between that of the homozygotes (Meyer-Lindenberg et al., 2006). Therefore,

we were able to model risk associated with *MAOA* genotype in a manner that also has received support in recent molecular and neuroscience literature.

Gender Differences in Risk for CD

Among females, the persistence of a main genetic effect of the low/low *MAOA* genotype while controlling for all other risk factors is striking and suggests that low activity *MAOA* confers greater risk for CD. The risk associated with the low activity *MAOA* genotype is modest (OR = 1.59) in the presence of low levels of childhood adversity and maternal ASP. Thus, low *MAOA* genotype does not predispose a female to CD, but suggests an increased risk for CD at lower levels of childhood adversity compared with the heterozygous and high/high genotypes.

The observation of a main genetic effect in females rather than males has been reported in twin studies of antisocial behavior. Significant additive genetic effects have been reported to account for a greater amount of variation of ASP in females as compared with males (Eley et al., 1999; Jacobson et al., 2002a). However, Gelhorn et al. (2005) demonstrated equal contributions of unmeasured environmental and genetic effects across gender after controlling for prevalence differences in CD symptoms between the two groups. While equating symptom prevalence across gender may alter these results, we should also consider the value in evaluating trends summarized in looking at gender separately rather than viewing CD as a disorder with common etiology across gender.

There was no significant GxE in females after transformation of the measure of childhood adversity using modified ridit scores. In models where GxE was significant, the interaction differed from that of males such that the low activity *MAOA* genotype conferred greater risk for CD at low levels of childhood adversity and high activity *MAOA* confers greater risk over the highest level of childhood adversity. In contrast, risk for CD increased with increasing exposure to childhood adversity among males with the low activity *MAOA* genotype. The opposite direction of GxE in males and females suggests a genotype-sex interaction. Further, Meyer-Lindenberg and colleagues (2006) also report a significant genotype-sex interaction for orbito-frontal cortex (OFC) structure and function. Males also had significantly reduced OFC connectivity with the amygdala when compared with females. The OFC and OFC-amygdala interaction has been implicated in the pathway of stimulus-reinforcement association learning and is important in assigning reward value to behavioral reinforcers (Meyer-Lindenberg et al., 2006). The current results and those of the neuroscience literature suggest that although males and females generally are similar in their exposure to genetic and environmental risk factors, these risk factors may be processed differently by gender as a function of genotype.

Passive rGE, as measured by maternal ASP symptoms was significant, though evocative rGE was not. While maternal ASP may be associated with childhood risk to CD, its effect is apparently not mediated by childhood adversity since the effect of maternal ASP remains significant in models that include maternal ASP as a covariate, while controlling for childhood adversity. However, the genotypic differences in

sensitivity to childhood adversity may relate to a general measure of family dysfunction rather than simply a specified measure of childhood adversity or maternal ASP as reported in a recent study of the interaction of family dysfunction and genetic effects in outcomes of antisocial symptoms (Button, Scourfield, Martin, Purcell, & McGuffin, 2005). Since there are significant associations in this sample between paternal and maternal ASP symptoms for both males and females, assortative mating for ASP among adults may result in a household with family dysfunction. Thus, it is plausible that children receive their genotypes from their parents as well as a genotypic sensitivity to the very environments (GxE) provided by the parents as a result of family dysfunction and those social cues in managing the environment through interpersonal interactions (passive rGE). Additionally, by differential processing of the home environment by gender, males and females may appear to exhibit symptoms of CD differently. This explanation may also explain why female CD is more likely to appear to result from disrupted relationships with caretakers or peers and females are more likely to engage in interpersonal violence against family members or intimate partners (Ehrensaft, 2005; Moffitt et al., 2001a).

Further analysis of the pattern of correlations between twins and their parents is required to resolve the role of childhood adversity in the correlation between parental ASP and CD and to disentangle the pathways between rGE, GxE and assortative mating. Also, studies of GxE should be expanded to determine whether *MAOA* sensitivity exists for other environmental exposures. It would be helpful to test the assumptions of X-linked inheritance on CD diagnosis to determine if they explain the

gender difference in CD prevalence. Gene-environment interaction as reported in this study serves to highlight the role of genetic risk on individual susceptibility to specific environments, but to also encourage family-centered prevention efforts interested in altering environmental exposure to childhood adversity and treating parental ASP, since no genotypic group is completely protected from the effects of household difficulty.

These results should be interpreted while acknowledging the following limitations. First, we were unable to estimate risk for CD among females with low *MAOA* activity also experiencing three or more exposures to childhood adversity due to a lack of affected observations (Figure 2.2). Consequently, GxE may be initially overestimated. Collapsing childhood adversity to reflect fewer exposures (ie: 0/1+ or 0/1/2+) did not adequately utilize the data and decreased our power to detect main genetic effects and GxE. The variable strength of GxE highlights the issue of scale in our measurement and treatment of environmental exposure towards the detection of GxE and genetic effects in humans, reinforcing the need for better measures of environmental risk for psychiatric disorders. Uncertainty in scale is ubiquitous in measures of human behaviors and thus no "right" scale exists for determining meaningful environmental exposure (Eaves et al., 1977). Additionally, the weak contribution of GxE in both males and females is expected since in general it is not anticipated to be a large contributor to total variance (Eaves et al., 1977). Thus, this finding of GxE in females requires either replication in another sample, studies using increased sample size, or consideration of the specific types of childhood adversity and how they may increase or decrease risk for CD with respect to *MAOA* genotype.

Second, this study is a cross-sectional analysis of longitudinal data from 4 waves of data. Therefore, these results reflect the risk associated with of childhood adversity, maternal ASP and *MAOA* for CD during the developmental period of adolescence. Furthermore, they do not lend insight into how GxE functions throughout development.

Third, these analyses treated CD as a categorical outcome and ignored the additional information that might be reflected by using indices of severity such as symptom counts or by differentiating subtypes such as aggressive and non-aggressive behaviors.

Fourth, the occurrence of X-inactivation in females has resulted in little attention to differences in enzyme function for *MAOA* in females. Finally, all participants are Caucasian and results may not generalize to populations of differing ethnicities and cultural norms.

CHAPTER 3 Testing Gender Differences in Conduct Disorder Resulting from the Effects of an X-Linked Gene

Abstract

Several twin studies have detected sex-specific genetic effects for conduct disorder (CD) and antisocial behavior. Further, X-linked genes have been implicated in explaining gender differences in antisocial behavior and brain structures responsible for aggressive behavior including the amygdala and prefrontal brain region. Assessing whether observed CD twin correlations arise from X-linked inheritance and detecting the presence of genotype-environment interaction (GxE) and sex-limitation may guide future inquiry into the role of *MAOA* in understanding gender differences in CD.

Two sub-samples of 1,124 same- and opposite-sex adolescent twin and maternal reports from the VTSABD were used to calculate expected twin correlations for CD under X-linked inheritance using the allele frequencies and genetic effects of *MAOA* on CD symptoms. The expected twin correlations were compared against those observed in the data to determine whether X-linkage could be used to explain the gender differences in CD prevalence. Twin correlations were also stratified by exposure to childhood adversity to test for GxE and gender was included as a covariate to test for sex-limitation on CD.

There was no evidence that X-linked inheritance or GxE explained risk for CD. Using maternal ratings of twin CD, there were significant genetic effects and genetic effects common to both males and females (non-scalar sex-limitation). Using twin ratings of CD, there was a significant interaction between genetic effects and gender,

suggesting the presence of genotype-sex interaction (scalar sex-limitation) with greater additive genetic effect in males. No significant main effects of childhood adversity or gender were found for either maternal or child reports. These results do not confirm the detection of GxE reported in previous studies (see chapter 2). However, the power to detect significant interactions with childhood adversity is low as a result of low sample sizes for exposure. Additionally, GxE may not have been detected because of the use of CD measured separately by raters rather than with multiple raters together. If an X-linked gene is functioning to produce gender differences or GxE, such effects are specific for each genetic and environmental combination and genetic study of gender differences for CD may benefit by focusing candidate gene efforts on genotype-sex interaction.

Introduction

X-linkage and its Role in Behavior and Cognitive Ability

Classic Mendelian inheritance of X-linked genes is suspected when higher prevalence rates are reported in males over females for a phenotype. X-linked inheritance is therefore a possible explanation of the consistent gender difference reported in conduct disorder (CD) prevalence.

An X-linked phenotype is defined as any trait that is dependent on a gene residing on the X chromosome. Genes on the X chromosome are of particular interest in understanding gender differences that exist for behavioral phenotypes, since (1) males have single X and Y chromosomes while females have two X chromosomes, (2) X-linked genes have been suggested in explaining gender differences in neural development (Skuse, 2005) and (3) X-linked genes have been suggested in explaining gender differences for behavior and cognitive ability (Craig, Harper, & Loat, 2004; Rutter, Caspi, & Moffitt, 2003; Skuse, 2005). However, dosage compensation in the form of X-inactivation is expected to equalize the expression of X-linked genetic effects on the phenotype between males and females. Further, a secondary form of dosage compensation involving the doubling of the active X chromosome expression in males and females is expected to result in no genetic effect by gender (Nguyen & Disteche, 2006b; Nguyen & Disteche, 2006a). As a result of the current understanding of dosage compensation, genetic studies using X-linked genes allow us to test the effects of X-linked inheritance on a phenotype, but such effects are not anticipated to account for

gender differences. Furthermore, the use of X-linked genes in the study of gender differences should identify and test other models of genetic effects including gene-environment interaction and gene-sex interaction.

X-Linked Genes as a Source of Phenotypic Variation

Sexually dimorphic traits refer to the differences in reproductive organs between gender, but can also be used to define all aspects of the differentiation of males and females such as body size and shape as well as physiology and behavior (Fairbairn & Roff, 2006). Normal sexual dimorphism of brain structure has been consistently reported in humans and animal studies (refer to Goldstein et al., 2001 for a comprehensive list of studies). Women were reported to have larger adult brain volumes in the frontal and medial paralimbic cortices compared with cerebrum size. In comparison, males had larger volumes in the frontomedial cortex, amygdala and hypothalamus, relative to cerebrum size (Goldstein, Seidman, Horton et al., 2001). Likewise, females perform better than males in various tasks assessing verbal skills, while males excel in tasks requiring spatial skills (Baron-Cohen, 2002; Fenson, Dale, & Reznick, 1994; Kramer, Delis, & Kaplan, 1997).

Genes residing on sex chromosomes are anticipated to facilitate the evolution of sexual dimorphism if their effects on phenotypic variance are associated with sex-specific fitness effects for that trait (refer to (Fairbairn et al., 2006)). Specialization of X-linked genes might regulate human cortical complexity and brain size. Thus, X-linked genes may play a role in the sexual dimorphism of some brain structures and

behavior (Skuse, 2006). The mechanisms by which genes of the X chromosome might produce differences in genetic expression by sex include (1) partial or complete escape from random X-inactivation, (2) differential expression of some X-linked genes in males and females, and (3) genomic imprinting.

X-Inactivation. X-inactivation is the mechanism by which X chromosome dosage (2 in females and 1 in males) is compensated between males and females (Lyon, 1963). X-inactivation is caused by methylation of the X-inactivation center on either one of the X chromosomes in each female cell and silences genes on that chromosome. X-inactivation is thought to occur randomly, with paternally and maternally derived X chromosomes equally as likely to be inactivated, resulting in functionally mosaic cell populations consisting of X chromosomes from both parents. Additionally, once an X chromosome is inactivated, it remains inactivated for the life of the cell and all the resulting daughter cells will also have the same inactive X (Heard et al., 2006; Nussbaum et al., 2001).

In general, X-inactivation in females is expected to be random, such that 50% of active X chromosomes are paternal and 50% maternal. Departure from this expectation is referred to as skewed X-inactivation (Heard et al., 2006; Nussbaum et al., 2001). Highly skewed inactivation patterns can result, for example, from an X-chromosome abnormality. Cells with an active damaged chromosome may have a significant survival disadvantage and so be underrepresented in the adult carrier (Amos-Landgraf

et al., 2006). This is a passive process, which occurs after inactivation itself, and may affect all daughter cells or only those in certain tissues.

Differential Expression of X-Linked Genes by Sex. Some genes are differentially expressed in male and female brains, irrespective of their X-inactivation status. Recently, six genes have been reported to have significantly higher levels of expression in adult female mice compared to males (Xu, Burgoyne, & Arnold, 2007). Though these differences have not been assessed in human brain and their cause within the mouse model is unclear, these differences may be similar across species and could have important implications in neural development (Skuse, 2005).

Genomic Imprinting. Differences in X-linked expression due to genomic imprinting are caused by the inheritance of an allele that differs in expression as a function of the parent of origin. Since males only inherit a maternal X chromosome, X-linked imprinted genes could cause sexually dimorphic traits. For example, X-monosomic females (Turner syndrome) had distinct hippocampal and amygdala morphology dependent on the parent of origin for the single X. Women inheriting an X chromosome from their mothers had significantly larger right hippocampal volumes than those women inheriting an X from their fathers (Cutter, Daly, Robertson et al., 2006; Kesler, Garrett, Bender et al., 2004; Skuse, 2005). Additionally, girls with Turner syndrome have lower social cognition if the single X is inherited from the mother rather than the father (Skuse, James, & Bishop, 1997), although this finding has not been

widely replicated. Therefore, genomic imprinting of some X-linked genes may contribute to sexual dimorphism in brain structure and function and may point to candidate genes for less severe conditions.

Monoamine Oxidase A as an X-Linked Genetic Risk Factor for Conduct Disorder

Monoamine oxidase-A (MAOA, EC 1.4.3.4) is responsible for the degradation of biogenic amines including the neurotransmitters epinephrine, norepinephrine, dopamine, and serotonin via deamination. *MAOA* is localized to Xp11.4-Xp11.3. A nonsense mutation in exon 8 (Gln296Stop) causes the truncation of the protein at codon 296, resulting in the loss of MAOA activity (Brunner et al., 1993). Males with the exon 8 mutation have engaged in impulsive/aggressive behaviors including rape, arson, and assault (Brunner et al., 1993). A mutation in transgenic mice results in the deletion of exons 2 and 3, resulting in a non-functioning enzyme that is associated with increased aggressiveness and injury among male mice and their cage-mates (Cases et al., 1995).

Gender Differences in the Genetic Risk for CD

Males are 2-4 times more likely to engage in activities related to CD compared to females (AACAP official action, 1997; Maughan et al., 2004). Conduct disorder is considered a heritable trait and several twin studies have detected sex-specific genetic effects for CD although the genetic contribution towards gender differences in risk for CD remains unclear (Eley et al., 1999; Graham et al., 1985; Hudziak et al., 2003; Jacobson, Prescott, & Kendler, 2002b; Rhee et al., 2002; Slutske et al., 1997). The X-

linked *MAOA* has been implicated in the gender difference in CD prevalence and in the direction of GxE for CD risk by gender (Chapter 2), thus making the X chromosome location of *MAOA* an ideal candidate gene for inquiry into whether CD may be due to X-linked inheritance. Recently, increased risk for violent behavior was associated with decreased limbic volume, hyperresponsive amygdala and diminished reactivity of the regulatory prefrontal brain region in males with the low activity *MAOA* allele. Additionally, there were significant genotype-sex interactions in (1) the left amygdala and hippocampus for emotional memory and (2) the cingulate region for inhibitory control (Meyer-Lindenberg et al., 2006). Therefore, *MAOA* may explain sexual dimorphism of neural development resulting in functional differences influencing gender-specific risk for aggression by gender.

The Present Study

Ultimately, if an X-linked gene were responsible for observed twin similarities in CD symptoms, it would provide an explanation for gender differences. However, in the absence of classic X-linked inheritance, an X-linked gene may still provide an explanation into the nature of gender differences if genotype-sex interaction is detected for CD. This work (1) calculates the expected means and twin correlations for CD symptoms by gender to test the assumptions of X-linkage and (2) tests for the presence of significant main effects of genetic and environment factors and gender as well as gene-environment interaction and genotype-sex interaction.

Methods

Study Population

This study is based on a sub-sample of 1,124 twin pairs and their parents from the Virginia Twin Study of Adolescent and Behavioral Development (VTSABD). The current sub-sample consists of those individuals and their parents for whom wave 1 CD measures and household neglect information were obtained.

Measures

Measure of Conduct Disorder. The data for the present study uses maternal and child symptom counts of CD from wave 1 of the VTSABD. Previous 3-month history of CD was assessed with the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al., 2000) and maternal or child self-report were included. Paternal ratings had low endorsement and were not included. Responses to the binary items were summed and used as a scale ranging from 0 to 12. Analyses were completed separately for maternal and child reports.

Measurement of Childhood Adversity. Three measures of negative family environment associated with CD indexed childhood adversity specifically parental neglect, exposure to inter-parental violence and inconsistent parental discipline. Childhood adversity was measured as a scale ranging from 0 to 7. Since this scale includes responses from each twin, some twins within a pair had different scores.

Therefore, the scale scores were averaged for each pair and the resulting score was rounded to the nearest whole number. This scale was then truncated as an ordinal measure of 0 or 1 or more exposures to address the issue of low frequency at the highest levels of exposure.

Genotyping of *MAOA*. Primer sequences previously described were used to genotype *MAOA* (Chapter 1), and classification of *MAOA* activity (high or low) was assigned to each allele resulting from previous work in the efficiency of transcription activity of the *MAOA* gene promoter (Sabol et al., 1998).

Results

Study 1- Using *MAOA* Genotype to Evaluate the Genotypic Effects of an X-Linked Gene on the Population Mean and Correlations of Conduct Disorder

Observed allele frequencies and the effect of *MAOA* genotype on CD were used to evaluate the expectations of X-linked inheritance on CD by (1) calculating the expected trait means for CD symptoms by gender and (2) calculating the expected twin correlations for CD symptoms.

Estimation of Mean CD Values by Gender Using *MAOA* Genotype and its Effects. The low and high activity *MAOA* alleles were represented as A and a, with frequencies of u (32.9%) and $v = 1-u$ (68.1%) respectively. These alleles correspond to three genotypes, AA, Aa, and aa in females with genotypic frequencies of u^2, $2uv$ and

v^2 respectively. Males will only have two genotypes representing their two alleles (A and a) with allele frequencies of u for the A allele and v for the a allele. The relationship between the female genotypes and a generic phenotype is represented in Figure 3.1.

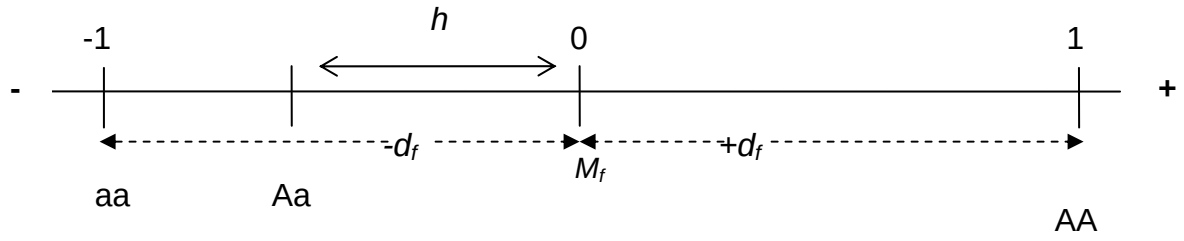


Figure 3.1 Genotypic Representation of a Continuous Trait

In Figure 3.1, the values $+d_f$ and $-d_f$ are the phenotypic differences of the homozygous females from the mean (M_f) and h represents the heterozygous deviation from M_f. The male mean is similarly defined as M_m and corresponding homozygous differences are as $+d_m$ and $-d_m$. In the absence of a heterozygous genotype for an X-linked gene in males, the value h is not included. The contributions of genotypes to the population means are summarized in Table 3.1.

Table 3.1 Summary of Genotypic Values and Frequencies for an X-Linked Gene

	Females			Males	
Genotype	AA	Aa	aa	A	a
Frequency	u^2	$2uv$	v^2	u	v
Value	d_f	h	$-d_f$	d_m	$-d_m$
Frequency x Value	u^2d_f	$2uvh$	$-v^2d_f$	ud_m	$-vd_m$

The genotypic contribution to the population mean of a trait is the sum of the products of the frequencies and the genotypic values (Falconer et al., 1996). This sum is $M_f = u^2d_f+2uvh-v^2d_f$ in females. This value can be simplified by noting that $u^2-v^2 = (u+v)(u-v) = (u-v)$. Further, in the absence of dominance ($h = 0$), the term $2uvh$ is zero. Therefore, the female mean is

$$M_f = (u - v)\, d_f$$

The male mean is $M_m = ud_m-vd_m$ and can be simplified to

$$M_m = (u - v)\, d_m.$$

Under X-linked inheritance, heterozygous females are carriers of an allele associated with a trait and often do not display the trait. Females homozygous for the allele associated with the trait will be affected and generally result from the pairing of a carrier mother and affected father. In comparison, males can display a trait through maternal inheritance of an allele from either an affected or carrier mother. Additionally, since homozygous females inherit an X chromosome from each parent, each of which is equally likely to undergo X-inactivation, the phenotypic variability associated with a

genotype is expected to be greater in females. Thus, the phenotypic mean associated with an X-linked inheritance is expected to be greater in males compared to females.

The expectation that X-linked inheritance results in a higher mean value of CD symptoms for males was assessed by calculating the expected means for CD symptoms by gender using *MAOA* allele frequencies (u and v) and sex-specific genetic effects (d_m or d_f) on CD symptoms. *MAOA* allele frequencies were obtained from Chapter 2 and used for this study (low activity allele, u = 32.9% and high activity allele, v = 68.1%). Regression models were used to estimate the effect of *MAOA* genotype on CD symptoms as measured by maternal and child reports in a manner similar to the models described in Chapter 2. The estimates of genetic effects on CD symptoms for maternal and child report and are summarized in Table 3.3.

Table 3.2 Genetic Effects of *MAOA* on CD Symptoms Used for the Estimation of X-Linked Twin Variances and Covariances

Maternal Measure			Child Measure		
d_x	d_m	d_f	d_x	d_m	d_f
0.14	0.12	0.24	0.14	0.10	0.25

For both maternal child reports, the expected mean value of CD symptoms for males (M_m) was 0.04 and the female mean (M_f) was 0.09. Used as a summary statistic, the larger mean value of CD symptoms in females compared to males does not follow the expectations of X-linked inheritance and suggests that X-linked genetic effects from *MAOA* alone do not account for the gender difference observed for CD.

Using *MAOA* Genotype to Estimate Twin Correlations Resulting from X-Linkage and X-Limitation. Under X-linked inheritance, the degree to which twins share X-linked genes is a function of sex. The genetic covariance of between brothers for alleles on X-linked genes is higher because their X-chromosomes are maternally inherited while females have both maternally- and paternally-derived X-chromosomes. Opposite-sex pairs share the fewest number of X-linked alleles. In contrast, under autosomal inheritance the correlation coefficients between sibling pairs would be the same for brothers and sisters as well as opposite-sex pairs. Table 3.2 summarizes the expected coefficients of covariance for autosomal and X-linked genes on a trait as defined by Mather and Jinks (Mather et al., 1982). The coefficients of ½ and ¼ refer to the autosomal additive genetic ($1/2\ D_R$) and dominance components ($1/4\ H_R$) and are derived in terms of the variance of inbred lines. These coefficients translate to the more commonly used notations of additive genetic effects (V_A) and dominance (V_D) (Falconer et al., 1996) as $V_A = 1/2\ D_R$ and $1/4\ H_R = V_D$.

Table 3.3 Expected Coefficients of Genetic Covariance for Siblings Resulting from Autosomal and X-Linked Gene Expression

	Autosomal			X-Linked		
Zygosity Group	Brothers	Sisters	Brothers-Sisters	Brothers	Sisters	Brothers-Sisters
MZM	1/2	0	0	1	0	0
MZF	0	1/2	0	0	1/2	0
DZM	1/4	0	0	1/2	0	0
DZF	0	1/4	0	0	3/8	0
ODZ	0	0	1/4	0	0	1/8

The effects of an X-linked gene on sibling resemblance for a trait of interest can be assessed using allele frequencies and genetic effects in a manner summarized in Mather and Jinks (Mather & Jinks, 1963). Under the expectations of X-linked inheritance, the estimates of the genetic contributions on sibling resemblance are:

Covariance of sisters $(W_{SS} \text{ or } \frac{3}{8} D_{RX}) = \frac{3}{8}\left\{4uv[d_f + h(u-v)]^2\right\} + 2u^2v^2h^2$ (3.1)

Covariance of opposite sex siblings $(W_{SB} \text{ or } D'_{xx}) = uvd_m[d_f + h(u-v)]$ (3.2)

Covariance of brothers $(W_{BB} \text{ or } \frac{1}{2} D'_X) = \frac{1}{2}(4uvd_m^2)$ (3.3)

Variance of females $(V_S \text{ or } \frac{1}{2} D_{RX}) = \frac{1}{2}\{4uv[d_f + h(u-v)]^2\} + 4u^2v^2h^2$ (3.4)

Variance of males $(V_B \text{ or } D'_X) = 4uvd_m^2$ (3.5)

The estimated effect of *MAOA* on CD symptoms was separately modeled for both maternal and child reports with each model specifying two situations, one where the effect of *MAOA* differed by sex ($d_m \neq d_f$) and another where it did not did not ($d_f = d_m = d_x$). The effects of *MAOA* on CD symptoms used for the estimation of twin variances and covariances are summarized in Table 3.3.

As addressed in Chapter 2, a model assuming additive genetic effects on CD diagnosis was used over one that included the effects of both additive and dominance effects. Therefore, estimates of *MAOA* on CD symptoms did not include dominance ($h = 0$). Further, in the absence of dominance, the variances and covariances associated with equations 3.1-3.5 are simplified to:

$$\text{Covariance of sisters } (W_{SS} \text{ or } 3/8\ D_{RX}) = \frac{3}{2} uvd_f^{\,2} \tag{3.6}$$

$$\text{Covariance of opposite sex siblings } (W_{SB} \text{ or } D'_{xx}) = uvd_m d_f \tag{3.7}$$

$$\text{Covariance of brothers } (W_{BB} \text{ or } \frac{1}{2} D'_X) = 2uvd_m^{\,2} \tag{3.8}$$

$$\text{Variance of females } (V_S \text{ or } \frac{1}{2} D_{RX}) = 2uvd_f^{\,2} \tag{3.9}$$

$$\text{Variance of males } (V_B \text{ or } D'_X) = 4uvd_m^{\,2} \tag{3.10}$$

The DZ twin variances and covariances can be used to calculate the correlations of X-linked genetic effects for brothers and sisters in the following manner:

$$r_{DZF} = \frac{W_{SS}}{V_S} \tag{3.11}$$

$$r_{DZM} = \frac{W_{BB}}{V_B} \tag{3.12}$$

$$r_{opp\text{-}sex} = \frac{W_{SB}}{\sqrt{V_S V_B}} \tag{3.13}$$

Under additive genetic effects MZ twins share 100% of their genes the MZ genetic correlation is expected to be equal to one, while DZ twins on average share 50% of their genes and the DZ genetic correlation is expected to be 0.5. Similarly, the expected MZ correlations due to X-linked effects will be equal to one. However, observed MZ twin correlations are generally smaller than 1, reflecting the proportional contribution of specific environmental effects in addition to genetic effects on the measure of twin similarity. In order to adequately estimate the effects of additive genetic inheritance on the expected twin correlations calculated using equations 3.11-3.13, it is also necessary to include and adjust for the effects of the environment. Expected DZ correlations under additive genetic inheritance were therefore adjusted to reflect the proportion of variance due to environmental effects by multiplying the expected DZ correlations with observed MZ correlations, since MZ correlations are expected to differ only as a result of MZ twin exposure to the environment. The assumptions of additive genetic inheritance were calculated by estimating the expected same-sex and opposite-sex DZ twin correlations. These correlations were calculated as:

$$\hat{r}_{DZM-additive(adj)} = \frac{1}{2} r_{MZM} \tag{3.14}$$

$$\hat{r}_{DZF-additive(adj)} = \frac{1}{2} r_{MZF} \tag{3.15}$$

$$\hat{r}_{opposite-sex(additive-adj)} = \sqrt{(\hat{r}_{DZM})(\hat{r}_{DZF})} \tag{3.16}$$

where r denotes an observed correlation and $\hat{r}$ identifies a calculated correlation.

Similarly, in order to adequately estimate the effects of X-linked inheritance on the expected twin correlations estimated using equations, it is also necessary to include and adjust for the effects of the environment using the observed MZ correlations. The adjusted DZ correlations were calculated as:

$$r_{DZF-X-linked(adj)} = (\hat{r}_{DZF})(r_{MZF}) \tag{3.17}$$

$$r_{DZM-X-linked(adj)} = (\hat{r}_{DZM})(r_{MZM}) \tag{3.18}$$

$$r_{DZO-X-linked(adj)} = (\hat{r}_{DZO})\sqrt{(r_{MZM})(r_{MZF})} \tag{3.19}$$

where r denotes an observed correlation and $\hat{r}$ identifies a calculated correlation that only includes X-linked genetic effects obtained from equations 3.11-3.13. Under the assumption of X-linked inheritance, the progression of the correlations is expected to be largest for the DZ female correlation, followed by the DZ male correlation and lastly, the opposite-sex pair correlation (Mather et al., 1963).

The χ^2-test was used to determine significant differences between the observed and expected correlations. This test statistic was weighted to address differences in

correlation variance as a function of sample size by including the asymmetric standard error (ASE) using the following formula:

$$\chi^2_{(DF=3)} = \sum_i w_i (O_i - E_i)^2 \qquad (3.20)$$

where $w_i = \frac{1}{S_i^2}$ and S is the ASE for the observed correlation, O_i is the observed polychoric correlation and E_i is the expected correlation.

Table 3.4 summarizes the expected twin correlations by zygosity group for maternal and child measures if (1) the genetic contribution for CD were X-linked following the allele frequencies and genetic effects of *MAOA* and (2) the genetic contribution for CD was due only to additive genetic inheritance. The observed child correlations suggested the role of X-linked genetic effects because $r_{DZF} > r_{DZM} > r_{\text{opposite sex}}$. Additionally, the X-linked observed maternal and child correlations for opposite-sex DZ pairs are lower than those of the same sex pairs. The observed CD correlations do not significantly differ from the correlations expected under X-linked or additive genetic inheritance. Therefore, neither X-linked nor additive genetic effects adequately explain gender differences in CD. This inability to distinguish between X-linked and additive genetic inheritance using the observed and expected twin correlations results from the relatively high values of ASE for the DZ correlations, indicating large variance in the observed correlations.

Table 3.4 Expected and Observed Twin Correlations for Conduct Disorder Symptoms

	Maternal Correlations					Child Correlations				
Zygosity Group	N	Observed (ASE)	Expected X-Linked $d_m = d_f$	Expected X-Linked $d_m \neq d_f$	Expected Additive	N	Observed (ASE)	Expected X-Linked $d_m = d_f$	Expected X-Linked $dm \neq d_f$	Expected Additive
MZM	261	0.83 (0.04)	-	-	-	265	0.58 (0.07)	-	-	-
MZF	321	0.86 (0.03)	-	-	-	326	0.37 (0.09)	-	-	-
DZM	151	0.62 (0.10)	0.41	0.39	0.42	153	0.29 (0.12)	0.29	0.28	0.29
DZF	142	0.61 (0.11)	0.65	0.65	0.43	146	0.49 (0.13)	0.28	0.27	0.19
ODZ	228	0.45 (0.10)	0.3	0.29	0.42	234	0.17 (0.12)	0.17	0.16	0.23
*$\chi^2_{(DF=3)}$			6.79	7.67	6.77			2.61	2.88	5.58

*no χ^2 values were significant at $p < 0.05$

Study 2- Detection of Gene-Environment Interaction and Gene-Sex Interaction for Conduct Disorder Using a Twin Sample

Twin Correlations. When the correlations for opposite-sex DZ pairs are less than for same-sex DZ pairs, X-linkage or sex limitation is expected (Eaves, 1982). However, since neither X-linked nor additive genetic effects adequately explained the observed CD twin correlations, alternate models including the contribution of genetic and environmental effects were used to detect the effects of sex-limitation and GxE.

Gene-sex interaction is generally defined as gender differences in genetic and environmental factors and is categorized as either scalar or non-scalar sex-limitation. Non-scalar sex-limitation indicates the extent to which different genetic effects occur in males and females. Under extreme non-scalar sex limitation, the correlation between opposite-sex twin pairs is expected to be zero, indicating that completely different loci are responsible for the variance in males and females (Eaves, Last, Young, & Martin, 1978). Scalar sex-limitation refers to a gender difference in genetic variance, such that the same factors affect both males and females but they may differ in magnitude for each gender. Under scalar sex-limitation, the correlation between the genetic effects in males and females is fixed to be equal to one, which indicates that the same genes affect both males and females.

As reported in Chapter 2, the direction of GxE differed in males and females, such that males with the low activity *MAOA* allele were at greater risk for CD when exposed to high levels of childhood adversity. In contrast, females with low activity

MAOA allele were at greater risk for CD when exposed to low levels of childhood adversity. A gender difference in the genetic contribution of CD may also result from the gender difference in GxE. A test of the genetic and environmental contributions to gender differences in twins should thus consider the effects of sex-limitation and GxE.

Polychoric twin correlations across zygosity groups were estimated for maternal and child scales of CD using PROC FREQ in SAS and invoking the PLCORR option (SAS, version 9.1). As a preliminary test of gender differences in the effect of gene-environment interaction (GxE) on twin similarity, polychoric correlations were generated across the levels of childhood adversity for maternal and child measures of CD. Gene-environment interaction may be present when there are significant differences in the correlations between groups with high and low exposure to an environmental risk factor in both MZ and DZ twins.

Tables 3.5 and 3.6 summarize twin correlations for maternal and child measures of CD across environmental exposures. Correlations across increasing levels of childhood adversity generally remained constant particularly in MZ twins, suggesting additive genetic effects and no significant genotype-environment interaction. For maternal and child ratings of CD, the twin correlations for opposite sex pairs increased as exposure to increasing childhood adversity increased. DZ males were more similar for CD symptoms as exposure to childhood adversity increased while DZ females were less similar (Table 3.5). However, the DZ child correlations reflecting one or more exposures to childhood adversity had high variation as demonstrated by the wide

confidence intervals. As expected with these sample sizes, the confidence intervals are larger for those correlations at the highest level of exposure to childhood adversity.

Table 3.5 Polychoric Correlations and 95% Confidence Intervals for Maternal Measures of Child Conduct Disorder by Levels of Exposure to Childhood Adversity

Childhood Adversity	MZM	MZF	DZM	DZF	Opposite Sex
0	0.86 (0.78-0.94) N=204	0.85 (0.76-0.93) N=232	0.47 (0.17-0.93) N=104	0.72 (0.51-0.93) N=104	0.29 (0.04-0.54) N=173
1 or more	0.78 (0.59-0.97) N=57	0.89 (0.80-0.98) N=89	0.80 (0.60-0.99) N=47	0.36 (-0.2-0.91) N=38	0.76 (0.57-0.96) N=55

Table 3.6 Polychoric Correlations and 95% Confidence Intervals for Child Measures of Child Conduct Disorder by Levels of Exposure to Childhood Adversity

Childhood Adversity	MZM	MZF	DZM	DZF	Opposite Sex
0	0.59 (0.42-0.75) N=205	0.36 (0.12-0.60) N=234	0.32 (0.05-0.58) N=108	0.55 (0.21-0.89) N=107	0.08 (-0.19-0.35) N=176
1 or more	0.53 (0.15-0.91) N=60	0.38 (0.06-0.71) N=92	0.27 (-0.21-0.74) N=45	0.36 (-0.26-0.97) N=39	0.42 (-0.07-0.91) N=58

Modeling the Effects of Genetic and Environment Influences, Gene-Environment Interaction and Gene-Sex Interaction

A linear model was fitted to the twin correlations using a weighted least-squares approach to test whether correlations of CD were due to genetic effects, gender, exposure to childhood adversity, GxE, scalar sex-limitation, non-scalar sex-limitation. First, twin correlations for maternal and child ratings of CD were each z-transformed to reflect a normal distribution and uniform variance with the r-to-z Fisher transformation:

$$z = \tfrac{1}{2} \log \{(1+r)/(1-r)\}$$

where *r* is the correlation coefficient. This transformation reduces the effects resulting from the skewed distribution of the CD scale scores such as heteroscedasticity and minimizes the effects of scale across levels of environmental exposure (Hotelling, 1953). Z-transformed scores were then weighted by their associated degrees of freedom, which was determined as a function of sample size (df = n-3, where n is the zygosity group sample size).

Dummy contrasts were created to assess the effects of childhood adversity, genetic effects and gender and their associated interactions and were defined as (1) the contrast between exposure or non-exposure to childhood adversity (2) The contrast between MZ and DZ pairs to measure the effects of genetic effects (A), (3) The contrast between males and females to measure the effects of gender (N), and (4) The contrast between like-sex and unlike-sex pairs to measure the effects of non-scalar sex-limitation (S) (Table 3.7). Models using maternal and child measures of CD were fitted separately to avoid the complications of non-independence between the two raters. Further, since

the overall difference between correlations by maternal and child report differ in their direction and magnitude, it would be more instructive to view the problem separately for each rater.

Table 3.7 Contrast Definitions for Parameters Predicting Conduct Disorder

Zygosity Group	Intercept	Childhood Adversity (E)	MZ vs.DZ Pairs (A)	Males vs. Females (N)	Like-Sex vs. Unlike-Sex Pairs (S)
MZM	1	1	1	1	0
MZF	1	1	1	-1	0
DZM	1	1	-1	1	1
DZF	1	1	-1	-1	1
ODZ	1	1	0	0	-2
MZM	1	-1	1	1	0
MZF	1	-1	1	-1	0
DZM	1	-1	-1	1	1
DZF	1	-1	-1	-1	1
ODZ	1	-1	0	0	-2

Model fitting using maternal and child measures of CD first assessed models of main effects separately and then together, followed by all estimable combinations of two-way interactions. Models comparisons were performed using goodness-of-fit measures and variable significance via (1) the F-value and its corresponding p-value

which indicates the degree to which the total variance is due to the model compared to chance alone; (2) the R^2, or the amount of the total variance explained by the mode where higher values suggest more of the variance explained by the model; (3) the root mean square error (RMSE), which measures the standard error associated with a given model, where lower values indicate a smaller portion of total variance due to error and improved fit; and (4) Akaike's Information Criterion (AIC) measuring model parsimony calculated as:

$$AIC = n \log\left(\frac{RSS}{n}\right) + 2p$$

where n = Number of observations, p = Number of parameters in the model and RSS = Residual sum of squares (Akaike, 1974; Maindonald & Braun, 2003). Lower values of AIC indicate more parsimonious models.

Table 3.8 summarizes the results from the model fitting strategy used to determine the contribution of genetic, environmental and gender influences to twin similarity of CD symptoms for maternal ratings. The estimates for the interactions between (1) A and S and (2) N and S were inestimable because they are confounded with one another and were not included. Further, two-way interactions were not tested because there were no significant one-way interactions.

For maternal ratings of CD, model 5 is most parsimonious as measured by AIC (10.87) and explains a significant amount of the total variance as expressed by the high R^2 value (0.74) and a low RMSE (2.48). This model consists of significant additive genetic effects (A) and genetic effects dependent on gender (S) (Table 3.9).

Table 3.8 Model Fit Summary of Maternal Measures of Child Conduct Disorder

Model	Model Specified	Model SS	Error SS	F-Value	P-Value	r^2	RMSE	Parameters	AIC
1	Genetic Effects (A)	73.70	91.88	6.42	0.04	0.45	3.39	1	13.81
2	Like-Sex vs. Unlike Sex Pairs (S)	21.15	144.43	1.17	0.31	0.13	4.25	1	16.75
3	Gender (N)	2.34	163.24	0.11	0.74	0.01	4.52	1	17.55
4	Childhood Adversity (E)	4.49	161.10	4.49	0.22	0.03	4.49	1	17.47
5	**A, S**	**122.54**	**43.04**	**9.97**	**0.009**	**0.74**	**2.48**	**2**	**10.87**
6	A, N	74.60	90.98	2.87	0.12	0.45	3.61	2	15.74
7	A, E	79.36	86.22	3.22	0.1	0.48	3.51	2	15.39
8	S, N,	23.51	142.07	0.58	0.59	0.14	4.51	2	18.65
9	S, E	25.01	140.57	0.62	0.56	0.15	4.48	2	18.58
10	N, E	6.67	158.91	0.15	0.87	0.04	4.76	2	19.38
11	A, S, E	127.35	38.23	6.66	0.02	0.77	2.52	3	12.09
12	A, S, N	123.23	42.35	5.82	0.03	0.74	2.66	3	12.76
13	A, S, N, E	127.95	37.63	4.25	0.07	0.77	2.74	4	13.99
14	A, S, E, A*E	128.15	37.44	4.28	0.071	0.77	2.74	4	13.96
15	A, S, E, S*E	138.87	23.72	6.50	0.032	0.84	2.31	4	10.99
16	A, S, N, A*N	123.32	42.26	3.65	0.094	0.74	2.91	4	14.75

Table 3.9 Parameter Estimates of Maternal Measure of Child Conduct Disorder

Parameter	Estimate	Standard Error	p
Intercept	0.89	0.08	<0.0001
Genetic Effects (A)	0.37	0.09	0.005
Like-Sex vs. Unlike Sex Twins (S)	0.21	0.08	0.03

Table 3.10 summarizes model fits for child ratings of CD. Model 16 had the greatest parsimony (AIC = 3.60) and includes a significant interaction between additive genetic effects and gender (A*N), or scalar sex-limitation. Like the models based on the maternal measures of CD, there is no significant effect of the environment. Table

3.11 summarizes the parameter estimates for this model. There was no significant main effect of gender (p = 0.89, Table 3.10).

Table 3.10 Model Fit Summary for Child Measures of Conduct Disorder

Model	Model Specified	Model SS	Error SS	F-Value	P-Value	r^2	RMSE	Parameters	AIC
1	Genetic Effects (A)	2.85	38.24	0.60	0.463	0.07	2.19	1	8.10
2	Like-Sex vs. Unlike Sex (S)	11.56	29.52	3.13	0.115	0.28	1.92	1	6.41
3	Gender (N)	1.62	39.47	0.33	0.58	0.04	2.22	1	8.30
4	Childhood Adversity (E)	0.04	41.05	0.01	0.94	0.00	2.27	1	8.56
5	A, S	18.46	22.62	2.86	0.12	0.45	1.80	2	6.68
6	A, N	4.76	36.32	0.42	0.65	0.12	2.28	2	9.76
7	A, E	2.89	38.19	0.27	0.77	0.07	2.34	2	10.09
8	S, N,	13.19	27.89	1.65	0.26	0.32	2.00	2	8.04
9	S, E	11.57	29.51	1.37	0.31	0.28	2.05	2	8.41
10	N, E	1.67	39.41	0.15	0.86	0.04	2.37	2	10.29
11	A, S, E	18.48	22.60	1.64	0.28	0.45	1.94	3	8.67
12	A, S, N	20.58	20.50	2.01	0.21	0.50	1.85	3	8.03
13	A, S, N, E	20.62	20.47	1.26	0.39	0.50	2.02	4	10.03
14	A, S, E, A*E	18.59	22.49	1.03	0.47	0.45	2.12	4	10.64
15	A, S, E, S*E	25.36	15.72	2.02	0.23	0.62	1.77	4	8.31
16	**A, S, N, A*N**	**33.46**	**7.63**	**5.48**	**0.05**	**0.81**	**1.24**	**4**	**3.60**

Table 3.11 Parameter Estimates for Child Measures of Conduct Disorder

Parameter	Estimate	Standard Error	p
Intercept	0.42	0.04	0.0001
Genetic Effects (A)	0.10	0.05	0.07
Like-Sex vs. Unlike Sex Pairs (S)	0.12	0.04	0.02
Gender (N)	0.01	0.04	0.89
Genetic Effects X Gender (A*N)	0.13	0.04	0.03

Discussion

It has been suggested that X-linked genes such as *MAOA* are associated with gender differences in antisocial behavior and brain structure for regions responsible for aggressive behavior. Furthermore, several twin studies have detected sex-specific genetic effects for CD, emphasizing the need to test the assumptions of X-linked genetic effects on twin correlations of CD as a possible cause for gender differences. Additionally, testing for the presence of genotype-environment interaction and genotype-sex interaction (scalar sex-limitation) using twin correlations may guide future inquiry of the role of *MAOA* in understanding gender differences of CD.

No Significant X-Linked Effects for Conduct Disorder

X-linked effects resulting from the genotype of the *MAOA* promoter region do not explain the sex differences for CD. This result is not surprising, since the estimation of heritability and thus of the importance of X-linked effects has been somewhat inconsistent. Extreme aggression in males resulting from Brunner's Syndrome is a consequence of a mutation on *MAOA* and its pattern of inheritance is clearly identified. This disorder is one exclusively of males, making the contribution due to additive genetic effects for this X-linked trait larger in males compared to females. Conduct disorder in contrast is much more complex since the variance due to additive genetic effects has been reported to be larger in females than males (Eley et al., 1999; Jacobson et al., 2002b; Vierikko, Pulkkinen, Kaprio, & Rose, 2004) and although

the opposite-sex pair correlation in this study is low, it has not been shown to function as an X-linked trait.

If X-linked effects were primarily responsible for gender differences in CD, traits related to CD would also be subject to X-linked effects (Fairbairn et al., 2006). Since antisocial behavior appears to manifest differently by gender and males have higher rates of CD than females, there may still be reason to study *MAOA* or other genes on the X-chromosome thought to contribute significant X-linked effects. The inclusion of parental genotype and CD data could also be used to evaluate the assumptions of X-linked effects with the correlations between parents and children. Correlations are expected to be greatest for mother-son pairs and father-daughter pairs followed by mother-daughter pairs, and lastly father-son pairs. However, it may be more fruitful to consider the effects of *MAOA* outside that of a solely X-linked effect (Mather et al., 1963).

While X-linked effects from *MAOA* are not responsible for genetic variation of CD, this gene may still provide insight into the genetic contribution towards gender differences of this disorder. An X-linked locus is at least three times more likely to be involved in sexual development than genes on an autosomal chromosome and sex-linked genes often exert their phenotypic effects by regulating the expression of autosomal genes (Fairbairn et al., 2006). It is therefore possible that this X-linked gene may moderate the expression of other genes by gender, moderate genotypic sensitivity to environmental exposure or moderate by gender.

Genetic Influences on the Gender Differences in Conduct Disorder

This study has identified the presence of gender-dependent genetic effects in both maternal and child reports of CD. Further, the most parsimonious model based on the child report identified a significant genotype-sex interaction. This finding is consistent with some twin studies (Eley et al., 1999; Jacobson et al., 2002b; Vierikko et al., 2004).

There is less clarity with respect to conclusions about the nature of the genetic contributions by gender because of the differences between the maternal and twin measures. Results from fitting models to the maternal measures do not show a significant effect of gender, but do show a significant effect of like vs. unlike sex pairs (non-scalar sex-limitation). This suggests that the same loci might be responsible for gender differences but might function differently in same sex and opposite-sex pairs. Research of opposite-sex versus same-sex twins has consistently reported significant differences in cerebral lateralization patterns such that female patterns from opposite-sex pairs are more masculine than same-sex females. This difference is often attributed to in utero differences in exposure to testosterone (Laffey-Ardley & Thorpe, 2006). From the best model fitted to the maternal measures, environmental and genetic effects are equal across sexes. However, the steps between genotype and phenotype cannot be addressed in these models.

Results from the child reports of CD indicate significant scalar sex-limitation and no significant effect of gender. This is consistent with two previous studies CD (Gelhorn et al., 2005; Jacobson et al., 2002a). Gelhorn and colleagues found no

significant effect of sex on CD while Jacobson and colleagues found a significant gene-sex interaction. However, this result differs with other studies, which have reported greater additive genetic effects in females (Eley et al., 1999; Vierikko et al., 2004). Ultimately, the gene-sex interaction identifies the child report as the source of the opposite direction of GxE reported in Chapter 2. This also suggests that the detection of GxE as well as any subsequent interpretations will be reporter dependent.

Absence of Significant Gene-Environment Interaction

As a result of the small sample size in twins exposed to childhood adversity, the power to detect significant interactions with environmental exposure is low and the conclusions regarding the environment have more heuristic than substantive value. Neither maternal nor child measures indicate the presence of gene-environment interaction. These results do not confirm the detection of GxE reported in previous studies (see Chapter 2). Additionally, GxE may not have been detected because of the use of CD measured by symptom count separately by rater rather than as a diagnosis including multiple raters as addressed in Chapter 2. There was a non-significant increase in the correlations for opposite-sex pairs as exposure to adversity increased in maternal and twin measures. This increase suggests that opposite-sex pairs may provide insight into the nature of genotype-sex interaction based on self-report measures and how males and females might respond to their environments as a result of genetic mediation. Females from opposite-sex twin pairs were reported to be more

masculine in their patterns of aggression than same-sex females despite similar testosterone levels (Cohen-Bendahan, Buitelaar, Van Goozen, Orleheke, & Cohen-Kettenis, 2005). Additionally, adolescent opposite-sex pairs were found to have higher levels of social competence and be more socially adaptive than individuals from same-sex pairs or singletons (Laffey-Ardley et al., 2006). Social learning differs in opposite-sex pairs by providing them alternative, gender-related responses to dealing with challenging environments (Cohen-Bendahan, Buitelaar, Van Goozen, & Cohen-Kettenis, 2000). Thus, the study of opposite-sex twin pairs compared with same-sex twins and singletons particularly in the presence of childhood adversity is anticipated to provide an avenue for understanding the genetic and environmental contributions to gender differences in conduct disorder.

Ultimately, if an X-linked gene is functioning to produce gender differences or gene-environment interaction, such effects are specific for each gene and environment combination and genetic study of gender differences for CD may benefit from focusing candidate gene efforts on genotype-sex interaction.

The following results should be interpreted while noting the following limitations. While this study provides insight into the nature of the X-linked genetic effects on CD using genotypes from a population-based sample of twins as well as measures from multiple raters, it provides only one level of inquiry into the role of genetic effects on gender differences. This study did not include inquiry into other known effects of molecular-level gender differences such as genomic imprinting or maternal effects.

Expected values of the effect of MAOA on twin similarity assume random X-inactivation in females. Although several reports suggest this is the case for MAOA, this point is still under debate. If X-inactivation for MAOA were non-random, even in a subset of individuals with a functional MAOA variant, a different set of predictions regarding the X-linked genetic effects would result.

Childhood adversity was the only measured environment and other parental measures such as traits related to genotype-environment correlation were not included in these models making the assessment of the environment incomplete. Additionally, the measure of childhood adversity only reflects two levels of exposure. In a model of 3 levels of childhood adversity (0, 1 and 2 or more exposures), a significant interaction between gender-dependent effects and childhood adversity was detected. However, the variance at the highest level of exposure was high due to low sample size. This work exemplifies the difficulty of detecting significant genotype-environment interaction and underscores the need to improve detection of interaction in population-based studies using information from multiple raters.

This study did not assess the role of age and thus CD is treated as a disorder of adolescence rather than one that may vary across this developmental period. However, the role of X-linked effects is not anticipated to vary as a result of age and in order to assess the role of the environment with some degree of confidence, age was not included as a covariate.

Finally, the VTSABD is a sample of Caucasian families and results from this sample may not apply to groups of other ethnicities or cultural norms.

CHAPTER 4 Detecting Genotype-Environment Interaction in Conduct Disorder Using a Markov Chain Monte Carlo Approach to Item Response Theory Modeling of Multi-Symptom Genetic Data

Abstract

The recent reports of significant gene-environment interaction (GxE) between monoamine oxidase-A genotype (*MAOA*) and childhood adversity for conduct disorder have been subject to criticism due to the treatment of measurement scale and environmental exposure, resulting in the possibility of false detection of GxE. It is imperative to address these issues if GxE is expected to improve insight into our understanding of how genetic and environmental risk factors function to increase risk for psychopathology. This study tested for the presence of GxE using a Markov Chain Monte Carlo approach that included a genetic item response theory (IRT) model to evaluate CD as a trait with continuous liability.

Among females, models that included GxE in the presence of the main genetic and environmental effects of *MAOA*, childhood adversity and age were appropriate in predicting risk for CD using both maternal and child ratings of CD. Among males, models of risk based on child reports of CD indicated that the inclusion of GxE as a predictor of risk for CD is justified, while maternal reports do not. Further, estimates of GxE and most of the corresponding main effects are weak.

When GxE was detected in males using child reports, the direction of the interaction was negative, suggesting that risk for CD increases among males with the low activity *MAOA* allele at low levels of exposure to childhood adversity, which is opposite of what has been previously reported (Caspi et al., 2002; Foley et al., 2004). Further, the susceptibility allele differs between models resulting from child and maternal reports of CD. The difference in the direction of GxE in males compared with previous reports may either be a consequence of using a latent trait to measure CD, a reflection of the weak effects of *MAOA* and childhood adversity or may result from rater differences in the measurement of CD.

Introduction

The recent detection of genotype-environment interaction (GxE) for susceptibility to antisocial behavior and conduct disorder using measured genotypes and environments (Caspi et al., 2002) as well as positive replications (Foley et al., 2004; Nilsson et al., 2005) and a positive meta-analysis (Kim-Cohen et al., 2006) have motivated reflection into the ability to detect significant interaction using current approaches. A recent simulation study demonstrated how the consistent false detection of GxE might occur as a result of the treatment of measurement scale (Eaves, 2006). Consequently, the use of alternative methods that minimize the effect of scale in detecting significant GxE should be considered and their outcomes compared against those reported using standard detection methods.

Classical Detection of Genotype-Environment Interaction

Genotype-environment interaction has been detected in plant and animal studies by comparing a continuous phenotype such as fruit production or height for different breeding lines in the presence of specific environments. The genetic effect on the phenotypic mean is specified using the single-gene system against the genetic background of multiple genes in a manner adopted by Fisher, Immer and Tedin (1932). This system defines the phenotypic midpoint between the two homozygous genotypes as *m*. The value *h* identifies the phenotypic departure of the heterozygote from the

mean phenotypic value. The values $+d$ and $-d$ are the phenotypic differences of the homozygotes from the mean (Figure 4.1) (Fisher et al., 1932).

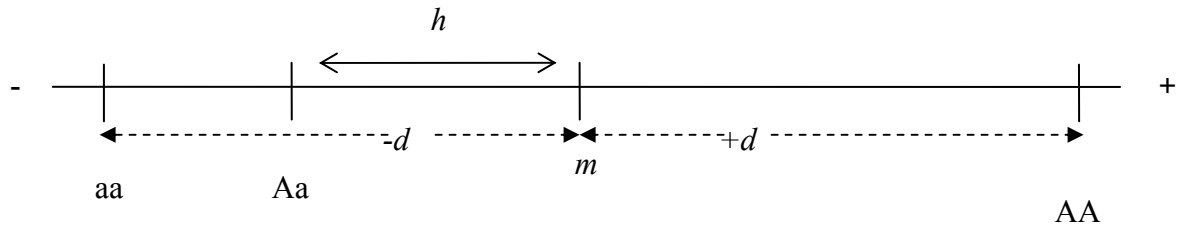


Figure 4.1 Genotypic Representation of a Continuous Trait

In the simplest case, the specification of GxE can be addressed by considering the effects of breeding lines differing by a single allele (A or a) to produce 3 different genotypes (AA, Aa and aa) that are grown in two different environments (X or Y). Parameters for genotypic (*d* denoting additive effects or *h* indicating dominance), environmental (*e*) and GxE (*i*) contributions are then estimated from the mean differences in phenotype using a least squares approach (Table 4.1).

Table 4.1 Parameter Coefficients and Phenotypic Values by Genotype and Environmental Exposure

Genotype	Environment	m	*d*	*h*	*e*	i	Expected Phenotype
AA	X	1	1	0	1	1	$m + d + e + i$
Aa	X	1	0	1	1	1	$m + h + e + i$
aa	X	1	-1	0	1	-1	$m - d + e - i$
AA	Y	1	1	0	-1	-1	$m + d - e - i$
Aa	Y	1	0	1	-1	-1	$m + h - e - i$
aa	Y	1	-1	0	-1	1	$m - d - e + i$

Note: Adapted from Mather and Jinks (1982)

Assuming no dominance, the model specifying the additive genetic and environmental contributions for an outcome of interest in the sample is summarized as

$$X_{ijk} = \mu + d_i + e_j + \delta_{ij} + \varepsilon_{ijk} \quad (4.21)$$

where μ is the overall phenotypic mean for a trait of interest, g_i is the additive genotypic effect for genotype, e_j is the effect of environmental exposure, δ_{de} is the effect of the genotype-by-environment interaction and ε_{ijk} is the associated random error term for each individual. The significance of GxE is tested by comparing the goodness-of-fit for a model that includes the interaction against a model without.

Using Twin Data to Estimate Additive Genetic Effects and GxE. Under the classical twin model having no measured genotype, estimates of the variances due to

additive genetic, shared environment, unique environment from twin studies depend on the variances and covariances for MZ and DZ twins. The phenotypic variance of a trait is represented as

$$\sigma_P^2 = \sigma_A^2 + \sigma_C^2 + \sigma_D^2 + \sigma_E^2 \tag{4.22}$$

Where σ_A^2 reflects the variance due to additive genetic effects, σ_C^2 is the variance due to the shared environment, σ_D^2 is the variance due to dominance, and σ_E^2 is the variance due to the unshared environment and measurement error. The estimation of additive genetic effects comes from the decomposition of the phenotypic variance into its respective variances using the covariances for monozygotic (MZ) and dizygotic (DZ) twin pairs. Their covariances are represented as

$$cov_{MZ} = \sigma_A^2 + \sigma_C^2 + \sigma_D^2 \tag{4.23}$$

$$cov_{DZ} = \frac{1}{2}\sigma_A^2 + \sigma_C^2 + \frac{1}{4}\sigma_D^2 \tag{4.24}$$

In practice, data on dominance and shared environmental effects for twin pairs reared together are confounded since σ_D^2 is not transmitted from parents to offspring. As an example, a substantial dominance genetic effect will lead to the covariance between DZ twins to be less than one-half the covariance of MZ twins. Consequently the dominance effect will lead to a negative estimate of σ_C^2 in a model including additive genetic, dominance, and shared environmental effects. Likewise, a substantial effect of σ_C^2 would result in a negative value of σ_D^2. Thus, models of additive genetic, shared environmental and unique environmental effects constrain the value σ_D^2 to equal zero

since the parameter estimates of genetic and environmental effects resulting from the twin covariances cannot be negative values, (Eaves et al., 1978; Martin, Eaves, Kearsey, & Davies, 1978).

Generally, twin studies that estimate the effects of the basic univariate model consisting of additive genetic, shared environmental and unique environmental effects assume no GxE or genotype-enivronment correlation. The variance due to GxE in twin studies is therefore tested by comparing a model that stratifies MZ and DZ covariances or correlations by environmental exposure against a model without environmental stratification.

Issues in the Measurement of Human Psychopathology Affects Detection of GxE

It has been widely recognized that the detection of interactions in general, and GxE in particular is especially sensitive to the scale of measurement (Mather et al., 1982), particularly the care of psychological measures for which definitions of the phenotype and units of measurement are more or less arbitrary. Thus, in evaluating the claim of detecting GxE in risk to psychopathology it is important to consider the extent to which the interpretation of data might vary as a function of choice of measurement and seek a model of analytical approach that clearly distinguishes the theoretically robust aspects of variation from that which depends on the instrument choices to measure it.

Most psychiatric diagnoses are the result of a threshold placed on symptom counts which are often arbitrary and do not take the underlying (latent) differences from where the symptom counts and diagnoses arrive. The distribution of symptom scores is often skewed, with the majority of respondents having few to no symptoms. As a result, the distribution of symptom scores may exhibit heteroscedasticity, or an unequal error variance across the range of measurement, and may lead to the false detection of GxE. This issue may be addressed by transforming the measure to remove the dependence of the mean variance, which often results in the loss of GxE. It might be possible to minimize the effects of hetereoscedasticity by creating a dichotomous variable and working within a logistic regression framework. However, dichotomizing a trait collapses multiple criteria into a single binary diagnosis and results in a loss of information. Additionally, the detection of GxE may be contingent on the more or less arbitrary placement of the threshold for diagnosis on the underlying, latent trait (Eaves, 2006).

The common approaches used to address the arbitrary measure of symptom counts results in an inadequate assessment of heritability and GxE. For example, the simulation of 100 samples, each consisting of 1000 observations demonstrated that significant additive genetic effects were detected in 100% of the samples, while GxE was detected in 15% of the samples when the outcome was modeled as a continuous variable. In contrast, additive genetic effects were detected in 55% of simulated samples and GxE was detected in 70% of samples when the outcome was modeled as a

dichotomous variable. Consequently, the strength of additive genetic effects and detection of GxE depends on how the measurement scale is treated (Eaves, 2006).

The detection of GxE in humans has also relied on the stratification of the environment in genetically informative samples. However, stratification treats environmental exposure as a fixed effect, since the levels of exposure are predetermined (ie: 0, 1 and 2 or more exposures) although environmental exposure is conceptualized as a random effect (Eaves & Erkanli, 2003b). Environmental exposure and genetic effects are also assumed to be independent for an outcome of interest. However, non-independence between genetic and environmental effects often results from gene-environment correlation, defined as the genetic control of environmental exposure (Eaves et al., 1977; Jinks et al., 1970). Further, the ability to detect GxE depends on the scale of the environment. For example, GxE is more often detected when increasing levels of environmental exposure are considered. Genotype-environment interaction was detected in 70% of simulated samples for a dichotomous outcome when four levels of exposure were considered. In contrast, 27% of samples detected significant GxE with 3 levels of environmental exposure.

Using Models Based in Item Response Theory to Address the Ability to Detect GxE

Item response theory (IRT) provides a framework for conceptualizing the relationship between categorical outcomes such as responses to multiple symptoms of a psychiatric diagnosis and liability for a disorder. Under the IRT paradigm, each item (g_1, g_2, …g_n) of an instrument is an index of a latent trait, θ. The regression of each

item score on the hypothetical, continuous latent trait is defined as the *item characteristic function*. Typically, the item characteristic function is assumed to be invariant for each respondent, meaning it is expected to be identical in separate respondents or across separate measurement conditions (Lord & Novick, 1968). For items with binary responses, the item characteristic function describes the probability that individuals will endorse the item given their value for the trait. Responses to multiple items are assumed to exhibit "local independence", or to be independent conditional on a latent trait value. Further, under the assumption of local independence an item is uncorrelated with other items in an instrument for subjects with the same trait value and items are only related to one another through the latent traits they measure (Lord et al., 1968).

The item characteristic function quantifies the probability of a respondent scoring an item (g) as 1 as a function of θ and may assume a number of forms. For this application we assume that the probability of endorsement of the g^{th} item is a logistic function of binary value with two parameters, a_g and b_g and defines the probability of a respondent scoring g with a binary response as 1, given their measure for a latent trait, Θ. (Birnbaum, 1968):

$$P_g(\theta) = \frac{1}{1 + e^{-b_g(\theta - a_g)}} \tag{4.25}$$

The item difficulty, a_g is the value of the latent trait at which the probability of item endorsement changes most rapidly. The item discrimination power (b_g) measures the rate of change in endorsement probability at a_g. Figure 4.2 illustrates the qualitative

role of the item parameters for a few hypothetical item characteristic curves. Each S-shaped line depicts the probability of positively endorsing a symptom with increasing levels of disease liability. Items with steeper slopes demonstrate greater discriminating power (b_g) as expressed by rapidly changing probabilities within small changes of liability. Additionally, item difficulty (a_g) is illustrated as the point of inflection on the S-curve, i.e. the point at which $P_g(\theta) = 0.5$ and reflects the corresponding level of θ at which the items discriminates most effectively (Lord et al., 1968).

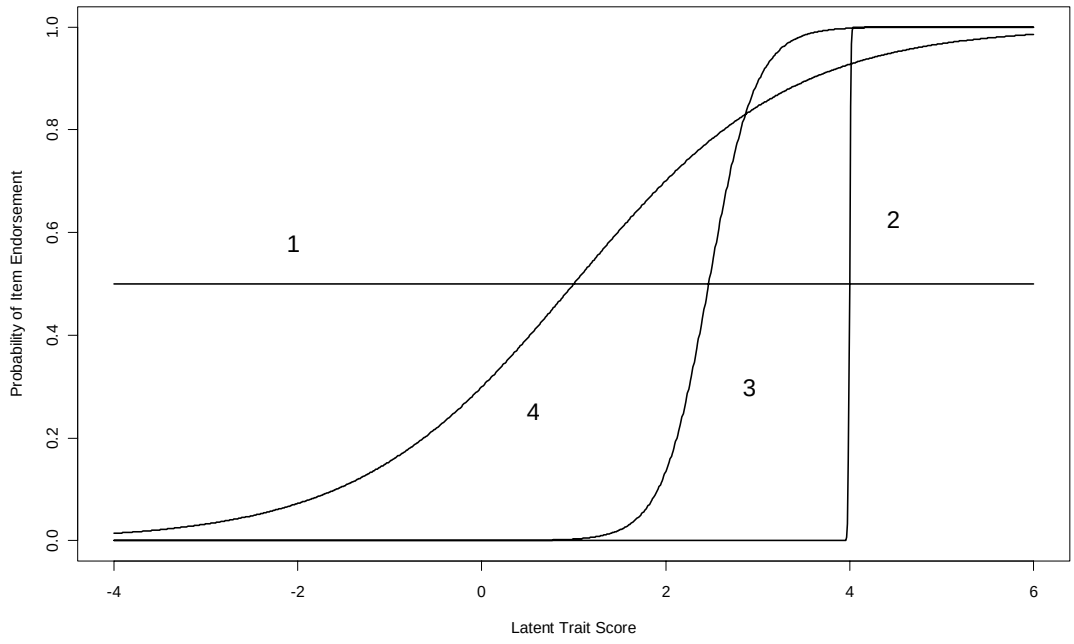


Figure 4.2 Hypothetical Item Characteristic Curves of the Logistic Function

Item 1 has a constant probability $P_1(\theta) = 0.5$ with $a_1 = 0.8$ and $b_1 = 0$. This item does not discriminate individuals at all across levels of the latent trait and provides no

information about the latent trait. Item 2 demonstrates a strong probability of endorsing an item at a specific level of the latent trait, with $a_2 = 4$ and $b_2 = 100$. The value of $P_2(\theta)$ is zero until reaching a latent trait measure of 4. This item perfectly discriminates an individual as affected or non-affected at this point on the latent trait scale. If however, the probability of endorsement is required at other measures of Θ, no additional information is available. Items 3 and 4 moderately discriminate across levels of the latent trait. Item 3 has parameter values of $a_g = 2.5$ and $b_g = 2.3$, and has a curve that appears to have more clear discrimination than item 4 ($a_g = 1$ and $b_g = 0.5$).

Using Genetically Informative Data within the IRT Framework to Estimate Genetic Effects. An IRT approach to estimating additive genetic effects and GxE provides the measure of θ as item difficulty and item discrimination parameters. This results in a unique endorsement pattern or liability score for each individual of a twin pair that is jointly organized on a common unit scale. Variation in the underlying trait is scaled to be N(0,1), with twin correlations ρ_{mz} and ρ_{dz} dependent on zygosity. The liability scores are then used to estimate the twin pair correlations for each twin type (MZ or DZ) and the variance due to additive genetic, shared environment and unique environment. Consequently, an IRT approach avoids the issues present when dealing with symptom scores since each individual in the sample has a specific measure of liability for a trait rather than a sum score with imposed constraints.

Using a Markov Chain Monte Carlo Approach and Bayesian Inference for Genetic IRT

Genetic IRT Using a Traditional Likelihood Approach. Until recently, it has been difficult to employ IRT models in the genetic analysis of twin data because of the computational difficulty maximizing the likelihood associated with this type of model. The likelihood of the response vector $\mathbf{X}_i$ for the i^{th} subject, given a trait value θ_i is

$$l(\mathbf{X}_i|\Theta_i) = \prod_{g=1}^{k} P_{gi}^{X_{ig}} [1 - P_{gi}]^{1-X_{ig}} \tag{4.26}$$

where P_{gi} is the probability of endorsing the gth item, given the subject's trait value θ_i. X_{ig} is the response (1 or 0) of the i^{th} subject to the g^{th} item (Edwards, 1984).

The unconditional likelihood of a subject response vector of individuals is the product of the likelihoods for each individual as the integral of Equation 4.6 over all values of θ as

$$l(\mathbf{X}_i) = \int_{-\infty}^{\infty} \varphi(\theta) l(\mathbf{X}_i|\theta_i) d\theta \tag{4.27}$$

where $\varphi(\theta)$ is the posterior distribution function of θ.

In order to utilize the measures of the latent trait derived from the IRT in any genetic application, the inclusion of trait values for related pairs are necessary. The likelihood of a twin pair with latent trait values of θ_1 and θ_2 (Eaves, Martin, Heath, & Kendler, 1987) is

$$l(\mathbf{X}_i, \mathbf{Y}_i) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \Phi(\theta_1, \theta_2) l(\mathbf{X}_i|\theta_1) l(\mathbf{Y}_i|\theta_2) d\theta_2 d\theta_1 \tag{4.28}$$

Where $\mathbf{X}_i$ and $\mathbf{Y}_i$ are the response vectors for each twin and $\Phi(\theta_1, \theta_2)$ is the bivariate frequency distribution of the trait values for the twin pair. The likelihood of all twin pairs in the sample is then assessed iteratively as

$$L = \prod_{i=1}^{N} l(\mathbf{X}_i \mid \mathbf{Y}_i) \tag{4.29}$$

Accurate numerical evaluation of the likelihood as a measure of model fit is computationally prohibitive especially when the number of dimensions is large. The estimation of the parameter values ρ_{MZ} ,$\rho_{DZ,}$ a_g, and b_g that maximize equation 4.9 distinguishes between the evaluation of likelihood for any set of parameter values and maximum likelihood estimates (i.e. obtaining the parameters that maximize the overall likelihood) maximum likelihood (Eaves, Erkanli, Silberg et al., 2005). Thus, the parameter values that are likely to explain a model yields a maximum likelihood estimate for that model, indicating optimal model fit. Generally, the calculation of the maximum likelihood cannot be explicitly solved and requires a computationally expensive trial-and-error numerical estimation approach using different parameter values.

Genetic IRT Using a Bayesian Approach. Recently, Bayesian approaches using Markov Chain Monte Carlo (MCMC) algorithms have provided an alternative approach which is computationally less demanding than maximum likelihood and yields helpful ancillary information (ie: 95% credibility regions) for evaluation. MCMC algorithms have been applied to twin models of GxE and gene-environment correlation; non-linear

developmental change; (Eaves et al., 2003a; Eaves et al., 2003b); estimates of additive genetic, dominance, shared environmental, and unique environmental variance (van den Berg, Beem, & Boomsma, 2006); survival analysis (Do, Broom, Kuhnert et al., 2000); and IRT(Eaves et al., 2005).

The interpretation of a model from the classical maximum likelihood paradigm relies on the conditional probability of observing the data given a particular model. In contrast, the Bayesian paradigm seeks the probability of observing a model given the data. More specifically, the Bayesian approach depends on the *posterior probability*, which is defined as the relative probability of one hypothesis versus another, taking all the available conditional information into account. The posterior probability is made up of the *joint probability* of a hypothesis, which is quantified as the product of the *prior probability* and the *conditional probability* and is defined as the probability of both the prior and conditional probabilities occurring. In the absence of a known distribution, as in the case of the estimation of a latent trait, Bayesian model inference utilizes the posterior *probability density function*, which is the aggregate of the probabilities of possible values for a parameter or set of parameters across all observations given the data. Within the Bayesian framework, observed data and model parameters are undistinguishable making all quantities random (Gilks, Richardson, & Spiegelhalter, 1996).

The distribution for determining of likelihood of a model will be the same as that necessary to calculate the conditional distribution. Therefore, $P(D|\Theta)$ is proportional to the product of model likelihood, $P(\Theta|D)$ (van den Berg et al., 2006).

The joint distribution of data and model parameters using a Bayesian approach is defined as

$$P(D,\Theta) = P(D|\Theta)P(\Theta) \tag{4.30}$$

where D denotes the observed data, $P(D|\Theta)$ is the likelihood conditional on θ, or the distribution that a hypothesis is true given the data and $P(\theta)$ is the prior distribution or the distribution of the model parameters given a hypothesis is true without having any additional knowledge. Since the prior distribution must be established before assuming any extra knowledge, Bayesian model inference will define prior distributions of a model in advance.

The posterior distribution or the distribution that results after taking all information into account is used to determine the distribution of Θ conditional on D and is identified as

$$P(\Theta|D) = \frac{P(\Theta)P(D|\Theta)}{\int P(\Theta)P(D|\Theta)d\Theta} \tag{4.31}$$

Generalizing this definition to any function $f(\theta)$ of interest yields a generic expectation of the posterior distribution as

$$E[f(\Theta)|D] = \frac{\int f(\Theta)P(\Theta)P(D|(\Theta)d\Theta}{\int P(\Theta)P(D|\Theta)d\Theta} \tag{4.32}$$

For high-dimensional distributions, $E[f(\Theta)|D]$ becomes

$$E[f(R)] = \frac{\int f(r)\pi(r)dr}{\int \pi(r)dr} \tag{4.33}$$

where $\pi(r)$ is the distribution of $f(R)$ and is also referred to as the *target distribution*.

The Markov Chain Monte Carlo Algorithm. The challenge of numerical integration of Equation 4.11 is overcome by using an algorithm of (1) the production of parameter distributions for multivariate models using a specific instance of a Markov chain, known as the Gibbs sampler and (2) the estimation of the posterior probability of the model using Monte Carlo integration of the distributions resulting from the Markov chain (Gilks et al., 1996).

One way to estimate integrals is to use the entire distribution and divide the area under a curve into rectangular segments, take the area of each segment and sum these values. Monte Carlo integration instead draws a sample from the posterior distribution and determines the integral and repeats this calculation over several draws of samples from the distribution. The estimates of the samples are then averaged to produce an estimate of the integral for the full distribution. More specifically, Monte Carlo integration draws samples $\{R_t, t = 1,2,3\ldots n\}$ from $\pi(r)$ and approximating

$$E[f(R)] \approx \frac{1}{n}\sum_{i=1}^{n} f(R_t) \qquad (4.34)$$

The value *t* is under the control of the analyst, and also refers to the number of *iterations* used to evaluate the posterior density function. When the samples R_t are independent, large numbers ensure that the approximation can be made as accurate as desired by increasing the sample size *t* by increasing the number of iterations. However, drawing independent samples R_t from a single distribution is not feasible because $\pi(r)$ is often non-standard. Thus, in samples of mental health disorders having

low prevalence, there is a higher probability of producing random samples of non-affected individuals. It is therefore necessary to draw random samples that each has the same proportions of $\pi(r)$ (Gilks et al., 1996).

A Markov chain for a model consisting of a *single* predictor variable produces random samples with a distribution having proportions of $\pi(r)$, which can be used for Monte Carlo integration. This is accomplished by producing a sequence of random variables $\{R_1, R_2, R_3 \ldots R_t\}$ with a conditional probability distribution of the future state R_{t+1} given the present state, R_t. Since the conditional distribution of the Markov chain, $P(R_{t+1}|R_t)$ only depends on the present state, the future state does not depend on the history of the chain. After a sufficient number of iterations, R_t will have a distribution similar to the posterior distribution of $f(R_t)$. The distribution from the Markov chain is then used to produce estimates of $E[f(R)]$ using Monte Carlo integration (Hastings, 1970).

Expanding $E[f(R)]$ to represent a *multivariate* model (2 or more predictor variables), the Markov chain is now produced using a Gibbs sampling algorithm whose goal is to create a sample based on the proportions of $\pi(r)$ for each parameter in the model conditional on the current values of the other parameters in the model. The Gibbs sampler functions by first generating a *proposal distribution*. The proposal distribution for the Gibbs sampler is

$$q_i(Y_i | X_i, X_{-i}) = \pi(Y_i | X_{-i}) \tag{4.35}$$

where $\pi(Y_i|X_{-i})$ is the full conditional distribution of a parameter at the present state given all other parameters in the model at the previous state , *-i*. At each time *t*, the next state X_{t+1} is chosen and the new distribution based on the conditional distribution.

A posterior distribution for each parameter is generated over several iterations of the MCMC algorithm and its corresponding estimates are calculated simultaneously. The mean of the posterior distribution is analogous to the parameter estimate of a model and the 95% credibility region is analogous to the 95% confidence interval. Ultimately, the Bayesian approach provides model values similar to those estimated under a maximum likelihood approach in a more time-efficient manner as a direct result of a decreased number of computations per model. Additionally, the Bayesian approach allows for greater flexibility in the treatment of parameters, because data and model parameters are considered random, thus compensating for some issues in the identification of a model that results from data quality.

Methods

Study Population

This study is based on a sub-sample of 255 male and 285 female same-sex twin pairs between the ages of 12 and 18 and their parents from the Virginia Twin Study of Adolescent and Behavioral Development (VTSABD). The current sub-sample consists of maternal and twin responses for whom twin *MAOA* genotype, wave 3 CD measures and household neglect information were obtained from at least one member of the twin

pair. Wave 3 responses were used to avoid issues of using repeated measures over multiple waves and to focus on an age range where the prevalence of CD is expected to be greatest. The average age of male pairs was 15.6 ± 1.7 years and consisted of 101 MZ and 154 DZ pairs. The average age of female pairs was 15.2 ± 1.7 years and consisted of 141 MZ and 144 DZ pairs.

Items

Measure of Conduct Disorder. Previous 3-month history of wave 3 CD as assessed with the Child and Adolescent Psychiatric Assessment (CAPA) was used (Angold et al., 2000) and included maternal or child self-report. Symptoms measured stealing without confrontation, running away from home, frequent lying, fire-setting, school truancy, breaking into a home or business, destroying property, cruelty to animals, use of weapons, initiating physical fights, stealing with confrontation, and physical cruelty to people. Items had binary responses (0 or 1), reflecting whether or not the individual engaged in a specific activity. Paternal ratings had low response rates and were not included. Table 4.2 summarizes the raw endorsement frequencies of items for males and females. Items that were endorsed by one percent or less of the sample were not included in further analyses.

Table 4.2 Frequency of Item Endorsement for Conduct Disorder Symptoms

	Maternal Report				Child Report			
	Male		Female		Male		Female	
Item	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Stealing without confrontation	10	2.4	13	2.7	30	6.5	32	6.2
Running away from home	0	0*	6	1.2	0	0*	5	1.0*
Often tells lies	31	7.9	26	5.7	64	14.6	74	14.7
Fire-setting	5	1.2	0	0*	9	2.0	7	1.4
Truancy	44	10.7	53	11.2	64	14.0	71	14.0
Breaking into others' property	2	0.5*	0	0*	3	0.7*	1	0.2*
Destroyed others' property	2	0.5*	0	0*	10	2.2	4	0.8*
Cruelty to animals	10	2.5	1	0.2*	43	9.6	10	1.9
Use of weapons	12	3.0	15	3.2	12	2.6	21	4.1
Initiates physical fights	10	2.6	18	4.0	13	2.9	19	3.7
Stealing with confrontation	4	1.0	1	0.2*	7	1.5	1	0.2*
Physical cruelty to people	20	5.1	13	2.8	24	5.3	13	2.5

* This item was not included in further analyses as a result of low endorsement
** Items in bold have been endorsed in both genders for all raters

Measurement of Childhood Adversity. Three measures of negative family environment associated with CD indexed childhood adversity, specifically parental neglect, exposure to inter-parental violence and inconsistent parental discipline. In this sample, females had significantly greater exposure to childhood adversity than males ($\chi^2_{df=2} = 11.65$). Among males, 266 individuals (67.7%) had zero exposures to childhood adversity, 51 (13.0%) had one exposure and 76 (19.3%) had 2 or more exposures. In comparison, 258 females (56.2%) had zero exposures, 87 (19.0%) had one exposure and 114 (24.8%) had 2 or more exposures.

Genotyping of *MAOA*

Primer sequences previously described were used to genotype *MAOA* and classification of *MAOA* activity (high or low) was assigned to each allele resulting from previous work in the efficiency of transcription activity of the *MAOA* gene promoter (Sabol et al., 1998).

Among males, 70.5% had the high activity allele and 29% had the low activity allele. Among females, 39.6% had the high/high *MAOA* genotype, 46.7% had the heterozygous genotype and 13.7% were found to have a low/low genotype.

Implementing the Genetic IRT Model. Model fitting and parameter estimates were evaluated in WinBUGS 1.4.1 (Speigelhalter, Thomas, Best, & Lunn, 2004). WinBUGS addresses the Bayesian model inference through the use of the Markov Chain Monte Carlo (MCMC) algorithm while invoking the Gibbs sampler. The genetic IRT model is implemented in WinBUGS using two general steps, (1) the estimation of the item response parameters, a_g and b_g and (2) the estimation of genetic and environmental contribution to CD using *MAOA* genotype and childhood adversity. Additionally, MZ and DZ twin correlations for CD measured as a latent trait were estimated using the Z-score. The Z-score was sampled from a normal distribution ranging from 0 to 4 and transformed using the r-to-z transformation. Specific genetic and environmental contributions were modeled using the specific effects of *MAOA*, childhood adversity and age. Models were initially run using a 5000 iteration "burn-in"

to achieve posterior probabilities (*Ef(R)*), which appropriately approximate their target distributions ($\pi(r)$). After the 5000 iteration burn-in, models were run for an additional 5000 iterations to produce point estimates for each parameter, or the average value of all sampled values for a parameter.

Testing the Significance of GxE Using a Genetic IRT Approach. Models testing the significance of GxE compared 2 sets of models. The first set tested the significance of GxE in the presence of only genetic and environmental effects. Therefore, the "full" model of this set simulated the main effects of *MAOA* genotype and childhood adversity, their interaction and CD measured as a latent trait. A second, nested model only included the main effects of *MAOA* and childhood adversity.

The second set of models tested the significance of GxE by including age (A) as a covariate to account for the often-reported developmental differences in the etiology of CD (Gelhorn et al., 2005). Models were assessed separately by gender and for maternal and child measures of CD to evaluate how genetic and environmental contributions might differ by rater. All models were compared against a "random effects" model which only included the effects of CD measured as a random trait to determine the extent to which the inclusion of genetic and environmental effects improved model fit over a model without such effects.

The model for the liability Z_{ij} of the j^{th} twin in the i^{th} pair is a function of the random effect on liability θ_{ij}, and the regression on the fixed effects of measured

genotype (g_{ij}), environment (e_{ij}), and age (a_{ij}). The full model for each subject from a twin pair was parameterized as

$$Z_{ij} = \theta_{ij} + \beta_1 g_{ij} + \beta_2 e_{ij} + \beta_3 a_{ij} + \beta_4 (g_{ij} \times e_{ij}) \quad (4.36)$$

The latent trait values (θ_{ij}) were simulated for each twin pair by simulating a trait value, θ_{i1} for the first twin on the assumption that θ_{i1}, is sampled from a normal distribution with a mean of zero and standard deviation of 1 (N[0,1]). The trait value of the second twin, θ_{i2} was sampled from a normal distribution, conditional on the first twin and with a specific residual variance [$r_{\theta i1, ,} \sigma_t^2$]. The term σ_t^2 denotes the variance for the MZ or DZ pair and was estimated as $\sigma^2_{MZ \text{ or } DZ} = 1 - r^2_{MZ \text{ or } DZ}$. The residual pair variance of the second twin conditional on the first was inverted to reflect a measure of precision (τ) for use in WinBUGS and was estimated as $\tau_{MZ \text{ or } DZ} = 1/\sigma^2_{MZ \text{ or } DZ}$.

Model Comparisons. Traditional model comparison assesses significant differences of the likelihood ratio chi-square statistic between nested models having differing numbers of parameters. The likelihood ratio statistic quantifies differences in model fit for "full" or more general models with nested or restricted models. Typically, model complexity is measured by using Akaike's Information Criterion (AIC) by evaluating the number of model parameters and goodness of fit simultaneously to provide a measure of parsimony that can be compared between models of varying complexity.

Model comparison under the Bayesian approach utilizes measures similar to those in the traditional framework. The overall model fit penalized for lack of

parsimony is measured using the Deviance Information Criterion (DIC) (Speigelhalter, Best, Carlin, & van der Linde, 2002). The DIC is used like the AIC in that it seeks to provide a comparison of models of varying complexity. Thus like AIC, lower values of DIC indicate model improved fit and parsimony. The DIC is calculated as $DIC = \overline{D} + pD$ and produced by the average deviance ($\overline{D}$), the deviance produced at the parameter averages over multiple iterations ($\widehat{D}$), and a measure of model complexity (pD). pD is defined as $pD = \overline{D} - \widehat{D}$, and is the complexity measure for the effective number of parameters in a model. pD yields an estimate of the number of parameters in a model. However, in this application the number of parameters also includes estimates of θ_{ij} for each individual. Therefore, pD will be large and reflect the parameters of the model as well as the individual measures of θ_{ij} (Speigelhalter et al., 2002).

Results

Female Genetic IRT

Figures 4.3-4.6 illustrate the performance of the MCMC algorithm using the simulation histories and autocorrelations for the item difficulty (a_1) and discrimination parameters (b_1) for the item reflecting "stealing without confrontation". Figure 4.3 and 4.4 illustrate the algorithm history of a_1 and b_1 for the last 5000 iterations. The simulation history is a trace of the parameter values sampled to produce the parameter estimate value obtained across iterations for each parameter.

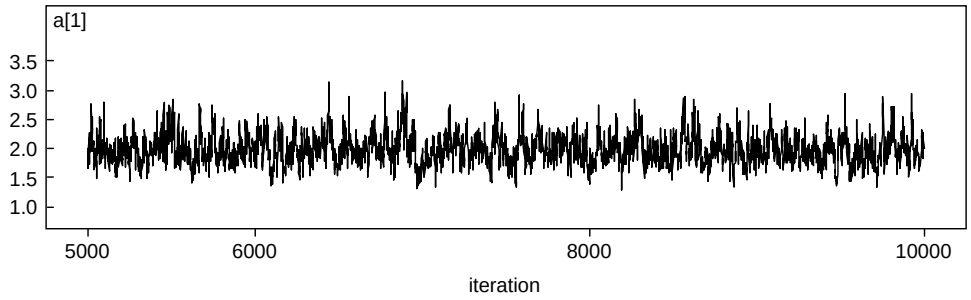


Figure 4.3 MCMC History of Sampled Values for Item Difficulty (a_1)

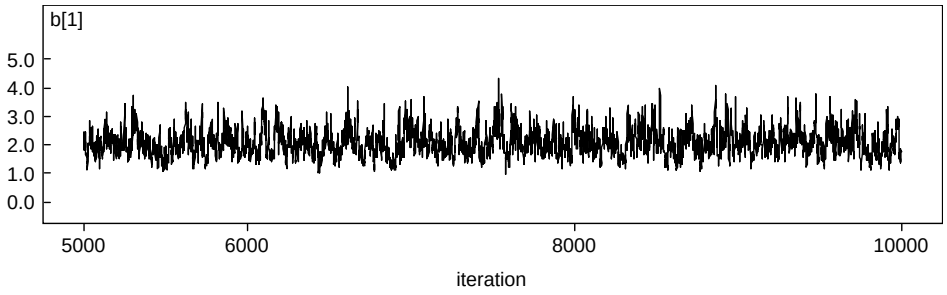


Figure 4.4 MCMC History of Sampled Values for Item Discrimination (b_1)

Figures 4.5 and 4.6 provide a visualization of the autocorrelations of a Markov chain for a_1 and b_1, which indicates how well the proposal distribution from the Gibbs sampler approximates the target distribution $\pi(r)$. When the proposal distribution approximates the target distribution, the two are said to have "converged". Further, the proposal distribution is considered to produce reliable estimates when the chain "mixes" rapidly around the target distribution. Large autocorrelations that decay slowly as a

function of lag suggest poor mixing of the MCMC algorithm which may indicate a high degree of co-linearity between parameters or lack of identification of the model. For the illustrated parameters, the autocorrelations decay quickly and are near zero, suggesting that the posterior distributions for the parameters provide reliable estimates.

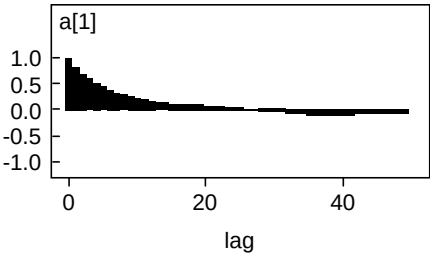


Figure 4.5 Autocorrelation of Item Difficulty (a_1)

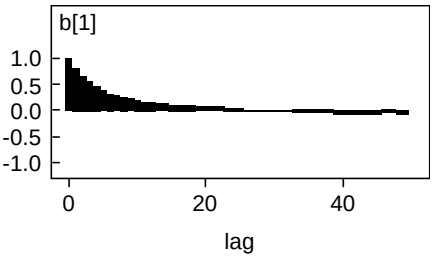


Figure 4.6 Autocorrelation of Item Discrimination (b_1)

Figure 4.7 provides graphic illustrations of the measurement properties for selected CD items according to the liability score distribution in females. Three items that had responses for both genders and across raters ("Often tells lies", "Truancy" and "Initiates physical fights") are highlighted. The liability ranges from -2 to 6, and is

assessed for a distribution N[0,1], with a score of 0 indicating “average” risk in the sample. Most items discriminate best at the upper tail of the distribution of liability.

Table 4.3 summarizes the parameter estimates and the 95% confidence region for each item as measured by maternal report. Table 4.4 summarizes the parameter estimates and 95% confidence region by child report. Figures 4.8 and 4.9 provide the kernel density plots based on 5,001 sampled values from the posterior distribution of the item “Stealing without confrontation” and were used to estimate the item parameters and the 95 % confidence region each parameter.

The item characteristic curves as well as the tables indicate some reporter differences for the items. Specifically, the items measured by child report generally have lower values of item discrimination and difficulty than maternal report. This difference may reflect the lag between children engaging in activities and parental knowledge of such behavior. Similarly, the higher values of item difficulty in the maternal report reflect parents respond to more extreme behavior than that reported by the child.

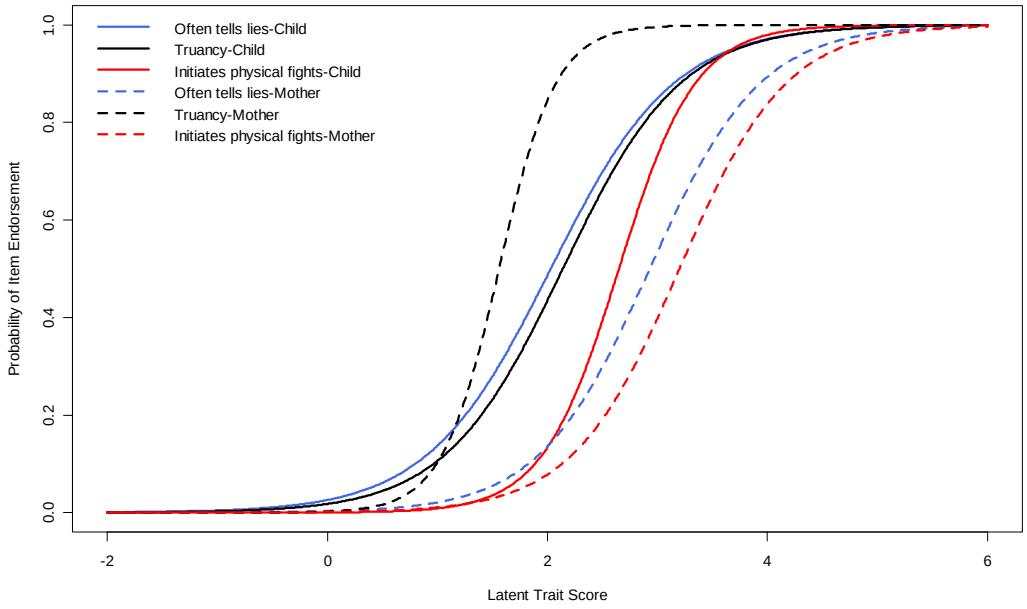


Figure 4.7 Item Characteristic Curves of Select Conduct Disorder Symptoms in Females

Table 4.3 Item Response Theory Parameter Estimates of Maternal Measures of Conduct Disorder Symptoms in Females

Item	Difficulty	2.5%	97.5%	Discrimination	2.5%	97.5%
Stealing without confrontation	**2.98**	**2.28**	**3.95**	**1.56**	**1.00**	**2.33**
Running away from home	2.69	2.18	3.44	2.63	1.60	3.93
Often tells lies	**2.93**	**2.14**	**3.98**	**1.17**	**0.73**	**1.76**
Truancy	**1.56**	**1.17**	**2.10**	**2.27**	**1.29**	**3.53**
Use of weapons	**2.96**	**2.17**	**4.00**	**1.44**	**0.89**	**2.29**
Initiates physical fights	**3.20**	**2.37**	**4.31**	**1.21**	**0.79**	**1.82**
Physical cruelty to people	**2.72**	**2.10**	**3.59**	**1.82**	**1.12**	**2.76**

**Items in bold have been endorsed in both genders for all raters

Table 4.4 Item Response Theory Parameter Estimates of Child Measures of Conduct Disorder Symptoms in Females

Item	Difficulty	2.5%	97.5%	Discrimination	2.5%	97.5%
Stealing without confrontation	**2.16**	**1.66**	**2.89**	**1.93**	**1.14**	**2.95**
Often tells lies	**2.03**	**1.42**	**2.84**	**1.05**	**0.66**	**1.55**
Fire-setting	3.04	2.35	4.02	1.92	1.19	2.87
Truancy	**2.14**	**1.52**	**3.06**	**1.10**	**0.67**	**1.67**
Cruelty to animals	3.16	2.40	4.19	1.55	1.00	2.32
Use of weapons	**2.46**	**1.93**	**3.22**	**1.86**	**1.15**	**2.88**
Initiates physical fights	**2.65**	**2.03**	**3.53**	**1.69**	**1.05**	**2.57**
Physical cruelty to people	**2.43**	**1.96**	**3.07**	**2.39**	**1.49**	**3.56**

** Items in bold have been endorsed in both genders for all raters

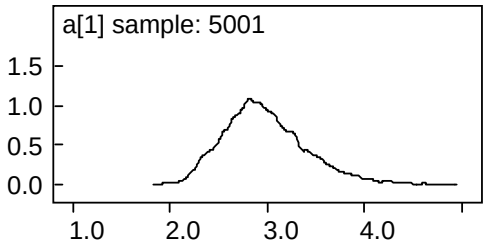


Figure 4.8 Kernel Density Plot of Item Difficulty (a_1)

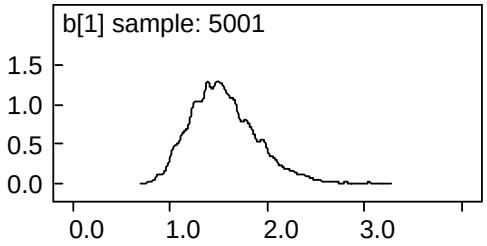


Figure 4.9 Kernel Density Plot of Item Discrimination (b_1)

Female Twin Correlations

The twin correlations from the maternal ratings of CD for female twins were estimated to be 0.86 for MZ pairs and 0.67 for DZ pairs. Similarly, estimates of twin correlations using item parameters from child ratings were $r_{MZ} = 0.64$ and $r_{DZ} = 0.50$, indicating the presence of additive genetic and shared environmental effects.

Male Genetic IRT

Figure 4.10 provides graphic illustrations of the measurement properties of CD items according to the liability score distribution in males. The items "Often tells lies" and "Truancy" have lower maternal item parameters than those of the child ratings. Thus, mothers have a higher probability of endorsing certain items at lower levels of CD over their male children. This trend is also reflected in Tables 4.5 and 4.6 for other items such as "Fire-setting" and "Physical cruelty to people". In comparison, other items in tables 4.5 and 4.6 such as "Stealing with confrontation", "Stealing without confrontation", "Use of weapons", and "Cruelty to animals" have lower levels of item difficulty in child report than maternal report.

There are two explanations for the relative inconsistency in trends for maternal and child measures. The first explanation is simply that male children are poor informants. However, the prevalence of item endorsements between maternal and child rating do not differ greatly and when differences occur, the prevalence of an item is often higher in the child rating (Table 4.2). A second explanation is that certain items, particularly those which have lower item parameters in the maternal measures, do not reflect the same severity for CD between maternal and child raters and do not discriminate well across the latent trait of CD using child reports. Maternal ratings of CD might be perceived to be "more reliable" because they reflect the endorsement of a behavior when it is brought to the attention of the mother (ie: child caught telling a lie, or school calling to follow-up on truancy) rather than through direct observation. However, male children and their mothers do not agree on what certain items mean as

evidenced by the fairly consistent item discrimination values in mothers and rather inconsistent values in children.

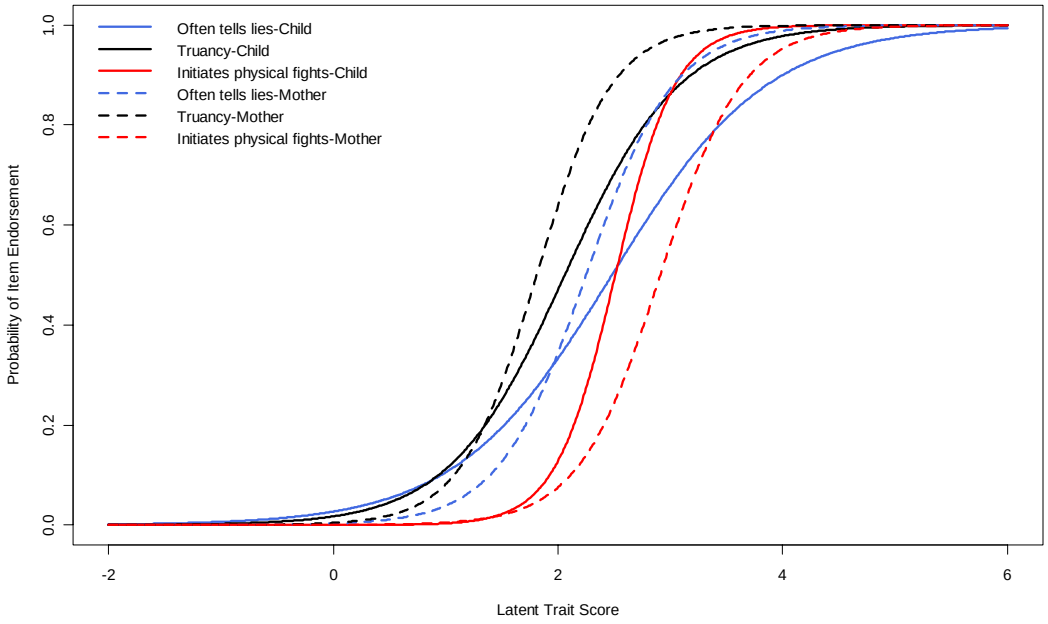


Figure 4.10 Item Characteristic Curves of Select Conduct Disorder Symptoms in Males

Table 4.5 Item Response Theory Parameter Estimates of Maternal Measures of Conduct Disorder Symptoms in Males

Item	Difficulty	2.5%	97.5%	Discrimination	2.5%	97.5%
Stealing without confrontation	**2.47**	**1.96**	**3.18**	**2.34**	**1.45**	**3.53**
Often tells lies	**2.25**	**1.66**	**3.12**	**1.51**	**0.92**	**2.35**
Fire-setting	2.63	2.12	3.31	2.81	1.72	4.12
Truancy	**1.81**	**1.38**	**2.49**	**1.76**	**1.07**	**2.69**
Cruelty to animals	3.19	2.37	4.22	1.39	0.89	2.09
Use of weapons	**2.75**	**2.12**	**3.70**	**1.84**	**1.10**	**2.83**
Initiates physical fights	**2.91**	**2.18**	**3.91**	**1.62**	**1.00**	**2.46**
Stealing with confrontation	3.08	2.38	4.09	2.04	1.25	3.10
Physical cruelty to people	**2.37**	**1.84**	**3.14**	**1.74**	**1.09**	**2.61**

**Items in bold have been endorsed in both genders for all raters

Table 4.6 Item Response Theory Parameter Estimates of Child Measures of Conduct Disorder Symptoms in Males

Item	Difficulty	2.5%	97.5%	Discrimination	2.5%	97.5%
Stealing without confrontation	**2.01**	**1.60**	**2.56**	**2.19**	**1.38**	**3.22**
Often tells lies	**2.48**	**1.69**	**3.61**	**0.85**	**0.52**	**1.29**
Fire-setting	2.96	2.30	3.93	1.96	1.21	2.99
Truancy	**2.06**	**1.43**	**3.02**	**1.15**	**0.67**	**1.80**
Destroyed others' property	2.73	2.14	3.54	2.19	1.36	3.33
Cruelty to animals	2.75	1.93	3.83	0.98	0.62	1.48
Use of weapons	**2.45**	**1.92**	**3.23**	**2.16**	**1.31**	**3.25**
Initiates physical fights	**2.51**	**1.98**	**3.30**	**2.21**	**1.34**	**3.35**
Stealing with confrontation	2.78	2.20	3.60	2.62	1.60	3.91
Physical cruelty to people	**2.49**	**1.92**	**3.35**	**1.59**	**0.99**	**2.33**

**Items in bold have been endorsed in both genders for all raters

Male Twin Correlations

The male twin correlations using the maternal ratings of CD for female twins were estimated to be 0.78 for MZ pairs and 0.44 for DZ pairs, indicating the contribution of additive genetic effects. Twin correlations using item parameters from child ratings were $r_{MZ} = 0.35$ and $r_{DZ} = 0.41$ and highlights a large environmental effect for male child ratings.

The Detection of GxE in Females

Table 4.7 summarizes the model comparisons using maternal and child measures to assess whether the inclusion of GxE is appropriate for risk of CD in females. For both maternal (DIC = 936.85) and child (DIC = 1359.07) raters, a model including the effects of *MAOA*, childhood adversity, age, and the interaction between *MAOA* and childhood adversity (model 5) most appropriately defines risk for CD although differences are marginal at best. This implies little supoort for an effect of the *MAOA* genotype either by itself as a "main effect" or in combination with environmental adversity as GxE.

Table 4.8 provides the point estimates of each parameter for the model identified as most appropriate for predicting CD by rater in females. The age parameter from the model produced by maternal report is significant ($\beta = 0.16$, 95% CR = 0.04-0.27), confirming risk for CD increases over time in females. The childhood adversity parameter was significant in the female model using child reports of CD ($\beta = 0.18$, 95%

CR = 0.01-0.36). The low activity *MAOA* genotype and a negative value GxE were weak, with the 95% confidence regions straddling zero.

Table 4.7 Summary of Model Comparisons of Contributions from *MAOA* and Childhood Adversity in Females by Rater

			Maternal Measure				Child Measure			
Model		**Parameters**	$\bar{D}$	$\hat{D}$	**pD**	**DIC**	$\bar{D}$	$\hat{D}$	**pD**	**DIC**
1	Random Effects	0	847.91	736.88	111.03	958.93	1223.97	1082.49	141.48	1365.45
2	G+E	2	852.73	750.17	102.56	955.30	1231.24	1099.88	131.36	1362.60
3	G+E+(G*E)	3	853.52	752.22	101.30	954.82	1230.73	1098.23	132.50	1363.23
4	G+E+A	3	842.21	735.51	106.70	948.91	1229.30	1098.08	131.21	1360.51
5	G+E+A+(G*E)	4	839.76	732.68	107.08	946.85*	1228.56	1098.04	130.52	1359.07*

*Best Fitting Model as Measured by DIC

Table 4.8 Parameter Estimates and 95% Confidence Region for Models Predicting Risk for CD in Females by Rater

	Maternal Rating			**Child Rating**		
Parameter	**Estimate**	**2.5%**	**97.5%**	**Estimate**	**2.5%**	**97.5%**
Age	0.16	0.04	0.27	0.09	-0.01	0.20
Childhood Adversity	0.16	-0.02	0.35	0.18	0.01	0.36
MAOA	0.14	-0.20	0.48	0.20	-0.09	0.51
GxE	-0.10	-0.38	0.17	-0.09	-0.33	0.15

The Detection of GxE in Males

Table 4.9 summarizes the model comparisons using maternal and child measures to assess whether the inclusion of GxE is appropriate for risk of CD in males. The best model with the lowest DIC value reflects the model that was determined to best predict risk for CD. For maternal reports, a model with the main effects of *MAOA* and childhood adversity (model 2) was determined to best predict risk for CD (DIC = 945.30). Model 3, which includes GxE, has a DIC value that is very similar to model 2 (DIC = 945.91). The most appropriate model of risk using child ratings of CD includes the main effects of *MAOA*, childhood adversity and age as well as the interaction between *MAOA* and childhood adversity (model 5, DIC = 1398.70), although the reduction on DIC is not compelling.

Table 4.10 provides the point estimates of each parameter for models identified as most appropriate for predicting CD by rater in males. The parameter estimates demonstrated weak effects for maternal and child measures as indicated by the 95% credibility region straddling zero. The negative value of the interaction suggests that risk for CD is higher among individuals with the high activity allele as exposure to childhood adversity increases. Likewise, risk for individuals with the low activity allele is greater at levels of low childhood adversity. The parameter estimate of *MAOA* using the maternal measure of CD is negative, while that of the child measure is positive. This discrepancy in the determination of the susceptibility allele between raters likely reflects the rater differences highlighted in the IRT. However, these models should be interpreted with caution since no single models offers large differences in DIC.

Table 4.9 Summary of Model Comparisons of Contributions from *MAOA* and Childhood Adversity in Males by Rater

			Maternal Measure				Child Measure			
Model		**Parameters**	$\overline{D}$	$\hat{D}$	**pD**	**DIC**	$\overline{D}$	$\hat{D}$	**pD**	**DIC**
1	Random Effects	0	833.27	718.83	114.44	947.71	1268.65	1132.33	136.32	1404.97
2	G+E	2	838.87	732.45	106.42	945.30*	1272.18	1144.84	127.35	1399.53
3	G+E+(G*E)	3	839.74	733.58	106.16	945.91	1272.16	1143.68	128.48	1400.64
4	G+E+A	3	842.04	736.89	105.15	947.19	1271.83	1143.51	128.32	1400.14
5	G+E+A+(G*E)	4	842.75	737.53	105.22	947.97	1272.02	1145.34	126.68	1398.70*

*Best Fitting Model as Measured by DIC

Table 4.10 Parameter Estimates and 95% Confidence Regions for Models Predicting Risk for CD in Males by Rater

	Maternal Rating			Child Rating		
Parameter	**Estimate**	**2.5%**	**97.5%**	**Estimate**	**2.5%**	**97.5%**
Age				0.02	-0.08	0.13
Childhood Adversity	0.04	-0.16	0.23	0.12	-0.10	0.32
MAOA	-0.05	-0.25	0.14	0.09	-0.13	0.32
GxE				-0.12	-0.33	0.09

Discussion

The recent reports of significant GxE between MAOA and childhood adversity for conduct disorder have been subject to criticism as a result of the treatment of measurement scale as well as environmental exposure (Eaves, 2006). The consequence of these treatments may result in the false detection of GxE. It is imperative to address these issues if GxE is expected to improve insight into our understanding of how genetic and environmental risk factors function to increase risk for psychopathology. This study tested for the presence of GxE using an MCMC approach that included a genetic IRT model to evaluate CD as a trait with continuous liability as well as the treatment of childhood adversity as a random effect.

Inclusion of GxE is Appropriate in Defining Risk for Conduct Disorder

Among females, models that included GxE in the presence of the main effects of *MAOA*, childhood adversity and age were most appropriate in predicting risk for CD using both maternal and child ratings of CD. There is less agreement between raters regarding the inclusion of GxE in males. Models of risk based on child reports of CD indicated that the inclusion of GxE as a predictor of risk for CD is justified, while maternal reports do not. This discrepancy is anticipated to result from rater differences. Heterogeneity between raters has been reported for this measure (Hewitt et al., 1997) and require appropriate investigation. Although a model that formally addresses the impact of rater effects on the latent trait are beyond the scope of this analysis, it is not beyond the scope of the MCMC method.

Despite model improvement by including GxE in the majority of models, the estimates of GxE and most of the corresponding main effects are weak. Breeding studies suggest that although GxE is widespread, it does not account for a large proportion of total variance of a trait. These results echo this sentiment, by suggesting that while the inclusion of GxE is important for defining risk for CD its associated effect is not overwhelming. Further, these results temper the enthusiasm for the degree to which detection of GxE will result in significant associations for candidate genes or for predicting risk of psychopathology, while providing practical insight into the interplay of genes and the environment. The detection of GxE with weak effect also suggests that the variables used to identify this specific interaction do not translate into significant risk. These results thus invite further study of alternative environments and genotypes to determine whether the definition of GxE can be optimized.

The Interpretation of GxE in Understanding Risk for Conduct Disorder

When GxE was detected in males, the direction of the interaction was negative, suggesting that risk for CD increases among males with the low activity *MAOA* allele at low levels of exposure to childhood adversity. This is opposite of what has been reported in the literature, where risk for CD increases in males with the low activity allele at high levels of exposure to childhood adversity. Further, the susceptibility allele differs between models resulting from child and maternal reports of CD. This difference in the direction of GxE compared with previous reports may be a consequence of addressing the issue of scale by measuring liability for CD rather than

diagnosis of CD or symptom count. In the model derived from the maternal measure of CD, risk was associated with the high activity allele as determined by the negative value of the parameter estimate. In contrast, risk for CD was associated with the low activity allele in the child measure. Alternatively, the inconsistency in the interpretation of GxE as well as the importance of the effects of genes and environment in males may result from rater differences in the measurement of CD. This discrepancy between raters did not occur in females and their point estimates are similar to those obtained using a maximum likelihood approach (see Chapter 2).

The Genetic Item Response Approach Identifies Rater Differences in the Measurement of Conduct Disorder by Gender

The female rater differences highlighted the decreased ability for parental observation of symptoms which are covert in nature. In general, the values associated with item discrimination for CD symptoms are generally similar between mothers and their daughters. For those items where they are not similar (ie: “Often tells lies”, “Truancy”), the item discrimination values are lower for child reports. The difference in item discrimination suggests that children consider these symptoms to reflect less severity than their parents. The lower item discrimination values for these two items as well two others (“Physical cruelty to others” and “Fire-setting”) were also observed for males using the child reports. However, the item difficulty parameters were actually lower in the maternal reports.

If the item difficulty value for maternal reports indicates maternal knowledge of a behavior having occurred, then maternal item difficulty also reflects the child getting caught for committing an act. Among females, the higher item difficulty values in females using maternal ratings compared to child ratings suggests that the child probably engaged in the behavior for a period of time without maternal knowledge. For the previous four items in males, mothers may know about these behaviors soon after they occur and males may simply get caught for these behaviors more often than females. However, the lower maternal values for item difficulty are not consistent across all symptoms of CD and for other items the values for item difficulty using child ratings are lower.

The variation of item difficulty in male item parameters using the child report provides one explanation in understanding rater differences and the consequent discrepancy in estimates for genetic and environmental effects as well as GxE interaction. Since it is reasonable to consider GxE in risk for CD, it may be worthwhile to study CD separately by informant to maximize on those risk factors for which they may provide more information. For example, the male child report could be used for the detection of environmental risk factors, while the maternal report could provide better insight into genetic effects. Similarly, the development of antisocial behavior may be better addressed using female data since there was a significant effect of age in females and the item parameters for CD symptoms show general differences in the detection of behaviors via item difficulty between maternal and child informants.

The results of this study should be interpreted while considering the following limitations. First, childhood adversity is measured as a scale score using arbitrary items from different measurement scales and may not appropriately assess environmental risk. Further, the issue of scale may still be present by using this measurement. However, the treatment of childhood adversity within the Bayesian framework is expected to attenuate any effect of scale by sampling from a normal distribution. Second, the effects of gene-environment correlation (rGE) were not included. While this work sought to detect and describe GxE, inclusion of rGE would provide a more complete understanding of genetic and environmental contributions to risk for psychopathology. Third, while gender differences have been highlighted, their effect sizes have not been specifically tested. A model of risk including gender differences is required and can be included in the MCMC framework. The current results thus serve to describe trends by gender rather than providing substantive gender differences.

CHAPTER 5 Additive and Epistatic Effects in Serotonin and Dopamine Models of Risk for Conduct Disorder and Attention Deficit Hyperactivity Disorder

Abstract

A sub-sample of 555 male and 683 female individual participants from the Virginia Twin Study of Adolescent Behavioral Development (VTSABD) were used to assess the presence of main genetic effects and genotype-genotype interaction (epistasis) for conduct disorder (CD) and attention deficit hyperactivity disorder (ADHD) using *MAOA*, *5HTTLPR* and *DAT1* genotypes to model serotonin and dopamine neurotransmitter systems.

Among females, there was a main effect of *MAOA* on CD diagnosis while controlling for either *5HTTLPR* or *DAT1* genotypes. However, this main effect on CD diagnosis was no longer significant after controlling for ADHD. In males, a significant main effect of the 9/9 DAT1 genotype for both ADHD and CD was detected. However, after controlling for comorbidity, the 9-repeat *DAT1* allele was only a significant risk factor for CD diagnosis. Comorbid illness may be genetically different from ADHD or CD alone.

There was no significant genotype-genotype interaction for ADHD or CD. The general lack of epistasis in these models is not surprising, since its detection requires large sample sizes or genes of large main effects or results when the effect of a gene in a system is lost as in knockout mouse studies. Estimates for detecting significant

epistasis resulted in prohibitively large sample sizes for human population studies and reinforces the need to incorporate other model systems or modeling approaches to address epistasis in the etiology of CD and ADHD in humans.

Introduction

Conduct disorder (CD) and attention deficit hyperactivity disorder (ADHD) are commonly co-occurring disorders (Acosta, Arcos-Burgos, & Muenke, 2004; Simonoff et al., 1997) that have been separately associated with genes of the serotonin and dopamine systems (Brookes, Xu, Chen et al., 2006; D'Souza & Craig, 2006; Murphy, Uhl, Holmes et al., 2003). However, the pathway from genotype to behavioral phenotypes remains unclear. The genes of the serotonin and dopamine systems are of particular interest in the study of behavior because they have been associated with aggression, impulsivity and hyperactivity in animal and human studies (Brookes et al., 2006; Brunner et al., 1993; Cases et al., 1995; Gainetdinov & Caron, 2003; Gaintedinov, Wetsel, Jones et al., 1999; Rodriguiz, Chu, Caron, & Wetsel, 2004; Winstanley, Theobald, Dalley, & Robbins, 2005). Modeling biologically meaningful neurotransmitter systems as main genetic and epistatic (genotype-genotype interaction) effects using current knowledge of differences in gene expression resulting from allelic variation at 3 susceptibility loci would provide insight into (1) whether genetic effects determine risk for CD using measured genotypes, (2) how polymorphisms modify a neurotransmitter system when assessed together and (3) whether any one gene product is more important over another in the function of the neurotransmitter system.

The Roles of the Serotonin and Dopamine Systems as Risk Factors for Conduct Disorder and Attention Deficit Hyperactivity Disorder

ADHD and CD as Comorbid Disorders. Attention Deficit Hyperactivity Disorder is characterized as a constellation of impulsive, inattentive and hyperactive behaviors often observed as fidgety and restless behaviors, such as having difficulty sitting still or having trouble maintaining focus on a particular task. Children with ADHD are often diagnosed with oppositional defiant disorder in childhood, defined as disruptive behaviors including engaging in arguments with adults, angry/intentionally annoying behavior and loss of temper. These individuals are at increased risk of being diagnosed with CD during adolescence (Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Lahey, McBurnett, & Loeber, 2000). Often, the behaviors associated with ADHD make affected individuals more likely to have learning difficulty, be disciplined in school through suspension or expulsion, be rejected by peers, and sustain physical injuries (Hinshaw, 2002).

Attention Deficit Hyperactivity Disorder and Conduct Disorder often co-occur (Simonoff et al., 1997), with an estimated 20% of children diagnosed with ADHD also having CD (Acosta et al., 2004). Additionally, in both males and females, children with ADHD are 2-4 times more likely to have a concurrent CD diagnosis (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). Like CD, ADHD occurs more often in males than females and is a risk factor for adult antisocial personality disorder, though females are

more likely to be comorbid for both disorders than males (Costello et al., 2003; Maughan et al., 2004).

Genes of the Serotonin and Dopamine Systems and their Relationships with ADHD and CD

Twin studies have highlighted substantial shared genetic risk between CD and ADHD as well as trait-specific genetic effects reported as a set of (anonymous) genes common to both disorders as well as a set of genes specific to each in the presence of shared/specific environments (Dick et al., 2005; Nadder, Rutter, Silberg, Maes, & Eaves, 2002; Silberg, Rutter, Meyer et al., 1996).

Studies of CD and ADHD as separate disorders have reported the roles of specific genes within several systems, particularly the serotonin transporter and monoamine oxidase-A in the serotonin system and the dopamine transporter in the dopamine system. The serotonin system plays an important role in the regulation of mood and affect cognition, satiety, and various autonomic functions when responding to stress. The dopamine system is involved in the reward and reinforcement pathways of behavior (Blum, Sheridan, Wood et al., 1996).

The Serotonin and Dopamine System Pathways. After release from the post-synaptic terminal, serotonin (5-HT) is transported from the synaptic cleft (extracellular) to the inside of the presynaptic terminal (intracellular) by the serotonin transporter (SERT). Monamine oxidase A (MAOA) removes an amine group from serotonin (5-

hydroxytryptamine, 5-HT) to produce 5-hydroxyindolacetaldehyde. Aldehyde dehydrogenase (ALDH2) and aldehyde oxidase (AOX) metabolize 5-hydroxyindolacetaldehyde to 5-hydroxyindolacetic acid (5-HIAA). 5-hydroxyindolacetaldehyde is unstable and uses both AOX and ALDH2 to form the carboxylic acid, 5-HIAA. Once 5-HIAA is produced it is moved into the cerebrospinal fluid by active transport in the choroids plexus by an H+/ATPase pump (Miyamoto, Uezu, Jiang, & Miyamoto, 1993).

After dopamine is released from the post-synaptic terminal, it is transported from the synaptic cleft into the presynaptic terminal by the dopamine transporter. Dopamine is then metabolized by either one of two pathways to eventually yield the metabolite homovanillate. The first pathway uses catechol O-methyltransferase (COMT) to move a methyl group from dopamine to produce 3-Methoxytyramine. Then, MAOA catalyzes the oxidation of an amine group in 3-Methoxytyramine to produce 3-Methoxy-4-hydroxy-phenylacetaldehyde. Afterwards, aldehyde dehydrogenase (ALDH) removes hydrogen to produce homovanillate.

The second pathway uses MAOA to produce 3,4-dihydroxyphenylacetaldehyde. Then aldehyde dehydrogenase and aryl-aldehyde dehydrogenase produce the unstable acid, 3,4-dihydroxyphenylacetate. Finally, COMT moves a methyl group to produce homovanillate. This metabolite is then moved into the cerebrospinal fluid and has been used to measure dopamine metabolism.

There are many proteins involved in the serotonergic and dopaminergic systems. However, the serotonin reuptake transporter, the dopamine transporter and monoamine oxidase-A have received particular interest in the study of ADHD and CD.

Monamine Oxidase-A. Monoamine oxidase-A (MAOA, EC 1.4.3.4) is responsible for the degradation of biogenic amines including the neurotransmitters epinephrine, norepinephrine, dopamine, and serotonin via deamination. *MAOA* is localized to Xp11.4-Xp11.3. A nonsense mutation in exon 8 (Gln296Stop) causes the truncation of the protein at codon 296, resulting in the loss of MAOA activity (Brunner et al., 1993). Males with the exon 8 mutation have engaged in impulsive/aggressive behaviors including rape, arson, and assault (Brunner et al., 1993). A mutation in transgenic mice results in the deletion of exons 2 and 3, resulting in a non-functioning enzyme that is associated with increased aggressiveness and injury among male mice and their cage-mates (Cases et al., 1995). The promoter region contains a variable number tandem repeat (VNTR) polymorphism with suggested effects on transcription level. Studies have reported low transcription activity for the 3- and 5-repeat elements while the 3.5- and 4- repeats had high transcription activity (Denney et al., 1999; Sabol et al., 1998), although these alleles do not confer differences in protein levels (Balciuniene et al., 2002).

Studies have reported a weak association between the low-activity *MAOA* alleles of the promoter region and CD/antisocial behavior. Samochowiec et al (1999) found a significantly greater frequency of low-activity *MAOA* alleles among antisocial

alcoholics compared with control participants and no significant differences among non-antisocial alcoholics and controls. On the other hand, Manuck et al (2000) found a decrease in aggression and impulsivity for males with the low-activity allele. Additionally, the low-activity allele has been associated with antisocial personality disorder and CD among males in adverse environments (Caspi et al., 2002; Foley et al., 2004; Kim-Cohen et al., 2006; Nilsson et al., 2005) though non-replications for the aforementioned gene-environment interaction are increasing (Haberstick et al., 2005; Young et al., 2006). The *MAOA* promoter polymorphism has been associated with increased risk for ADHD. In one study, the 4-repeat allele was reported to have increased maternal transmission among cases in a transmission disequilbrium test (Manor, Tyano, Mel et al., 2002). However, two other studies identified an association between the 3-allele and ADHD (Lawson, Turic, Langley et al., 2003; Domschke, Sheehan, Lowe et al., 2005).

Serotonin Transporter. The serotonin transporter (5-HTT or SERT) is responsible for the presynaptic transport of 5-HT from the synaptic cleft to the inside of the presynaptic terminal after release from receptors on the post-synaptic terminal. The gene encoding this protein, *SLC6A4* consists of 14 exons and is localized to chromosome 17q11.1-q12. The promoter region of the gene contains a polymorphic VNTR (*5HTTLPR*) with a repeat element consisting of 20-23 base pairs. The *5HTTLPR* polymorphism is a deletion of 44 base pairs between repeat units 6-8 that confers reduced transcription of of *SLC6A4* (Heils, Teufel, Petri et al., 1996). The most

frequently observed alleles are the short allele, consisting of 14 repeat elements and the long allele, containing 16 repeat elements. The short allele has been generally associated with lower SERT mRNA transcriptional efficiency, resulting in lower serotonin reuptake while the long allele has been associated with increased serotonin reuptake (Lesch, Bengel, Heils et al., 1996). Several other alleles have been identified, including the 15-, 18-, 19-, 20-, and 22-repeat (Collier, Stöber, Li et al., 1996; Heils et al., 1996; Lesch et al., 1996; Mortensen, Thomassen, Larsen, & Wiborg, 1999). These alleles have been further examined and reported to have several other sequence variants. The 14-repeat allele is further categorized to include 4 allelic variants (14-A, 14-B, 14-C, and 14-D) while the 16-repeat has 6 variants (16-A, 16-B, 16-C, 16-D, 16-E, and 16-F) (Nakamura, Ueno, Sano, & Tanabe, 2000). Further, these variants have been reported to function as silencers, decreasing transcription of *SLC6A4* in raphe nucleus cells (Sakai, Nakamura, Ueno et al., 2002). The 14-A, 14-B, 16-A, 16-B, 16-C, and 16-D variants had significantly higher levels of silencing, or greatly decreased transcriptional efficiency while the 15, 19, 20, and 22 alleles had low silencing activity or only slightly decreased transcriptional efficiency (Sakai et al., 2002). Despite being categorized as high activity silencer alleles, the most frequently occurring variants, 14-A and 16-A, commonly referred to as the "short" and "long" *5HTTLPR* alleles have been reported to have significantly different transcription activities, with the 16-A variant having increased transcription over the 14-A allele (Mortensen et al., 1999). However, this finding has not been positively replicated (Sakai et al., 2002), and requires further investigation.

The short *5HTTLPR* allele has been associated with human anxiety, depression, and aggression-related personality traits (Bennett, Lesch, Heils et al., 2002). In addition, it has been reported that dysfunction of the serotonin transport mechanisms is associated with specific CD behaviors among children (Stadler, Schmeck, Nowraty, Müller, & Poustka, 2004). Early studies reported significant associations between the short allele and CD as well as ADHD in males (Cadoret, Langbehn, Caspers et al., 2003; Retz, Retz-Junginger, Supprian, Thome, & Rosler, 2004). Recently, one study reported a significant association between the short *5HTTLPR* allele and teacher-reported aggression in a community twin sample of children ages 7-9 (Haberstick, Smolen, & Hewitt, 2006). Another study reported a significant association between the short *5HTTLPR* allele and CD using a case-control design of adolescents between the ages of 13 and 19 in a drug treatment setting (Sakai, Young, Stallings et al., 2006).

In contrast, the long *5HTTLPR* allele is often reported to be associated with ADHD. Among children with hyperkinetic disorder, a designation similar to ADHD, children with CD were more likely to have the long *5HTTLPR* allele compared to controls (Retz, Thome, Blocher, Baader, & Rosler, 2002). Further, a case-control study reported a significant association between the long *5HTTLPR* allele and hyperkinetic disorder in children with and without CD (Seeger, Schloss, & Schmidt, 2001). Additionally, a significant association was reported between aggressive children with ADHD and the long *5HTTLPR* allele (Beitchman, Davidge, Kennedy et al., 2003). One study reported significant associations between the long allele and a combined-type definition (inattention and hyperactive-impulsive) diagnosis of ADHD using family-

based analysis (Manor, Eisenberg, Tyano et al., 2001). Another study found a non-significant trend for an association between the long allele and ADHD (Kent, Doerry, Hardy et al., 2002). However, Langely and colleagues noted no association between *5HTTLPR* and ADHD in either a combined ADHD/CD group or a sub-sample of individuals with only ADHD, using a case-control and transmission disequilibrium test approach (Langley, Payton, Hamshere et al., 2003).

Dopamine Transporter. The dopamine transporter (*DAT1*) is responsible for the transport of dopamine from the synaptic cleft into the presynaptic terminal after release from receptors on the post-synaptic terminal. The gene encoding the dopamine transporter (*DAT1* or *SLC6A3*) resides on 5p15.3 and consists of 15 exons (Kawarai, Kawakami, Yamamura, & Nakamura, 1997; Vandenbergh, Persico, Hawkins et al., 1992). The 3' untranslated region (UTR) contains a VTNR polymorphism ranging from 3-11 copies of a 40-base pair repeat element which is thought to affect (1) *DAT1* regulation and gene expression (Fuke, Suo, Takahashi et al., 2001; Miller & Madras, 2002), (2) dopamine transporter availability (Heinz, Goldman, Jones et al., 2000; Jacobsen, Staley, Zoghbi et al., 2000), or (3) *DAT1* mRNA stability (Greenwood & Kelsoe, 2003) (Mignone, Gissi, Liuni, & Pesole, 2002).

The confusion surrounding the functional significance of this polymorphism results from several conflicting studies. For example, some studies report a general decrease in *DAT1* expression in the presence of either the 9- or 10- allele compared to constructs without these inserts (Mill, Asherson, Craig, & D'Souza, 2005), while

another study reported either allele to enhance transcription (Michelhaugh, Fiskerstrand, Lovejoy, Bannon, & Quinn, 2001). Additionally, some studies have observed increased transcription with the 9-repeat allele (Fuke et al., 2001) while others have observed greater transcription with the 10-repeat allele (Miller et al., 2002) (Mill, Asherson, Brownes, D'Souza, & Craig, 2002). Further, a significant association was reported between the 9-repeat allele and decreased DAT protein availability (Heinz, Saunders, Kolachana et al., 1999), while another study reported such an association with the 10-repeat allele (Jacobsen et al., 2000). Thus, the 3' untranslated region (UTR) polymorphism may play a role in the regulation of *DAT1* expression, though the mechanism by which it occurs is not clear.

Studies of the *DAT1* 3' UTR polymorphism have mainly utilized the 9- and 10-repeat alleles, since they are most frequent (Vandenbergh et al., 1992; Doucette-Stamm, Blakely, Tian, Mockus, & Mao, 1995). Consequently, other alleles, including the 3-, 5-, 7-, 8-, and 11-repeat alleles have been largely unstudied in the molecular literature, making it difficult to include these alleles in *DAT1* candidate gene studies. *DAT1* knockout mouse studies have demonstrated increased rates of reactivity and aggression when exposed to social contact either in group or isolation settings. In addition, these mice display enhanced aggression in the presence of a novel environment, suggesting a lower tolerance for social contact when compared with wild-type controls (Rodriguiz et al., 2004).

The *DAT1* 3' UTR polymorphism has received a great deal of interest in the study of ADHD since the most frequently prescribed medications for ADHD,

methylphenidate and dextroamphetamine, inhibit the dopamine transporter and keep extracellular dopamine in the synaptic cleft for a longer period of time (Amara & Kuhar, 1993). Studies of the *DAT1* 3'UTR polymorphism have investigated whether the polymorphism plays a role in mediating individual differences of dopamine transmission via transporter reuptake. There have been several reports of an association between the 10-repeat allele and ADHD (Barr, Xu, Kroft et al., 2001; Chen, Chen, Mill et al., 2003; Cook, Jr., Stein, Krasowski et al., 1995; Curran, Mill, Tahir et al., 2001; Daly, Hawi, Fitzgerald, & Gill, 1999; Gill, Daly, Heron, Hawi, & Fitzgerald, 1997; Waldman, Rowe, Abramowitz et al., 1998a) (Swanson, Flodman, Kennedy et al., 2000). Recently, a large-scale association study of single nucleotide polymorphisms (SNP) in the 3'UTR produced a non-significant overtransmission of these alleles from parents to their affected offspring (Brookes et al., 2006). However, several non-replications of this association have been reported as well (Holmes, Payton, Barrett et al., 2000; Palmer, Bailey, Ramsey et al., 1999; Todd, Jong, Lobos et al., 2001) (Bakker, van der Meulen, Oteman et al., 2005). Additionally, a meta-analysis resulted in a non-significant pooled odds ratio estimate, suggesting no significant relationship between *DAT1* and ADHD (Maher, Marazita, Ferrrell, & Vanyukov, 2002).

There has also been increasing interest around the relationship between *DAT1* and CD and externalizing behaviors, with varying results. The 9-repeat allele has been associated with externalizing behaviors in children ages 4 and 7. Externalizing behaviors are defined as aggressive, destructive, oppositional, impulsive and delinquent behavior, which have been implicated in later development of more serious

psychopathology and is considered an effective screen for clinical diagnoses of CD (Young et al., 2002). Further, Barkley and colleagues reported a relationship between behavioral and neuropsychological measures of ADHD and externalizing behaviors with the 9/10 genotype in an ADHD case-control study (Barkley, Smith, Fischer, & Navia, 2006). In contrast, another study reported no significant associations between externalizing behaviors, CD or ADHD and *DAT1* in a longitudinal population-based study of children ages 4 months to 16 years (Jorm, Prior, Sanson et al., 2001). Further, Rowe and colleagues found no significant association between *DAT1* and parental self-reports of lifetime CD in a clinic population of children receiving treatment for ADHD (Rowe, Stever, Chase et al., 2001).

Interaction of the Serotonin and Dopamine Neurotransmitter Systems in the Development of ADHD and CD

The interplay between the serotonin and dopamine systems has been highlighted in mouse knockout, pharmacological and neuroscience approaches. There are 3 major dopamine pathways in the brain, consisting of the (1) nigrostriatal pathway, which originates in the substantia nigra pars compacta and ends in the dorsal striatum, (2) mesolimbic pathway, connecting the ventral tegmental area (VTA) to the nucleus accumbens and (3) mesocortical pathway, starting in the VTA and ending in the prefrontal cortex. The mesolimbic pathway is responsible for the mediation of natural and drug induced reward, while the mesocortical pathway is responsible for selective attention and working memory, both important in the decision making/learning process

and recall for future responses. Serotonin (5-HT) neurons originate in the medial and dorsal raphe nuclei and have direct synaptic contact with dopamine cells and terminals in the midbrain. Consequently, it has been thought that 5-HT could regulate dopamine function in the mid brain dopamine cell bodies or terminals (Alex & Pehek, 2006).

DAT1 knockout mice display hyperactive behaviors (Gaintedinov et al., 1999) and were more likely to initiate reactive and aggressive behaviors with cagemates (Rodriguiz et al., 2004). However, these mice became calm after treatment with several serotonergic drugs and selective serotonin reuptake inhibitors (SSRIs), independent of any changes in dopamine levels (Gaintedinov et al., 1999).

MAOA knockout mice have increased levels of extracellular 5-HT (Cases et al., 1995; Murphy et al., 2003). Additionally, extracellular dopamine clearance is affected by the administration of parglyine, a monoamine oxidase inhibitor (Gaintedinov-2003-Ann Rev Pharmacol Toxicol). *SERT* x *MAOA* double knockout mice have high 5-HT accumulation in the dopaminergic neurons of the substantia nigra, resulting from aberrant uptake of increased extracellular 5-HT. This accumulation appears to be the result of a compensatory pathway for 5-HT metabolism using the dopamine transporter to substitute for the loss of *SERT* (Murphy et al., 2003) and *DAT1* knockout mice do not show this compensatory pathway. Rather, loss of *DAT1* activity results in an accumulation of extracellular dopamine in the striatum (Rodriguiz et al., 2004).

These studies provide preliminary evidence for epistatic interaction between genes encoding proteins within the serotonergic and dopaminergic systems in development of behavior. Given our current understanding of how these two

neurotransmitters function with one another as a result of genotypic differences as well as our ability to detect behavioral differences resulting from epistasis using the mouse model (Murphy et al., 2003), it is worthwhile to test the role of main genetic and epistatic effects as risk factors for ADHD and CD in humans. No known research has studied epistasis using measured genotypes in human or non-human primate aggression/CD or ADHD. If detected, epistasis may provide additional information on the mechanism and pathways involved with CD and ADHD. Furthermore, in the absence of significant epistasis, the pathway from genotype to phenotype might be elucidated by studying the effect of allelic variations for genes within the serotonin system. Including current knowledge regarding the functional significance of specific genotypes may ultimately provide a simple framework for understanding how genetic risk might translate into individual etiology.

Epistasis Defined and its Use to Improve Understanding of the Serotonin and Dopamine Systems

Defining Epistasis.

Epistasis or "epistacy", as it was classically defined from the biometrical perspective, refers to an interaction of alleles at different loci resulting in differences in a phenotypic outcome (Fisher, 1918). Epistasis was initially observed as a deviation from the expected Mendelian F_2 segregation ratio of 9:3:3:1 demonstrating independent assortment of discrete traits (ie: comb color in fowl or fur color in house mice) and described as an allele at a locus preventing an allele at another locus from manifesting its effect for discrete traits (Bateson, 1909). The biometricians

Karl Pearson and W.F.R. Weldon then approached the issue from the perspective of continuous variation (Phillips, 1998). Later, R.A. Fisher (1918) bridged the gap between continuous variation and discrete traits, by suggesting that predicting a quantitative phenotype would be better approached by considering the interaction between loci, rather than their additivity. Additionally, Fisher partitioned the total genetic variance of quantitative traits to reflect additive, dominance, and epistatic variance.

The single-gene system adopted by Fisher, Immer and Tedin (1932) defines the phenotype corresponding to the heterozygote as *m*, or the mean phenotypic value. The value *h* is used to identify the phenotypic departure of the heterozygote from the mean value. The values $+d$ and $-d$ are the phenotypic differences of the homozygotes from the mean. Adapting the continuous phenotype framework to that of a dichotomous phenotype (ie: affected or unaffected), the contribution associated with one homozygous genotype (AA) to the phenotype can be denoted as 1, while the contribution of the other homozygous genotype (aa) is defined as –1 and the heterozygote as 0 (Figure 5.1) (Fisher et al., 1932).

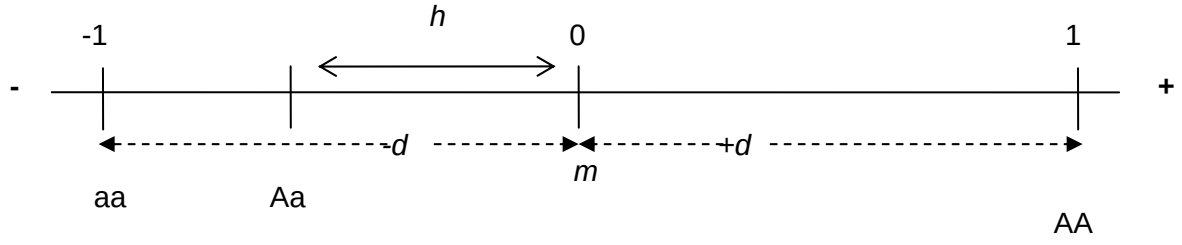


Figure 5.1 Genotypic Representation of a Continuous Trait

The definition of epistatic variance by Hayman and Mather (1955) produce nine genotypes from two different alleles at each locus, A/a and B/b (Hayman & Mather, 1955). Using Robson definitions of non-allelic interactions, multiple genotypes can be defined to reflect expected phenotypes measured as continuous traits (Eaves, 1994; Mather et al., 1982) (Table 5.1). m is the mean of the inbred population of the four possible outcomes (AABB, AAbb, aaBB, and aabb) from the two true breeding lines (AABB x aabb and AAbb x aaBB). d_a refers to the additive deviations of the homozygotes from the mean for the A/a locus, while d_b refers to the additive deviations at the B/b locus.

Table 5.1 Expected Phenotypic Values for the Nine Genotypes Resulting from Two Epistatic Loci

Genotype	Expected Phenotype
AABB	$m + d_a + d_b + i_{ab}$
AABb	$m + d_a + h_b + j_{ba}$
AAbb	$m + d_a - d_b - i_{ab}$
AaBB	$m + h_a + d_b + j_{ab}$
AaBb	$m + h_a + h_b + l_{ab}$
Aabb	$m + h_a - d_b - j_{ab}$
aaBB	$m - d_a + d_b - i_{ab}$
aaBb	$m - d_a + h_b - j_{ba}$
aabb	$m - d_a - d_b + i_{ab}$

(Adapted from Mather and Jinks, 1982)

The heterozygous differences from the mean for the A/a and B/b loci are h_a and h_b respectively. Mather and Jinks parameterized epistasis to include the classes of (1) homozygote X homozygote interaction (d_a x d_b or i_{ab}), (2) homozygote X heterozygote interaction (d_a x h_b or j_{ba} and d_b X h_a or j_{ab}), utilizing the different heterozygotes at each loci and (3) heterozygote X heterozygote interaction (h_a x h_b or l_{ab}) (Kao & Zeng, 2002; Mather et al., 1982; Phillips, 1998).

Several departures from the classical Mendelian ratio of 9:3:3:1 have been detected, highlighting specific types of genotype-genotype interaction, including dominant epistasis (12:3:1), recessive epistasis (9:3:4), epistasis of duplicate genes with cumulative effect (9:6:1), and dominant and recessive interaction (13:3) (Phillips, 1998; Stansfield, 1991; Bateson, 1909). Classical duplicate genes epistasis (also known as duplicate dominant epistasis) and classical complementary genes epistasis (also referred to as duplicate recessive epistasis) are easily parameterized within the biometrical genetic framework to provide representations of pathways with biochemical and evolutionary implications (Eaves, 1994; Mather et al., 1982). Duplicate genes epistasis (15:1) has been classically defined as a situation where the dominant alleles of both loci each produce the same phenotype without cumulative effect. With respect to biochemical pathways, this type of epistasis might be conceptualized as two proteins functioning in parallel and would be observed when the high-risk allele on either locus produces the same phenotype. Complementary genes epistasis (9:7) is defined as a situation where one phenotype is produced by both homozygous recessive genotypes and the dominant alleles produce another distinct phenotype. The pattern of epistasis is

conceptualized as a biochemical pathway in series, where the high-risk allele at the first locus has subsequent implications for the second protein and results in a distinct outcome from a system where the first locus has a low-risk allele.

The 9:3:3:1 ratio describes a situation with no interaction and occurs when $d_a = h_a$, $d_b = h_b$ and $i_{ab} = j_{ab} = j_{ba} = l_{ab}$. When parameterized to reflect additive and dominance effects using an approach summarized by Mather and Jinks (1982), duplicate gene epistasis (15:1) is defined as $d_a = d_b = h_a = h_b = -i_{ab} = -j_{ab} = -j_{ba} = -l_{ab}$. Complementary epistasis (9:7) is parameterized as $d_a = d_b = h_a = h_b = i_{ab} = j_{ab} = j_{ba} = l_{ab}$. Complementary and duplicate epistasis are the easiest types of genotype-genotype interaction that can be detected because (1) the homozygous genotypes (*d*, or additive effects) and in turn the heterozygous genotypes (*h*, or dominance effects) in *h* are equal in sign and magnitude and are defined as having a value of either +1 or –1 (Table 5.2). Additionally, alternate forms of epistasis cannot be detected using this framework because they require knowledge of the individual signs and magnitudes of *h*. The value *h* cannot be estimated in a community-based sample of humans because it represents the deviation of the heterozygotes from the mean of the two true breeding lines (AABB x aabb and AAbb x aaBB) and would require additional multi-generational family data (Table 5.2).

Table 5.2 Expected Coefficients of Epistatic Interactions Resulting from Genotypic Differences

Genotype	Expected Phenotype	d_a	d_b	d_a X d_b
AABB	$m + d_a + d_b + i_{ab}$	+1	+1	+1
AABb	$m + d_a + h_b + j_{ba}$	+1	0	0
AAbb	$m + d_a - d_b - i_{ab}$	+1	-1	-1
AaBB	$m + h_a + d_b + j_{ab}$	0	+1	0
AaBb	$m + h_a + h_b + l_{ab}$	0	0	0
Aabb	$m + h_a - d_b - j_{ab}$	0	-1	0
aaBB	$m - d_a + d_b - i_{ab}$	-1	1	-1
aaBb	$m - d_a + h_b - j_{ba}$	-1	0	0
aabb	$m - d_a - d_b + i_{ab}$	-1	-1	+1

The Implications of Epistasis on System Complexity. The characterization of epistasis is associated with genomic complexity, such that epistasis detected in *D. melanogaster* often has a negative value (duplicate gene epistasis), while vesicular somatitis virus (VSV) often has positive values of epistasis (complementary gene epistasis). Thus, epistasis in complex systems has been suggested to accommodate genomic or environmental perturbations and that complexity arises as a response to maintain a system. For example, a small number of deleterious mutations of genes with products functioning in a system might be buffered by functional mutations for other gene products elsewhere in the pathway. An organism with few genes would display fewer alternate pathways, and less buffering, than an organism with a more complex genome. This system buffering could produce a positive feedback mechanism by which new functions and increased genetic complexity emerge which in turn results in a selective advantage for robust systems (Sanjuan & Elena, 2006).

The Implications of Epistasis on Natural Selection. Fitness is the reproductive success of a genotype across generations and fitness-related traits are subject to natural selection. An example of a direct fitness-related trait is the total number of offspring born to a parent. Other less-direct traits related to this characteristic include offspring viability, mating success, predator survival, and disease resistance (Falconer et al., 1996). Similarly, conduct disorder may be one measure of the overall fitness-related trait of aggression. Aggression has been studied in many species using other measures including proactive or reactive aggression, and human studies have addressed aggression with measures such as life-course persistent or adolescent limited antisocial behavior, interpersonal aggression, and physical aggression against strangers. While it is unlikely that CD is a direct indicator of fitness, the presence of epistasis using CD as an outcome estimating aggression may yield insight into the nature of natural selection for this trait.

Under stabilizing and directional selection, a single trait optimum is favored and any departures from that optimum are increasingly penalized as their magnitude increases. Further, when the character optimum is similar to the population mean expression of the trait, there will be a stabilization of the trait at the mean value. In contrast, disruptive selection always acts to disrupt the distribution of expression of a trait in a population. Under disruptive selection two or more optimal trait levels may be bound in their functioning, such as the number of males and females in a breeding

population, and their levels will be adjusted to one another across generations (Mather, 1966).

Natural selection acts on a trait if phenotypic differences of individuals result in differences in fitness for the trait. Traits with a direct and obvious relation to fitness are expected to be under directional selection while traits less obvious to fitness undergo stabilizing selection. In order to maintain directional selection for traits with strong fitness-related implications, genetic variance for the trait would no longer be due largely to additive genetic effects, but rather to either dominance or epistatic interaction. Thus under continuous directional selection, one allele responsible for a more favored expression of a trait would have a permanent and unconditional advantage over all others and would be expected to occur most frequently. If multiple genes were also responsible for the expression of the trait, the frequency of the alleles at the other contributing loci would also be favored. Thus, directional selection would be expected to favor unidirectional genotype-genotype interaction and would be characterized by the presence of duplicate gene epistasis (Mather, 1966). Ultimately, if significant duplicate gene epistasis were found to contribute to CD it would indicate the importance of this phenotype for population fitness.

The Multiple Definitions and Uses of "Epistasis"

Epistasis is thought to be important in our understanding of evolutionary biology and to have profound clinical implications (Templeton, 2000). However, the appreciation for its detection and characterization is often lost in the multiple uses of the

very term. The definition of "epistasis" can also refer to the term "biological epistasis" or "physiological epistasis" in an attempt to characterize any biomolecular interactions, which also includes genotype-genotype interaction (Moore & Williams, 2005).

Detecting epistasis using population-based methods. The fields of evolutionary biology and biometrical genetics refer to epistasis with the goal of understanding the mechanistic effects of alleles on individual differences and ultimately population variation (Brodie, 2000). From these two disciplines, epistasis has a rich history in breeding and some wild population studies. Breeding studies have classically studied the means of parental, F_1, F_2, and first backcross (B_1 and B_2) generations of a cross between two inbred lines (Hayman, 1958). These studies have been able to detect and describe the interactions in alleles of natural populations and, to a lesser degree, determine the importance in mapping genotype to phenotype and ultimately to the evolutionary process (Templeton, 2000).

Epistasis has been reported for several complex traits in many plant and animal populations. One such example is flowering time in *Arabidopsis thaliana*. Flowering time is a fitness-related trait and has been associated with size at reproduction and fecundity. Several loci have been identified to control this complex phenotype. Recently, the genes *FLC* and *FRI*, whose products function in the pathway responsible for response to vernalization (exposure to prolonged cold treatment), were identified to be associated with this phenotype. Further, any association between *FLC* and flowering time is only observed in epistatic interaction with *FRI*. The interaction was

characterized as complementary gene epistasis, with functional *FRI* alleles upregulating *FLC* activity and resulting in *FLC*-related variation in flowering time. Thus, epistasis among genes in pathways of regulatory function is important in phenotypic variation.

Studies of wild and laboratory rats have reported varying degrees of epistasis for different traits. For example, significant additive genetic variation, dominance variation, and duplicate gene epistasis was reported for escape-avoidance behavior. Additionally, in later trials of the 60 total trials of avoidance, there was increasing heritability and the detected epistasis reinforced a trend toward high avoidance among heterozygotes. These results suggested increasing genetic control for conditioning to avoidance behavior. Also, epistasis was reported for a behavior known as "raising-up". This behavior is observed as rat standing on its rear legs in response to a stressor (fluorescent lighting) in a small rearing compartment. However, for other measures of stress-response to an open-field test, such as frequency of open-field defecation, only some additive genetic effects were detected. Another measure of emotional reactivity to stress is ambulation, or exploration of an unknown area. This trait is measured as the amount of surface area covered in waste. The greater the amount of surface area covered in waste implies more ambulation and more exploration under stress. This trait was only subject to dominance. These studies of behavior demonstrated that the detection of epistasis is dependent on the phenotype measured and may not be important to the phenotype itself but to other behaviors related to it (Broadhurst & Jinks, 1966; Hewitt & Fulker, 1981; Hewitt & Fulker, 1983; Hewitt & Fulker, 1984).

Detecting epistasis using laboratory-based methods. Transgenic animal models must use sequences that provide new functions (knock-in), inhibit expression (knockdown) or completely ablate (knockout) expression of endogenous genes. These new sequences, also known as exogenous DNA, are then used in the development of a transgenic zygote. The typical transgenic zygote is produced first either by (1) selectively transferring DNA into germ cells or into the egg immediately after fertilization so that it can integrate prior to the first cell division of the zygote or (2) non-selectively transferring DNA into the totipotent embryonic stem cells, which can then become any type of cell in the embryo. Selective transfer via microinjection of DNA into the cytoplasm of a newly fertilized egg is a more established and efficient method and most often used in generating transgenic animals. After the fertilized egg is injected, the desired DNA is integrated immediately throughout nicks in the chromosomal DNA of the male pronucleus. The zygote is then transferred to the oviducts of a pseudo-pregnant female (a female mouse which has been mated with a vasectomized male to initiate the physiological changes necessary to maintain the pregnancy). The zygote will consist of both transformed (DNA integrated) and non-transformed (DNA not integrated) cells, including germ cells. The germ cells, which will eventually become gametes, maintain the transgene and can be passed on to future generations of mice. Once several different strains of transgenic mice are produced, these mice can then be mated with one another to produce mice that have more than one transgenic modification such as double knockout mice (Strachan & Read, 2004b).

Transgenic animal models have found single, double and even triple locus effects for many phenotypes including aggression, drug use and hyperactivity that can be reproduced across species or strains (Murphy et al., 2003; Schork, Nath, Lindpainter, & Jacob, 1996). These models readily identify changes in systems of interest, which can mimic a disease of interest when one or more genes are altered. Consequently, the animal model is particularly useful in identifying important genes in disease etiology. Further, in studies of behavior, the neurobiology of the animal can be studied to determine differences in function from wild-type animals.

Although a mouse model of epistasis can easily detect such interaction due to the loss of function of one or more genes, this type of "knockout" is not generally realistic and may not easily translate to human studies of similar genes or breeding studies of epistasis using unmodified mice. Aggression in mice is not necessarily the same as aggression in humans though the same genes might be important in both systems. For example, the human version of the *MAOA* knockout mouse is seen in individuals with Brunner's syndrome but this disorder has only been found in one extended Dutch family. The loss of gene function or control of genetic background is not normal in a general population of humans. Mouse models are able to control environmental exposures, something not easily or ethically accomplished in human studies. Therefore, reports of epistasis in mouse models should be used as only a guide to improve understanding of biochemical pathways and the complexities of the multiple genes controlling the respective proteins in such pathways for humans (Williams, Haines, & Moore, 2004).

The yeast two-hybrid system uses a yeast strain (YS1) encoding a protein of interest and the DNA-binding domain of a transcription factor. YS1 is mated to a second yeast strain (YS2), with each cell containing a different complementary DNA (cDNA) sequence for a specific protein, the coding sequence for the transactivation domain, a reporter gene, and/or a selectable marker gene that is activated when a transcription factor is assembled. YS2 represents a library of proteins that could potentially interact with the protein expressed in YS1. If the protein expressed in YS1 interacts with a protein from the library of proteins in YS2, this interaction can be visually identified and/or viewed as a selective propagation of cells containing the YS2 protein of interest (Strachan & Read, 2004a). This particular system detects protein-protein molecular binding interactions which may or may not display epistatic interactions on the trait. Also, since studies of genetic polymorphisms attempt to distinguish functional differences in protein expression and interactions may be detected between polymorphisms with defined protein function and a phenotype of interest, it is possible that interaction on a protein level may be equated to interaction on a genetic level and vice versa. However, detecting and characterizing epistasis using the yeast two-hybrid system suffers from some limitations. Current models for detecting protein-protein interaction focus on single genes and their products occurring in simple systems that are easily manipulated by the researcher and may not reflect complete human biology or disease etiology. For example, the yeast two-hybrid system does not include mammalian post-translational modification, thus making interactions in yeast difficult to relate to humans. Even though the advent of the mammalian two-hybrid system

offers some insight into protein-protein interaction in a mouse model, translation to genotype-genotype interaction in humans is difficult (Strachan et al., 2004a). Finally, protein-protein interactions may be detected that often do not occur in the normal environment, leading to false positive results (Figeys, 2004).

The definition of epistasis differs across disciplines. Any genotype-genotype interaction is detected due to an effect on a phenotype of interest and only represents a small portion of a biological pathway. Genotype-genotype interaction may not occur with respect to other phenotypes or disorders suggesting a single specific interaction within a larger framework consisting of multiple pathways of risk. In comparison, "biological interaction," as defined by Moore and Williams (2005) is "the physical interactions among proteins or other molecules that impact phenotype". Further, biological interaction is understood to occur at any level of etiology, from interactions between transcription factors to non-linear interactions between enzymes within a metabolic pathway. Biological interaction highlights pathways, and has been hypothesized to result in the detection of substantial epistasis for complex phenotypes (Moore, 2003). However, animal models detecting epistasis and yeast models detecting protein-protein interaction focus on single genes and their products, which occur in simple systems that are easily manipulated by the researcher and may not reflect complete human biology or disease etiology.

Though the definitions of epistasis differ across perspectives, there is a common desire to detect and characterize the path from genotype to phenotype. Here, the term "epistasis" will be used interchangeably with genotype-genotype interaction and any

significant genotype-genotype interaction will be further defined. Despite the supposed ubiquitous nature of epistasis from mouse and yeast models (Moore, 2003), genotype-genotype interaction is not easily detected in human behavior and mental health disorders (Eaves, 1994; McClay & van den Oord, 2006). The vast majority of behavior-related phenotypes in humans only detect significant additive genetic effects, although few studies actually attempt to detect epistasis. Additionally, several examples of epistasis have been identified in the development of other complex disorders in humans including triglyceride levels, sickle-cell anemia, Alzheimer's disease, and breast cancer (Culverhouse, Suarez, Lin, & Reich, 2002), encouraging the study of epistasis in psychiatric and behavioral genetics. It has been suggested that the general lack of epistasis in behavior may be a result of the phenotypes themselves. As previously discussed, breeding studies of rat behavior reported epistasis to be phenotype-specific. Additionally, epistasis is often reported for fitness-related traits. In the absence of significant single candidate gene associations, the inclusion of epistasis for loci functioning in the same biochemical pathway may also improve detection of main effects for psychiatric disorders. Complex phenotypes are understood to be the result of interplay between multiple genes and environmental exposures (Culverhouse, Klein, & Shannon, 2004). Therefore, modeling genetic risk for CD and ADHD using main genetic and epistatic effects has the potential to inform the development of future systems biology models with the anticipation of clarifying how genes moderate biochemical pathways such as those involved in the serotonin and dopamine neurotransmitter systems.

Methods

Study Population

The current study comprises a sub-sample of 555 male and 683 female individual participants from the Virginia Twin Study of Adolescent Behavioral Development (VTSABD). This sub-sample consists of individuals for whom genotypes were successfully obtained for *MAOA* and either *SERT* or *DAT1*. The age range of eligible participants upon entry into the study was 8 -17 years (males- 11.15 ± 2.31 years, females- 11.17 ± 2.49 years).

Items

Diagnosis of Conduct Disorder. Previous 3-month history of CD was assessed using the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al., 2000) by maternal, paternal or child self-report and diagnosis was assigned using a symptom-OR rule at any wave of data collection.

Diagnosis of Attention Deficit Hyperactivity Disorder. Previous 3-month history of ADHD was assessed using the CAPA (Angold et al., 2000) by maternal or paternal report and diagnosis was assigned using a symptom-OR rule at any wave of data collection. Child report for ADHD symptoms was determined to be unreliable for this disorder and was not assessed in this study (Eaves, Maes, Foley, & Silberg, 1999).

DNA Extraction and Genotyping of *MAOA*, *5HTTLPR* and *DAT1* Polymorphisms

MAOA. Primer sequences previously described were used to genotype *MAOA* and classification of *MAOA* activity (high or low) was assigned to each allele resulting from previous work in the efficiency of transcription activity of the *MAOA* gene promoter (Sabol et al., 1998).

5HTTLPR. Primer sequences previously described (Caspi, Sugden, Moffitt et al., 2003) were used, specifically *5HTTLPR*-F labeled with the FAM-6 fluorophore (5'TGAATGCCAGCAGCACCTAACCC3') and *5HTTLPR*-R (5'TTCTGGTGCCACCTAGACGC3'). The two fragments measured were the short allele, consisting of 484 base pairs and the long allele, measuring 528 base pairs.

DAT1. Primer sequences previously described (Gill et al., 1997) were used, specifically *DAT1*-F labeled with the FAM-6 fluorophore (5'TGTGGTGTAGGGAACGGCCTGAG3') and *DAT1*-R (5'CTTCCTGGAGGTCACGGCTCAAGG3'). Several fragments were measured including the common 9- and 10-repeat alleles as well as the rare 3-, 5-, 7-, 8-, and 11-repeat alleles.

Data Analysis

Genotype/Allele Distribution and Test for Hardy-Weinberg Equilibrium.

Genotypic and allelic distributions were assessed using a randomly selected individual from each twin pair having both genotypic data for a particular marker and CD and ADHD diagnoses. Tests of Hardy-Weinberg Equilibrium (HWE) were performed separately on each of the three markers. For *DAT1* and *5HTTLPR*, calculation of HWE utilized allele frequencies of both males and females and tested the expected allele distribution of the total sample.

Since human males are not diploid on the X-chromosome, the calculation of HWE differed for *MAOA* to reflect the genotypic differences between males and females. Hardy-Weinberg equilibrium (HWE) was first tested in the female genotypes. Male and female allele frequencies were then tested for significant differences in distribution as a population-level evaluation of HWE. If a population is determined to be in HWE, it is not subject to assortative mating, population bottleneck, mutation, or population admixture due to inmigration.

CD and ADHD Prevalence by Gender and Genotype. Gender differences in CD and ADHD diagnoses as well as single genotype distributions were assessed using the χ^2-test for association. Prevalence of CD and ADHD was also measured by *MAOA* x *5HTTLPR* and *MAOA* x *DAT1* for each gender. Distributions of CD and ADHD by

MAOA genotype utilized all individuals with a diagnosis for each disorder and an *MAOA*, *5HTTLPR* or *DAT1* genotype (male N=555, female N=683). CD and ADHD distributions by *5HTTLPR* genotype and *MAOA* x *5HTTLPR* utilized all individuals with a diagnosis for CD or ADHD as well as both *5HTTLPR* and *MAOA* genotypes (male N= 526, female N= 584). Likewise, distributions of both disorders by *DAT1* genotype and *MAOA* x *DAT1* used all individuals with diagnoses for either disorder as well as *DAT1* and *MAOA* genotypes (male N = 488, female N=574).

Testing Main Genetic and Epistatic Effects on Risk of CD and ADHD. Models separately tested risk for CD and ADHD by including parameters for main genetic and epistatic effects. Models of the serotonin and dopamine system were utilized for both disorders. A model of the serotonin system used *MAOA* and *5HTTLPR* genotypes, while the dopamine system utilized *MAOA* and *DAT1* genotypes. The genetic architecture of the epistatic interaction (complementary or duplicate gene epistasis) was tested across a series of additive and dominance models. One model defined both loci in an additive fashion where heterozygotes had a phenotypic risk mid-way between the two homozygotes. Since risk associated with homozygote was defined as either 1 (d_a and d_b for increasing risk) or –1 ($-d_a$ and $-d_b$ for decreasing risk), heterozygotes were assigned as having risk equal to 0 ($h_a = h_b = 0$). A second additive model included the interaction between the 2 genotypes. Dominance models, both with and without epistasis were also tested. Dominance for each of the heterozygous genotypes was

defined as risk associated with one of the homozygous genotypes, as either 1 or –1 (ie: $d_a = h_a = 1$ if dominance is in the direction of the homozygote increasing risk), and varied depending on how dominance was defined for each model. Goodness of model fit was assessed as significant differences in deviance from the null and additive models. Goodness of fit and parsimony were assessed using deviance and Akaike's Information Criterion (AIC) respectively. Models determined to best describe risk for CD and ADHD were determined to have (1) the lowest values of AIC and (2) significant differences in deviance from an additive model.

Models were tested using logistic regression within PROC GENMOD in SAS (SAS version 9.1.3; SAS Institute, Cary, NC). Random residual effects of twin resemblance and repeated measurement were accommodated using the Generalized Estimating Equation (GEE) algorithm incorporated in the GENMOD procedure. GEE accounts for the constant correlations between twin pair responses that result from the genetic similarity associated with being a member of either a monozygotic or dizygotic pair to produce unbiased regression estimates (Ballinger, 2004).

Controlling for Comorbidity in Tests of Main Genetic Effects for ADHD and CD. After determining the models best describing genetic risk for ADHD and CD, these models also controlled for the effect of its respective comorbid disorder in order to determine whether comorbid and non-comorbid forms of the disorders are genetically different. As an example, a model estimating the main genetic effect of *MAOA* and *SERT* on ADHD also included and controlled for the number of CD symptoms.

Assessing the Power to Detect Epistasis. In an effort to determine the ability of the current study to detect the effect of epistasis on CD, the allele frequencies of the systems used to model the serotonin and dopamine systems were included to estimate (1) The proportion of the total variance due to genetic effects without epistasis, (2) The proportion of the total variance due to epistasis and (3) The sample sizes necessary to detect the variance due to epistasis significant at the 5% level with an 80% chance of detection for each genotype at both loci. The model of the serotonin system utilized the minor allele frequencies of *MAOA* and *5HTTLPR* with frequencies of 32% and 49%, while the evaluation of the dopamine system used the minor allele frequencies for *MAOA* and *DAT1* with frequencies of 32% and 27%. These analyses treated CD as a continuous variable having a mean equal to one.

The proportions of the total variance due to main and epistatic genetic effects were estimated by (1) Calculating the expected genotypic sample sizes for all locus combinations of epistasis using a simulated sample size of 1000 observations and the estimated genotypic frequencies using the minor allele frequencies for each marker in a system, (2) Estimating the genetic effects weighted by genotypic sample sizes and having a within-genotype variance equal to 10, (3) Estimating the proportion of variance due to main genetic and epistastic effects by calculating the difference between the total sums of squares (TSS) for model containing no genetic effects (TSS_{null}) and the TSS for the model containing main and epistatic genetic effects ($TSS_{main + epistatic}$), (4) Estimating the proportion of variance due main genetic effects by calculating the

difference between the TSS for a model with only main genetic effects (TSS_{main}) and that of a model with no genetic effects (TSS_{null}).

The sample size necessary for the detection of the variance due to epistasis was estimated by multiplying the probability of detecting significant epistasis at the 5% level with an 80% chance of detection by the simulated sample size (1000).

Results

Genotypic and Allelic Distribution and Test for Hardy-Weinberg Equilibrium

Table 5.3 summarizes distributions of the *5HTTLPR* and *DAT1* genotypes by gender. These frequencies were used to test the assumption of HWE and revealed no significant departures. The genotypic distribution of *MAOA* in females and the allelic distribution between males and females also revealed no significant departures from HWE at this locus. Additionally, there were no significant associations between the genotype distributions, indicating non-random association of the genotype distributions.

Table 5.3 *5HTTLPR* and *DAT1* Genotype Frequencies

	Males		Females		Total		Total Expected	
Genotype	N	%	N	%	N	%	N	%
5HTTLPR								
ss	73	25.4	77	25.9	150	25.6	144.5	24.7
sl	146	50.7	136	45.8	282	48.2	292.5	50.0
ll	69	24.0	84	28.3	153	26.2	148.0	25.3
DAT1								
9/9	13	4.8	24	8.7	37	6.8	37.1	6.8
9/10	98	36.4	112	40.6	210	38.5	210.4	38.6
10/10	158	58.7	140	50.7	298	54.7	297.6	54.6

Table 5.4 summarizes the allele distributions of *MAOA*, *5HTTLPR* and *DAT1* by gender. There were no significant differences by gender for any of the markers, despite a trend for the increased frequency of the short *5HTTLPR* allele in females. The 3- and 4-repeat *MAOA* alleles occurred most frequently (31.5% and 64.9 % respectively). The *5HTTLPR* alleles were almost equally distributed (51% short allele), and the *DAT1* alleles showed the highest frequency for the 9- (26.6%) and 10-repeats (71.9%). The allele frequencies for *5HTTLPR* do not agree with those previously reported (Kent et al., 2002; Volk, Neuman, & Todd, 2005) although their associated genotypic frequencies are similar to those of previous studies and demonstrate the population to be in Hardy-Weinberg equilibrium. Approximately 2% of the total distribution consisted

of rare *DAT1* alleles. These alleles were not included in the remainder of the analyses as a result of their low frequency and the lack of information regarding functional significance.

Table 5.4 Allele Frequencies of *MAOA*, *5HTTLPR* and *DAT1*

			Males		Females		Total	
Allele	Repeat	Activity	N	%	N	%	N	%
MAOA								
1	3	low	151	31.7	164	31.4	315	31.5
2	3.5	high	13	2.7	12	2.3	25	2.5
3	4	high	307	34.4	342	65.4	649	64.9
4	5	low	3	0.6	5	1.0	8	0.8
5	2	low	3	0.6	0	0.0	3	0.3
5HTTLPR								
1	14	low	235	49.0	276	52.8	511	51.0
2	16	high	244	50.9	247	47.2	491	49.0
DAT1								
1	9	low-density	119	24.8	147	28.1	266	26.6
2	10	high-density	355	74.1	365	69.8	720	71.9
3	11	NA*	5	1.0	6	1.2	11	1.1
4	3	NA*	0	0	2	0.4	2	0.2
7	6	NA*	0	0	3	0.6	3	0.3

*Rare alleles with unknown functional significance

Prevalence of CD and ADHD by Gender and Genotype

The prevalence of CD was 9.0% and was 4.1% for ADHD within the total sample. The prevalence of CD was 11.2% among males and 7.3% in females, while 6.3% of males and 2.3% of females were diagnosed with ADHD. For both disorders, males had significantly higher rates than females (CD, $p = 0.02$; ADHD, $p = 0.0005$).

Among individuals with ADHD, 24.5% were also diagnosed with CD, while 12.5% of participants with CD also had an ADHD diagnosis.

Table 5.5 summarizes the prevalence of CD and ADHD by genotype for each gender. The low activity *MAOA* genotype was significantly associated with CD in females ($p = 0.04$). The 9/9 *DAT1* genotype was significantly associated with CD diagnosis in males ($p = 0.03$) and females ($p = 0.0004$). This genotype was also significantly associated with ADHD in males only ($p = 0.006$). There were no significant associations between alleles at other markers and either disorder. Subsequent analyses were performed separately for males and females in order to (1) determine any genetic differences in risk by gender and (2) to address differences in the analysis of models using *MAOA*, which resides on the X-chromosome.

Table 5.5 Prevalence of Conduct Disorder and Attention Deficit Hyperactivity Disorder by Gender and Genotype

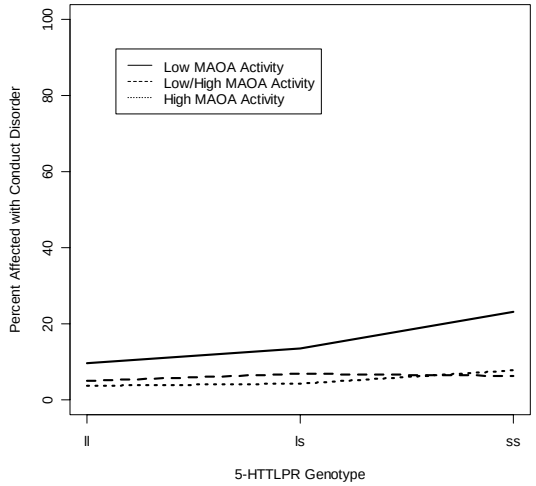
	Conduct Disorder				ADHD			
	Males		Females		Males		Females	
Genotype	N	%	N	%	N	%	N	%
MAOA								
low	19	10.3	14	14.1*	9	4.9	3	3.0
low/high	NA	NA	24	7.1	NA	NA	10	3.0
high/high	54	12.8	18	5.8	27	6.4	5	1.6
5HTTLPR								
ll	14	10.2	8	5.2	9	6.6	4	2.6
ls	33	12.6	18	6.6	18	6.9	8	2.9
ss	10	7.8	13	8.4	8	6.3	2	1.3
DAT1								
9/9	6	21.4*	6	12.5*	5	17.9*	1	2.1
9/10	26	14.8	9	3.9	11	6.3	3	1.3
10/10	23	8.1	30	10.2	13	4.6	9	3.1

* Significant association between disorder and genotype at $p<0.05$

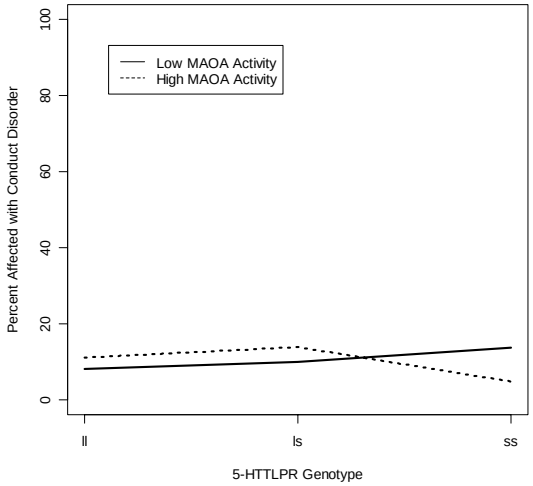
Figures 5.2 - 5.5 detail differences in the prevalence of CD by *MAOA* x *5HTTLPR* and *MAOA* x *DAT1* genotypes. In both males and females, the highest prevalence of CD was in those individuals homozygous for the short *5HTTLPR* allele with the low activity *MAOA* genotype although there was no significant association between this genotypic combination and CD diagnosis (figures 5.2 and 5.3). There were no significant trends for CD diagnosis among *MAOA* x *DAT1* combinations. Figures 5.6 – 5.9 detail differences in the prevalence of ADHD by *MAOA* x *5HTTLPR* and *MAOA* x *DAT1* genotypes. Prevalence across genotypic combinations is small,

resulting in few significant trends for ADHD diagnosis. Tables 5.6 and 5.7 summarize the prevalence of CD and ADHD by genotype combinations used to generate these figures and highlights their small cell sizes.

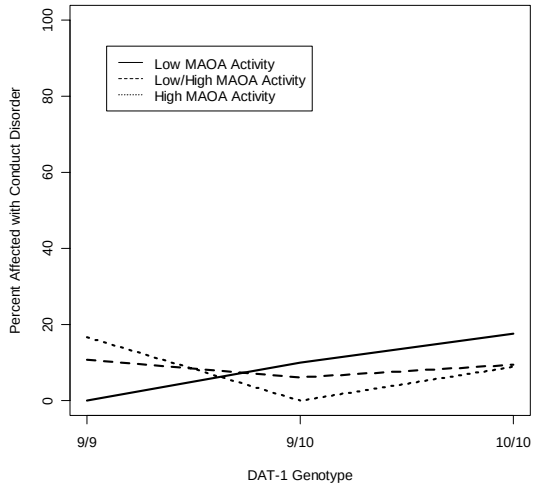
5.2



5.3



5.4



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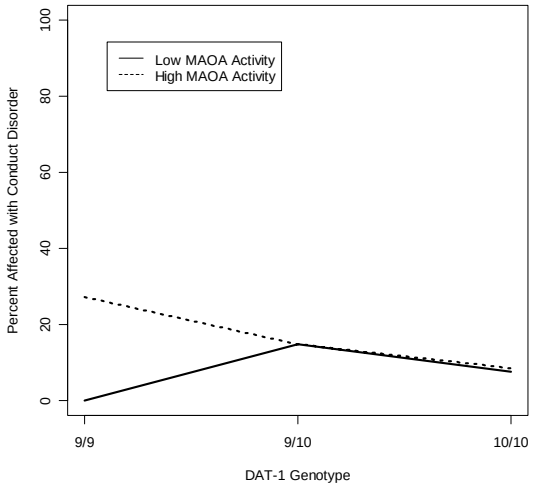

Figure 5.2 *MAOA* x *5HTTLPR* in Females

Figure 5.3 *MAOA* x *5HTTLPR* in Males

Figure 5.4 *MAOA* x *DAT1* in Females

Figure 5.5 *MAOA* x *DAT1* in Males

Figures 5.2 - 5.5. Prevalence of Conduct Disorder by *MAOA* x *5HTTLPR* and *MAOA* x *DAT1* by Gender

Table 5.6 Sample Sizes of Individuals Affected with Conduct Disorder Having Specific Genotypic Combinations

	MAOA- males (N= 526)		*MAOA*- females (N=584)		
5HTTLPR	low	high	low/low	low/high	high/high
ll	3/37	11/100	2/21	4/80	2/54
ls	8/81	25/180	5/37	8/119	5/119
ss	6/44	4/84	3/13	4/64	6/77

	MAOA- males (N=488)		*MAOA*- females (N=574)		
DAT1	low	high	low/low	low/high	high/high
9/9	0/6	6/22	0/2	3/28	3/18
9/10	9/61	17/115	3/30	6/99	0/104
10/10	7/93	16/191	7/40	12/128	11/125

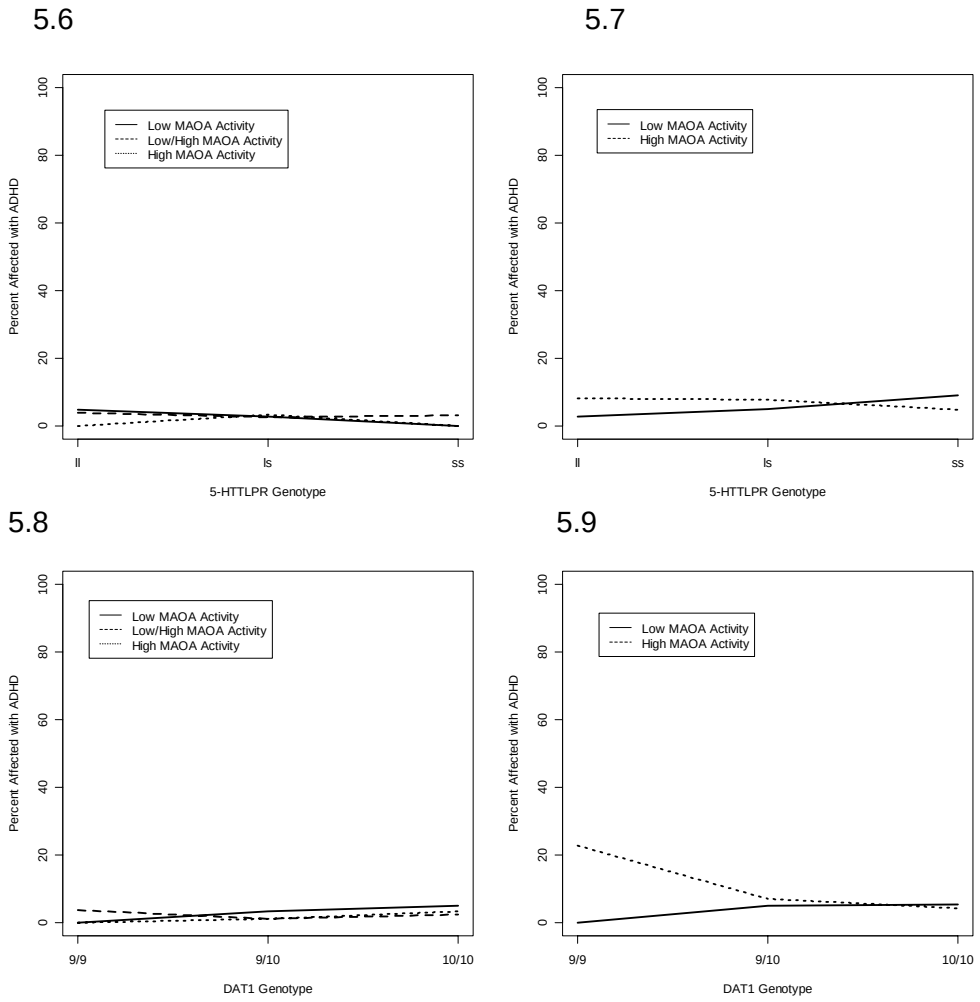

Figure 5.6 *MAOA* x *5HTTLPR* in Females

Figure 5.7 *MAOA* x *5HTTLPR* in Males

Figure 5.8 *MAOA* x *DAT1* in Females

Figure 5.9 *MAOA* x *DAT1* in Males

Figures 5.6 – 5.9. Prevalence of Attention Deficit Hyperactivity Disorder by *MAOA* x *5HTTLPR* and *MAOA* x *DAT1* by Gender

Table 5.7 Sample Sizes of Individuals Affected with Attention Hyperactivity Disorder Having Specific Genotypic Combinations

	MAOA- males (N=526)		*MAOA*- females (N=584)		
5HTTLPR	low	high	low/low	low/high	high/high
ll	1/37	8/100	1/21	3/80	0/54
ls	4/81	14/180	1/37	3/119	4/119
ss	4/44	4/84	0/13	2/64	0/77

	MAOA- males (N=488)		MAOA- females (N=574)		
DAT1	low	high	low/low	low/high	high/high
9/9	0/6	5/22	0/2	1/28	0/18
9/10	3/61	8/115	1/30	1/99	1/104
10/10	5/93	8/191	2/40	3/128	4/125

Testing Main Genetic and Epistatic Effects on Risk of CD and ADHD

Tables 5.8 – 5.11 summarize models of risk for CD by gender using the serotonin and dopamine models. Few significant main genetic effects were observed. A significant effect of the low activity *MAOA* allele in the additive model among females was detected using the serotonin model for CD (Table 5.8, $\beta = 0.53$, $p = 0.05$). There was marginally significant effect of low activity *MAOA* allele in the dopamine models of risk for CD (Table 5.10, $\beta = 0.46$, $p = 0.06$). Among males, there was a significant effect of the 9-repeat *DAT1* allele in an additive model of risk for CD ($\beta = -0.59$, $p = 0.01$) and ADHD ($\beta = -0.64$, $p = 0.04$) (Table 5.10 and Table 5.11).

Controlling for Comorbidity in Tests of Main Genetic Effects for ADHD and CD

Maternal reports of ADHD and CD were significantly associated in males ($r = 0.15$, $p = 0.02$) and females ($r = 0.24$, $p < 0.0001$) and the two disorders were considered comorbid. Models of risk for each disorder thus controlled for the presence of the other disorder to determine whether comorbid and non-comorbid forms of the disorders are genetically different. After controlling for CD diagnosis, the main effect of *DAT1* on ADHD diagnosis in males was no longer significant ($p = 0.07$). However, controlling for ADHD diagnosis did not change the significant main effect of the 9/9 genotype for CD diagnosis in males. Additionally, the main effect of the low activity *MAOA* genotype on CD in females was no longer significant after controlling for ADHD diagnosis.

Assessing the Power to Detect Epistasis and Main Genetic Effects

In a model of the serotonin system, it was estimated that in order to explain 8.6% of the total variance to be the result of genotypic effects and 0.8% of the total variance to be the result of epistatic effects with 80% power, a sample size of 8.1×10^{35} individuals would be required. Within the dopamine system, in order to explain 7.6% of the total variance to be the result of genotypic effects and 3.3% of the total variance to be a result of epistatic effects, 3.6×10^{31} individuals would be necessary. Therefore, the weak interaction highlighted in Table 5.8 suggesting increasing risk for CD in males with low activity *MAOA* and the short *5HTTLPR* allele ($\beta = 0.43$, $p = 0.07$) must be interpreted cautiously since this study is underpowered to detect any significant epistasis. Several

models that included interactions could not be tested as a result of cells with a zero count for individuals diagnosed with CD or ADHD who lack all genotype combinations.

Table 5.8 Summary of Serotonin Models of Risk for Conduct Disorder

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction
Null	360.92	0	360.92				286.43	0	286.43			
Additive, without Epistasis	360.55	2	364.55	0.67	0.25		279.80	2	283.80	0.05	0.32	
Additive, with Epistasis	358.67	3	364.67	0.67	0.58	0.16	279.78	3	285.78	0.06	0.29	0.83
COMPLETE DOMINANCE WITHOUT EPISTASIS												
MAOA- low activity *5HTTLPR*- long allele	359.20	2	363.20	0.66	0.10		283.62	2	287.62	0.28	0.41	
MAOA- low activity *5HTTLPR*- short allele	360.82	2	364.82	0.70	0.99		283.81	2	287.81	0.29	0.49	
MAOA- high activity *5HTTLPR*- long allele	359.20	2	363.20	0.66	0.10		279.25	2	283.25	0.01	0.39	
MAOA- high activity *5HTTLPR*- short allele	360.82	2	364.82	0.70	0.99		279.73	2	283.73	0.01	0.51	

[1] P- Number of Parameters in Model

Table 5.8 Summary of Serotonin Models of Risk for Conduct Disorder (continued)

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction
Null	360.92	0	360.92				286.43	0	286.43			
Additive, without Epistasis	360.55	2	364.55	0.67	0.25		279.80	2	283.80	0.05	0.32	
Additive, with Epistasis	358.67	3	364.67	0.67	0.58	0.16	279.78	3	285.78	0.06	0.29	0.83
COMPLETE DOMINANCE WITH EPISTASIS												
MAOA- low activity *5HTTLPR*- long allele	355.21	3	361.21	0.14	0.19	0.07	283.21	3	289.21	0.35	0.41	0.81
MAOA- low activity *5HTTLPR*- short allele	360.59	3	366.59	0.94	0.85	0.66	283.81	3	289.81	0.38	0.54	0.95
MAOA- high activity *5HTTLPR*- long allele	355.21	3	361.21	0.14	0.19	0.07	279.01	3	285.01	0.01	0.22	0.47
MAOA- high activity *5HTTLPR*- short allele	360.59 360.90	3 1	366.59 362.90	0.94	0.85	0.66	279.65 281.77	3 1	285.65 283.77	0.05	0.35	0.57

[1] P- Number of Parameters in Model

Table 5.9 Summary of Serotonin Models of Risk for Attention Deficit Hyperactivity Disorder

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction
Null	360.92	0	360.92				286.43	0	286.43			
Additive, without Epistasis	256.84	2	260.84	0.52	0.90		130.89	2	134.89	0.35	0.46	
Additive, with Epistasis	254.52	2	258.52	0.41	0.54	0.16	130.79	2	134.79	0.39	0.46	0.58
COMPLETE DOMINANCE WITHOUT EPISTASIS												
MAOA- low activity 5-HTTLPR- long allele	256.81	2	260.81	0.52	0.74		129.85	2	133.85	0.33	0.35	
MAOA- low activity 5-HTTLPR- short allele	256.84	2	260.84	0.51	0.91		130.88	2	134.88	0.27	0.95	
MAOA- high activity 5-HTTLPR- long allele	256.81	2	260.81	0.52	0.74		130.88	2	134.88	0.86	0.31	
MAOA- high activity 5-HTTLPR- short allele	256.84	2	260.84	0.51	0.91		132.04	2	136.04	0.80	0.87	

[1] P- Number of Parameters in Model

Table 5.9 Summary of Serotonin Models of Risk for Attention Deficit Hyperactivity Disorder (continued)

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction	Deviance	P[1]	AIC	*MAOA*	*5HTTLPR*	Interaction
Null	360.92	0	360.92				286.43	0	286.43			
Additive, without Epistasis	256.84	2	260.84	0.52	0.90		130.89	2	134.89	0.35	0.46	
Additive, with Epistasis	254.52	2	258.52	0.41	0.54	0.16	130.79	2	134.79	0.39	0.46	0.58
COMPLETE DOMINANCE WITH EPISTASIS												
MAOA- low activity 5-HTTLPR- long allele	254.52	3	260.52	0.98	0.85	0.17	*					
MAOA- low activity 5-HTTLPR- short allele	255.83	3	261.83	0.28	0.50	0.34	*					
MAOA- high activity 5-HTTLPR- long allele	254.52	3	260.52	0.98	0.85	0.17	*					
MAOA- high activity 5-HTTLPR- short allele	255.83	3	261.83	0.28	0.50	0.34	131.68	3	137.68	0.69	0.59	0.54

*- Model cannot be estimated due to missing data

[1] P- Number of Parameters in Model

Table 5.10 Summary of Dopamine Models of Risk for Conduct Disorder

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction
Null	343.68	0	343.68				315.51	0	315.51			
Additive, no Epistasis	336.02	2	340.02	0.85	0.01		309.50	2	313.50	0.06	0.55	
Additive, with Epistasis	335.56	3	341.56	0.66	0.04	0.57	309.47	3	315.47	0.28	0.51	0.87
COMPLETE DOMINANCE WITHOUT EPISTASIS												
MAOA- high activity DAT1- 9-allele	336.55	2	340.55	0.79	0.02		307.35	2	311.35	0.09	0.09	
MAOA- low activity DAT1- 9-allele	336.55	2	340.55	0.79	0.02		307.76	2	311.76	0.16	0.08	
MAOA- high activity DAT1- 10-allele	340.87	2	344.87	0.89	0.16		310.12	2	314.12	0.06	0.20	
MAOA- low activity DAT1- 10 allele	340.87	2	344.87	0.89	0.16		311.35	2	315.35	0.19	0.28	

[1] P- Number of Parameters in Model

Table 5.10 Summary of Dopamine Models of Risk for Conduct Disorder (continued)

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction
Null	343.68	0	343.68				315.51	0	315.51			
Additive, no Epistasis	336.02	2	340.02	0.85	0.01		309.50	2	313.50	0.06	0.55	
Additive, with Epistasis	335.56	3	341.56	0.66	0.04	0.57	309.47	3	315.47	0.28	0.51	0.87
COMPLETE DOMINANCE WITH EPISTASIS												
MAOA- high activity DAT1- 9-allele	336.50	2	340.50	0.80	0.03	0.96	307.35	3	313.35	0.13	0.16	0.96
MAOA- low activity DAT1- 9-allele	336.50	2	340.50	0.80	0.03	0.96	306.30	3	312.30	0.11	0.04	0.27
MAOA- high activity DAT1- 10-allele	*						*					
MAOA- low activity DAT1- 10 allele	*						309.50	3	315.50	0.97	0.14	0.22

*- Model cannot be estimated due to missing data

[1] P- Number of Parameters in Model

Table 5.11 Summary of Dopamine Models of Risk for Attention Deficit Hyperactivity Disorder

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction
Null	343.68	0	343.68				315.51	0	315.51			
Additive, no epistasis	214.87	2	218.87	0.62	0.04		122.35	2	126.35	0.47	0.36	
Additive, with epistasis	212.19	3	218.19	0.24	0.36	0.16	121.88	3	127.88	0.24	0.43	0.46
COMPLETE DOMINANCE WITHOUT EPISTASIS												
MAOA- high activity DAT1- 9-allele	217.36	2	221.36	0.58	0.11		121.35	2	125.35	0.27	0.21	
MAOA- low activity DAT1- 9-allele	217.36	2	221.36	0.58	0.11		122.27	2	126.27	0.75	0.20	
MAOA- high activity DAT1- 10-allele	214.54	2	218.54	0.66	0.03		123.06	2	127.06	0.24	0.99	
MAOA- low activity DAT1- 10 allele	214.54	2	218.54	0.66	0.03		124.06	2	128.06	0.74	0.92	

*- Model cannot be estimated due to missing data

[1] P- Number of Parameters in Model

Table 5.11 Summary of Dopamine Models of Risk for Attention Deficit Hyperactivity Disorder (continued)

	Males						Females					
				p-values						p-values		
Model	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction	Deviance	P[1]	AIC	*MAOA*	*DAT1*	Interaction
Null	343.68	0	343.68				315.51	0	315.51			
Additive, no epistasis	214.87	2	218.87	0.62	0.04		122.35	2	126.35	0.47	0.36	
Additive, with epistasis	212.19	3	218.19	0.24	0.36	0.16	121.88	3	127.88	0.24	0.43	0.46
COMPLETE DOMINANCE WITH EPISTASIS												
MAOA- high activity DAT1- 9-allele	215.84	3	221.84	0.58	0.32	0.32	121.29	3	127.29	0.26	0.34	0.81
MAOA- low activity DAT1- 9-allele	215.84	3	221.84	0.58	0.32	0.32	121.77	3	127.77	0.57	0.17	0.49
MAOA- high activity DAT1- 10-allele	*						*					
MAOA- low activity DAT1- 10 allele	*						*					

*- Model cannot be estimated due to missing data

[1] P- Number of Parameters in Model

Discussion

Main Genetic Effects Differ by Gender in the Etiology of ADHD and CD

Among females, the presence of a main effect of *MAOA* after controlling for *5HTTLPR* genotype as well as the main effect of the low activity genotype approaching significance after controlling for *DAT1* genotype suggests the importance of this gene within the serotonin and dopamine systems for CD diagnosis. The use of *5HTTLPR* and *MAOA* genotypes to model the serotonin system demonstrated the low activity *MAOA* genotype to be a moderate risk factor for CD diagnosis (OR = 1.70). A similar effect was observed in the model of the dopamine system (OR =1.58). In order to model genes functioning within specific neurotransmitter systems the joint rather than single effects of the multiple genotypes was tested. Modeling *MAOA* in conjunction with other genes in a neurotransmitter system underscores the weak role of *MAOA* genotype in individual vulnerability to CD.

In males, a significant main effect of the 9/9 *DAT1* genotype for both ADHD and CD was detected. Significant associations between the 9-repeat allele and externalizing disorders have been reported in other samples (Barkley et al., 2006; Young et al., 2002). This result is in contrast to several reports of the more frequent 10-allele having a significant association with ADHD (Barr et al., 2001; Chen et al., 2003; Cook, Jr. et al., 1995; Curran et al., 2001; Daly et al., 1999; Gill et al., 1997; Waldman et al., 1998a) (Swanson et al., 2000) and CD (Rowe et al., 2001). However, many of these reports are association studies using clinical samples undergoing pharmacological treatment. It has been suggested that some of the significant associations between the

10-allele and ADHD may actually reflect responsiveness to methylphenidate treatment as evidenced by changes in cortical activity (Barkley et al., 2006).

No significant main effects were detected for *MAOA* or *5HTTLPR* in males. It is possible that these genes are not as important to the etiology of CD in males compared to females, or that we simply do not have the power to detect significant main effects given the current sample size and their relative weak effects as evidenced by the female results.

The lack of significant relationship between *5HTTLPR* and CD or ADHD diagnosis has been reported elsewhere (Beitchman et al., 2003; Davidge, Atkinson, Douglas et al., 2004). However, these results contradict those of previous studies which reported significant associations between the short allele and CD as well as ADHD in males (Cadoret et al., 2003; Retz et al., 2004) and which utilized highly selected samples such as individuals at high risk for aggression/violence but also suffered from low sample size. Another study reported a significant association between the short *5HTTLPR* allele and aggression in children ages 7-9 within a community twin sample. However, this association was for teacher reports for children at age 9 only, and was not replicated in parental reports of aggression (Haberstick et al., 2006). It is difficult to determine whether the CD reported in the aforementioned study can be compared to the CD used here, since the current diagnosis relied on parental and child report and relates to a different developmental period. Recently, a study using a case-control design reported a significant association between the short allele and conduct disorder. However, the allele frequency of the case-control study was not in HWE, possibly due

to assortative mating which might result in population stratification of this sample. After using a transmission disequilibrium test (TDT) to control for population stratification by including parental genotypes and producing a "pseudo-sibling" as a control for each case used, no significant association between CD diagnosis and *5HTTLPR* was detected (Sakai et al., 2006).

Comorbid and Non-Comorbid Forms of CD and ADHD are Genetically Different

The 9/9 *DAT1* genotype was a risk factor for CD and ADHD in males. However, after controlling for comorbidity, the 9-repeat *DAT1* allele was only a significant risk factor for CD diagnosis. Additionally, after controlling for ADHD the main effect of *MAOA* on CD diagnosis in females was no longer significant in either serotonin or dopamine systems.

These results indicate that comorbid illness may be genetically different from ADHD or CD alone. *DAT1* in males and *MAOA* in females could highlight externalizing behaviors common to both disorders, since externalizing behaviors are often measured as symptoms similar to CD (Young et al., 2002). Alternatively, CD diagnosis is a confounder in the association of *DAT1* and ADHD, and should be controlled for in association studies of ADHD and *DAT1*. Another possibility is that the distinction between an ADHD+CD subtype is necessary to identify "pure" ADHD in association studies. Both issues have been identified (Thapar et al., 2001; Waldman, Rowe, Abramowitz et al., 1998b) but have not been addressed in previous association studies of candidate genes for ADHD.

Common additive genetic effects between CD/oppositional defiant disorder and ADHD symptoms have been reported in the VTSABD (Nadder et al., 2002; Silberg et al., 1996) as well as other samples (Dick et al., 2005; Thapar et al., 2001) and this result adds to the previous work on candidate genes for liability to comorbidity for CD and ADHD by gender. The previous studies demonstrating common genetic risk also reported a gender difference in genetic control. Consequently, future research on molecular genetic and environmental risk factors of externalizing behaviors may benefit from the use of a combined ADHD/CD phenotype and comparing it to the dimensions of ADHD (inattention and hyperactivity) alone since it is still unclear whether ADHD and CD can be viewed as a single or separate phenotypes (Volk et al., 2005).

No Significant Genotype-genotype Interaction in the Etiology of ADHD or CD

No significant epistasis was detected in either system for ADHD or CD. The general lack of epistasis in these models is not surprising, since its detection requires large sample sizes or genes with large effects. Estimates for detecting significant epistasis resulted in prohibitively large sample sizes for human population studies and reinforce the need to incorporate other model systems or modeling approaches to address epistasis in the etiology of CD and ADHD in humans. For example, modeling of the 5-HT presynaptic terminal while simulating genetic deletions also demonstrated significant epistasis with respect to presynaptic firing rates (Stoltenberg, 2005). McClay and van den Oord (2006) demonstrated that genotype-genotype interaction is statistically significant and readily reported when the transcriptional effects resulting

from allelic variation, as additive and dominance variance, are close to zero (McClay et al., 2006). However, in the presence of additive and/or dominance variance, epistasis will not be detected. Similarly, Culverhouse and colleagues demonstrated the inability to detect pure epistasis in the absence of additive and dominance variance using association studies (Culverhouse et al., 2002). Thus the absence of significant epistasis is not surprising since it is difficult to detect in systems of average human function compared to knockout systems, and the genes used might not be appropriate for study since they display weak effects. Moreover, as previous studies have stated, epistasis will be difficult to detect and describe with reference to human phenotypes.

These results should be interpreted while keeping several considerations in mind. First, the allele frequencies for *5HTTLPR* do not agree with those previously reported and make it difficult to compare our results to other studies (Kent et al., 2002; Volk et al., 2005). However, these genotypic frequencies are similar to those of previous studies and demonstrate the population to be in Hardy-Weinberg equilibrium. Additionally, given recent literature regarding other rare variants as well the uncertainty regarding the repressor versus enhancer effects of the *5HTTLPR* polymorphisms, it is difficult to draw conclusions about the functional significance of the polymorphism.

Second, genotypes were modeled using previous molecular work on mRNA transcription efficiency, protein function or protein density as well as previous association studies for ADHD and/or CD. The mean differences between genotypes thus may not reflect biological differences as a function of scale.

Third, the models of risk for CD and ADHD dichotomized these outcomes to reflect affected or non-affected status, while the assessment of power assumed a continuous outcome. Dichotomizing a clinical outcome as was done for CD and ADHD diagnoses is an arbitrary division of a truly continuous trait and leads to a major loss of power due to a loss of information. Thus, larger sample sizes would be necessary to detect significant effects for the binary outcome of CD and ADHD diagnoses used in these analyses.

Fourth, the low prevalence of ADHD decreases the power to detect main genetic effects especially in the presence of CD. Therefore, future studies of a common gene approach to ADHD and CD would benefit from the use of more data to better control for comorbidity.

Fifth, these analyses tested several models of genetic risk for CD and ADHD and any significant effects have not been corrected for multiple comparisons. However, any significant main effects that have been detected have been marginal and are expected to be non-significant after correction for multiple testing. Lastly, this sample consists only of Caucasian participants, and these results are not anticipated to generalize to other ethnic populations, since the distribution of *5HTTLPR* alleles vary widely between different ethnic groups. For example, a Caucasian sample allele distribution had 40% short and 60% long alleles (Kent et al., 2002). In a Chinese sample and another of Korean participants, the allele distribution was approximately 72% short allele and 28% long (Chong, Lee, Tay, Chan, & Tan, 2000) (Kim, Badner, Cheon et al., 2005). Further, these two studies eventually reported conflicting results,

with the long *5HTTLPR* allele significantly associated with ADHD in the Korean sample (Kim et al., 2005), while the short allele had a significant association with ADHD in the Chinese sample (Li, Wang, Zhou et al., 2006). Further investigation of these polymorphisms using other populations is necessary to understand the etiology of ADHD and CD in non-Caucasian populations.

CHAPTER 6 Screening a Community-Based Sample of Adolescents for Environmental Exposures Related to Conduct Disorder Using Random Forest Classification

Abstract

The identification of environments for conduct disorder outside of childhood adversity expands the definition of environmental risk and could result in the opportunity to test whether GxE is detectable using other environmental risk factors. Large-scale genetic studies such as the VTSABD have measured several different environments at various levels of risk, for which certain aspects have often been associated with CD in the literature. However, identifying alternate environmental risk factors measured in a sample of single item measures is difficult using traditional methods because of the prohibitively large number of items assessed. Additionally, when presented with a variety of environmental risk factors, most of which have been included for study because of their associations with psychopathology, it is difficult to know which environments would be best for the detection of GxE. It would therefore be instructive to take a dataset of environmental risk factors and reduce the number of variables to the ones that are most important to the classification of an outcome using supervised learning methods.

Identification of environmental exposures that classify individuals for CD status consisted of determining the most important variables and assessing how well they classified observations for CD diagnosis. A random forest consisting of 2000

classification trees was produced to rank the most important environments classifying individuals by CD diagnosis.

The variables considered to be most important for the classification of CD were validated by building a series of separate random forests and comparing the out-of-bag estimate of each against that of the original random forest. The variables considered to be most important were assessed to determine if they produced meaningful CD classifications using Multidimensional Scaling using proximity measures obtained from random forest algorithm.

The random forest approach identified several environments that have been previously identified as risk factors in the literature. However, variables determined to be "important" for CD classification require careful examination prior to inclusion in models of GxE since no items were found to be strong classifiers of CD. This weak ability to classify individuals into CD diagnosis groups is not surprising since "environment" functions simultaneously at various levels in a variety of ways. Small effects for any particular risk factor are sensible since clear risk for antisocial behavior has been reported to accrue only when a person accumulates a large number of risks (Rutter, Giller, & Hagell, 1998), each of which each may have a small effect (Daniels & Plomin, 1985). Further, this study assessed the effects of environments alone. Once included in an appropriate model, the effect of the environment conditional on genotype may increase the effect of the environment (Moffitt, 2005).

Introduction

The identification of multiple environments could improve the definition of the environment and detection of genotype-environment interaction (GxE) in psychopathology. In the case of CD, the identification of other environments in addition to childhood adversity would allow us to test whether GxE is detectable using other environments. Additionally, the use of other environments would assess how the effect of GxE may be influenced by the definition of the environment. Examples of proximal environmental risk factors associated with CD include poor parenting, physical/sexual abuse, parental neglect, parental antisocial personality disorder, maternal prenatal smoking, birth complications, lead exposure, and negative child temperaments (ie: negative emotionality, intense/reactive responding and inflexibility) . Distal, or community level risk factors have also been reported including community disorganization, unemployment, neighborhood violence, and community availability of drugs (Bassarath, 2001; Burke et al., 2002; Raine, 2002; Simonoff, 2001).

Large-scale genetic studies such as the VTSABD have measured several of these environmental risk factors. The use of single items to reflect a specific environmental exposure for GxE has been successfully implemented in previous studies of CD(Foley et al., 2004; Haberstick et al., 2005; Nilsson et al., 2005), encouraging the systematic review of the environment to identify single items within measurement scales that are related to CD classification. In an effort to replicate the initial report of GxE, many studies chose variables of environmental exposure that were similar in content to the original items used. However, these items were often part of and

optimized for use in other measures. As a result, the definition of environmental exposure utilizes individual items that approximate certain aspects of environmental risk. The use of single items in recent studies of GxE suggests that certain features of the environment, measured by a few items might appropriately estimate environmental risk. Systematic evaluation of single environmental items using supervised learning approaches may provide alternate measures of the environment to test GxE. However, when multiple measures of environmental exposure are available, the task of environment identification using conventional approaches is prohibitively large and is subject to the prior knowledge of the analyst.

Linear models are often used to assess the predictive strength of variables of interest. This is accomplished by picking those variables or scales that will minimize the residual sum of squares and in turn do an adequate job of predicting an outcome. Variables are often chosen for a model inclusion prior as a result of an interest in testing a relationship that has been previously addressed in the literature. When multiple variables are present, it is necessary to select those variables that will best predict the outcomes. Methods that rely on p-values to assess variable importance such as forward selection or backwards elimination are limited in their ability to handle large amounts of data to produce predictive models. Forward selection allows the user to introduce variables one at a time and include those variables that significantly predict the outcome as determined by the p-value. Variables that are not significant predictors of the outcome are not included in the model. As more variables are included in a model, the model does a better job in explaining an outcome, as evidenced by increasing values of

R^2 or decreasing values of deviance or likelihood which indicate improved model fit. The backwards elimination approach uses a "full" model or one with a full complement of predictors, and iteratively eliminates a single non-significant item until a simplified model with only significant predictors. Both of these methods are largely dependent on the degree of correlation between the predictor variable and the outcome. Further, as the number of parameters in the model increase, it becomes unnecessarily complex resulting in "overfitting", where a model with many predictors has too many free parameters to estimate given the number of observations in the data and will not likely be applicable for use in other samples. Additionally, the number of variables to be included in a model is not infinite because of its dependence on the sample size. In general, a fitted regression model is likely to be reliable when the number of predictors is less than the limiting sample size, which is a factor of the total sample size and varies by data types. Therefore traditional linear models cannot be built using datasets with more measures than observations, and are often forced to test a limited number of items which are driven by the literature. This results in the study of the same variables and constructs due to bias towards the most frequently reported results (Harrell, 2001).

For a large-scale dataset with several hundred observations and an equal number of items that include a variety of data types, the time required to test an environmental exposure using a traditional linear modeling approach for a set of single items would be prohibitively long. Additionally, the measurement of environmental exposure within large datasets is based on theoretical insight and the associated measurement scales have been optimized from previous studies to measure a specific construct of the

environment. As a result of the pointed refinement of the environmental measures, many of the environments measured included in a study of environmental risk for psychopathology are expected to be associated with the an outcome of interest. However, specific genetic and environmental risk factors are expected to result in the detection of significant GxE (Moffitt et al., 2005). When presented with a variety of environmental risk factors, most of which have been included because of their associations with psychopathology, it difficult to know which environments would be best for the detection of GxE and the prospect of testing each variable for an outcome of interest seems inefficient. It would therefore be instructive to take a dataset of environmental risk factors and reduce the number of variables to the ones that are most important to the classification of an outcome and follow those up with the detailed linear model fitting approaches. One such an approach could be addressed using supervised learning methods, which predict the value of an outcome measure based on a number of input measures. The advantage of using such methods is the ability of the algorithms to make decisions that are based on the similarity between a given outcome and a variable of interest.

Supervised Learning Approaches

Supervised learning refers to the various statistical approaches used to identify relevant patterns from large amounts of data using machine learning algorithms. Data that are appropriate for use with these approaches have a high number of dimensions (variables), a mixture of data types, a non-standard data structure, and are

heterogeneous for different aspects of the data. Supervised learning can produce accurate classifiers, which can be used to reduce the volume of data for more detailed consideration for an outcome of interest. Additionally, these approaches can also determine the predictive structure for an outcome of interest when select variables are used. The random forest algorithm is a powerful supervised learning tool that can be used for the identification of classification variables, which in turn can be used for data reduction of large datasets. Random forests are based on classification and regression trees. Additionally, the current work focuses on the use of variables to classify individuals in to CD diagnosis groups. Therefore, classification tree construction will be discussed first.

Classification Trees. Classification trees, introduced by Leo Breiman have been used for identifying variables that characterize an outcome from datasets with a high number of dimensions and/or having a mixture of data types (ie: categorical, ordinal or binary) (Breiman, Friedman, Olshen, & Stone, 1984). Classification trees are generally constructed by first taking a single variable ("root node") that best partitions all of the data into 2 subgroups ("daughter nodes") with respect to an outcome of interest. The data are partitioned a second time for each of the 2 subgroups to produce 2 additional daughter nodes. These partitions, or "splits", are recursively produced until the subgroups either reach a minimum size (ie: 5 observations per node) or until no improvements can be made to each subgroup, resulting in "terminal nodes". The tree is then trimmed back to identify the variables that are most important to the classification

of the outcome. Thus, the construction of a classification tree is dependent on (1) the selection of the splits, (2) a goodness-of-split criterion to evaluate how well the split distinguishes the resulting nodes from one another, (3) the decisions required to declare that a node needs further partitions or whether no extra partitions are necessary (a "stop-splitting rule"), and (4) the assignment of a class label to the terminal nodes.

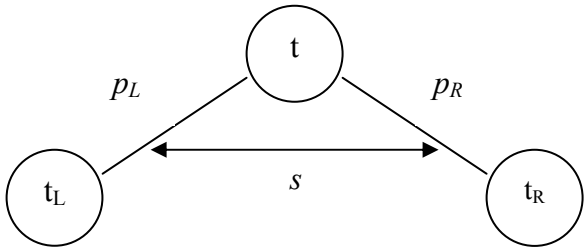

Figure 6.1 Example of a Single Classification Split

Figure 6.1 visualizes how a single split is constructed. The root node *t* is split into 2 daughter nodes t_L and t_R by the candidate split *s*. A proportion (p_L) of the cases of *t* go to node t_L and a proportion (p_R) goes into t_R. p_L is defined as $p_L = \frac{p(t_L)}{p(t)}$ and p_R is defined as $p_R = \frac{p(t_R)}{p(t)}$ and their sum is equal to one ($p_L + p_R = 1$). Thus, the node proportions for p_L and p_R reflect the "predicted class probability"($p(j|t)$), or the proportion of the cases in a node t_L *or* t_R that belong to a particular class *j*.

Splits are selected by taking each variable in a measurement vector **X,** where **X** = (x_1, x_2, …, x_m) and determining the optimal cut points for each variable based on that which classifies observations into the outcome categories. Splits for categorical

variables identify the optimal partitioning of the nominal classes into two groups. Splits for ordinal and continuous variables identify the optimal cut point that best classifies the observations. The best split for each variable is then compared to determine which variable produces the best classification such as the minimum impurity in the descendent nodes.

The goodness-of-split measure determines how well a node splits the data into descendant nodes by quantifying the decrease in node impurity between nodes. Goodness-of-split is defined as:

$$\Delta i(s,t) = i(t) - p_L i(t_L) - p_R i(t_R) \tag{6.37}$$

where $i(t)$ is a measure of node impurity of the parent node t, $p_L\, i(t_L)$ is the impurity measure for node t_L and $p_R\, i(t_R)$ is a measure of impurity at node t_R. Node impurity measures the heterogeneity of classes within a node. Further, node impurity is largest when all classes are equally mixed together in a node and smallest when the node contains only one class. Generally, node impurity is defined as a function of the class probabilities within a specific node t for two classes ω_1 and ω_2 by some impurity function, φ. Thus,

$$i(t) = \varphi(p(\omega_1|t), p(\omega_2|t) \tag{6.38}$$

The measure of impurity for an entire classification tree ($I(T)$) is defined as the sum of the values of impurity for each node in the tree, or

$$I(T) = \sum_{t \in T} i(t)\, p(t) \tag{6.39}$$

where *i(t)* is the impurity measure for node *t* and *p(t)* is the probability an observation is in node *t*. The Gini index is a commonly used function (φ) for measuring impurity because it improves the construction of multiple splits in a tree and is defined as

$$i(t) = \sum p(j \mid t) p(i \mid t) \tag{6.40}$$

The classification tree will stop splitting when the value of the node impurity is low or when a node is homogeneous within a class or when there are too few observations for further splitting. This results in an overly-large tree with many terminal nodes, each containing a few observations. In order to produce a smaller sub-tree that appropriately classifies cases in a node, it is necessary to "prune" the overly-large tree upward to minimize misclassification. Pruning a tree consists of estimating the re-substitution error for each node in the overly-large tree (T_{max}) and progressively pruning T_{max} at each node upward to its root node, so that the re-substitution rate at each stage of pruning is as small as possible. The pruned sub-tree reflects the tree with the fewest nodes that also produces the lowest misclassification error.

The proportion of cases that are misclassified in a node is defined as the re-substitution estimate, *r(t)*. The general re-substitution estimate for a single node *t* is calculated as 1 minus the predicted class probability for a class *j*, or

$$r(t) = 1 - \max_j p(j \mid t) \tag{6.41}$$

The cost of misclassification must be included in the re-substitution estimate because the probability of classifying groups is dependent on the probability of the groups

occurring in the sample. Therefore, the use of the misclassification estimate allows the construction of the classification tree to include the actual proportion of cases in a sample and to penalize the node for misclassified observations. The re-substitution estimate from equation 6.21 is then modified to include misclassification cost for node *t* as

$$r(t) = \min_i \sum_j c(i|j)\, p(j|t) \tag{6.42}$$

where *c(i|j)* reflects the cost of misclassifying a class *j* object as a class *i* object and is either $c(i|j) \geq 0$ if $i \neq j$ or $c(i|j) = 0$ if $i = j$.

The re-substitution rate for a tree classifier (T) is the sum of the proportion of misclassified cases for each class at every node including the terminal nodes ($\tilde{T}$), or

$$R(T) = \sum_{t \in \tilde{T}} r(t)\, p(t) \tag{6.43}$$

The estimation of misclassification error cost is obtained by using an independent test dataset or by employing a bootstrap or cross-validation approach. The independent test dataset approach takes a fixed number of cases from the dataset and withholds them from the tree growing procedure. After the tree is derived, an independent test dataset is run through the classifier, the predicted class is obtained and the error rate is estimated. Alternatively, cross-validation estimates misclassification through the construction of a number of sub-samples, each containing the same proportion of cases as the original sample. Each sub-sample can then be used to construct separate classification trees. The misclassification error of each sub-sample is separately estimated and then summed to produce an estimate of overall misclassification.

Random Forests. Random forests extend the classification tree algorithm by growing a series of several hundred non-pruned classification trees. Maximal sized trees are built by drawing bootstrap samples from the original data with replacement to form a learning set. A tree is then derived for each bootstrap sample. The aggregated trees then yield a vote for the predicted class for each observation (Breiman, 2001).

Random forests utilize the out-of-bag (OOB) observations to estimate misclassification error. OOB error for a single tree is calculated by producing a classification tree from a bootstrap sample of the data using approximately 2/3 of the original data (the test set). A second sample, the "out-of-bag" sample is produced using observations remaining in the original dataset (the training set). The OOB sample is then used to test the tree produced by the first 2/3 of the data and estimate how well the tree resulting from the test sample classified groups from the OOB sample. The proportion of times the classification of the OOB observation did not match the classification from the tree was averaged across all trees to produce the overall OOB error estimate.

The OOB sample is also used to estimate variable importance, which also determines how well a variable predicts classification groups. Variable importance is estimated by permuting a single variable in the OOB sample and then running the OOB observations down the tree produced by the training set. The proportion of correct classifications from the variable-permuted OOB sample is compared against the proportion of correct classifications in the OOB sample to produce the variable

importance score (Importance Score = error OOB permuted – error OOB). Therefore, variables that cause the largest Importance Score difference are considered to be the most important variables. Each classification tree provides its "vote", for variable importance and votes for each variable are averaged across trees. Importance scores for all variables are assessed in this fashion, and the variable with the highest importance score across trees is determined to be the most important predictor variable.

The generation of proximity values is a useful by-product of random forests. Proximity values offer a measure of how often pairs of observations lie in a terminal node of the classification trees. Since each classification tree produced using random forest is unpruned, the number of observations in each terminal node is low. The proximity value between two observations increases as the number of instances in which they occur together at a terminal node also increases. The proximity measure is estimated by running observations from the test and OOB samples down a classification tree and assigning a value of 1 for terminal nodes containing both observations from the test and OOB samples. This procedure is repeated for all trees produced by the random forest.

The proximity measure can be transformed to produce a measure of similarity between two observations, which can be used to assess outliers in the data and clustering of observations. The squared distance between two observations is calculated as 1- proximity measure. This squared distance reflects the similarity between the two observations.

Multidimensional Scaling. Classical mutidimensional scaling (MDS) is a method for displaying high dimensionality data in low dimensional space. The goal of MDS is to map classes to observations through a measure of dissimilarity for an item between two observations. Dissimilarity is quantified using the distance measure, for two different observations (x_i and x_j) in a given space, v (Gower, 1966).

$$d_{ij}^2 = \sum_{r=1}^{v} (x_{ir} - x_{jr})^2 \tag{6.44}$$

Therefore, lower values of squared distance reflect observations that are more similar. It is anticipated that predictor variables that produce distinct classification groups will produce small distances for observations within the same class, while having larger distances for observations in different classes. Similarly, poor classification variables will have similar distances for observations in every class.

Methods

Data Analysis

Identification of environmental exposures that classify individuals for CD status consisted of determining the most important variables and assessing how well they classified observations for CD diagnosis. First, a random forests approach was used to rank the most important environments classifying individuals by CD diagnosis. A random forest consisting of 2000 classification trees was produced across the entire sample. The predicted classes were weighted to reflect the expectation that 10% of the

population was affected with CD and 90% of the population was unaffected to decrease misclassification error. The variables considered most important for the classification of CD were determined by using variable importance scores, with higher scores indicating greater importance.

Once all the variables were ranked by importance from the initial random forest, a select group of the most important variables were identified for future study. This group was chosen through an iterative process, which consisted of (1) Using the two most important variables identified in the first run in a second random forest, (2) Comparing the OOB estimates from the random forest using only the two most important variables against that of the original random forest, (3) Adding the third most important variable to the previous list and assessing the OOB estimate. The new random forests were compared against the original random forest, and the new random forest that had an OOB estimate producing the smallest difference with the original OOB estimate was considered to have the most important variables (Breiman, 2001). Thus, it is anticipated that the smaller list of highly important variables also having an OOB estimate similar to the original random forest will result in CD classifications comparable to that of the full list of variables.

Second, the final list of most important variables were assessed to determine if they produced meaningful CD classifications by Multidimensional Scaling using proximity measures obtained from random forest. It is expected that strong classifiers would place observations into two distinct groups by CD diagnosis.

All analyses were performed in R 2.3.1: A Language and Environment for Statistical Computing (R Development Core Team, 2006). Random forests were conducted using the randomForest function within the randomForest package (Liaw & Wiener, 2002). Classical Multidimensional Scaling was conducted using the cmdscale function within the stats package.

Study Population

The current study uses a sub-sample of the VTSABD for whom environmental data was collected at any of the four waves and for whom *MAOA* genotype, CD diagnosis, maternal ASP and childhood adversity were determined, resulting in a sample of 1299 individuals. However, participation varied across waves as a result of either loss to follow-up or aging-out of the sample at different waves. Further, a disadvantage of Random Forests results from the algorithm's inability to manage missing data. Although imputation via use of (1) the sample median for a variable (for continuous data), (2) the most frequent category (for categorical data), or (3) a measure of predictor variable proximity are acceptable means of handling missing data, it may distort the true nature of the data, especially if there is a systematic reason for the occurrence of missing data (Breiman, 2001). Therefore, each measure of the environment only includes those observations for whom *MAOA* genotype was measured and had no missing data for a particular environmental measure. Additionally, each measurement scale was assessed separately in an effort to identify the aspects within each level of environmental exposure that are important for the classification of CD. Consequently,

the sample sizes used for each environmental measure vary between measures. Finally, gender was also included in all evaluations of CD classification because gender is a common risk factor for CD and certain environments are likely to result from gender (ie: peer group composition). *MAOA* genotype was not included in these analyses in order to identify those environments that are most important to CD classification. Additionally, since the detection of statistical interactions is improved in the presence of a significant main effect (Chapter 2), and main genetic effects for an outcome are not often expected to be large (Chapter 5), the identification of risk environments alone is anticipated to result in better models of GxE.

Data Collection

Life Events. The Life Events Questionnaire consisted of 39 items that determined the occurrence of common life events within the previous 12 months (N = 1258). Examples of items included death of a parent, special recognition for achievement, moving to a new neighborhood, and a close friend moving away. Twin responses to the binary items were measured across four waves for twins. The sum over the four waves was calculated to determine the total number of times a particular life event occurred for an individual.

Parental Psychopathology. Measures of mental disorders in parents were obtained using (1) an assessment protocol developed for the study of adult twins. The July 1, 1985 draft version of the Structured Clinical Interview for DSM-III-R was adapted to measure major depression, generalized anxiety disorder, antisocial

personality disorder, panic disorder, alcoholism, (2) the Diagnostic Interview Schedule for DSM-III to measure phobias and (3) the Adult Personality Functioning Assessment to measure antisocial personality disorder. Responses to these items produced a diagnosis for each disorder (Foley, Pickles, Simonoff et al., 2001). Additionally, the number of disorders in an individual was summed to reflect a total number of disorders. Both the individual diagnoses and the sum of the number of disorders was used to assess CD classification (N =910).

Peers. Twenty-six peer variables were obtained from section R (Peer Relations) of the VTSABD protocol (N = 956). Items asked respondents to report the number of peers from both close and wide peers groups that engaged in certain activities such as skipping class in the previous 3 months, getting drunk more than once in the previous 3 months, or getting in trouble with the police. The number of male and female peers engaging in each activity was assessed separately. Additional items measured the composition of the group through peer group size (number of peers) for close and wide peers groups as well as the gender of the peer group (ie: mostly male, female or a mixture of both genders).

Demographic/Census Variables. Two types of variables were used to assess socioeconomic status (N = 905). Sixty-nine variables were obtained from the 1990 United States block group census data. A block group is defined as the immediate area around a household, and these variables measure the neighborhood surrounding a twin family. Examples of items measured are block group educational attainment, income, housing type (ie: high-rise apartments or single family homes), and average household

income. Another 18 variables were collected during the first wave regarding parental assessment of their own socioeconomic status, including education, occupation, and home description (number of rooms, owning vs. renting and value of home) (Meyer et al., 1996).

Pre-/Perinatal Environment. Maternal reports of pregnancy and the first year of life were obtained from section A of the VTSABD protocol (N = 1126). Items included questions on gestational age, smoking/alcohol consumption during pregnancy, type of birth, and hospitalization after birth. Items were measured at wave 1.

Family Environment. Ninety-seven variables reflecting the parent interaction with the twins were assessed using items from the Early Home Environment Measure (Robins, Schoenberg, Holmes et al., 1985) (N= 828). Items included time spent with twins, parental affection or anger towards twin and parental drug use. The items used to measure family environment were obtained from twin responses collected at wave 3. Twin reports of family environment were used over parental reports of the same measures to reflect the environmental exposure as perceived by the child.

Diagnosis of Conduct Disorder. The sample consisted of data on individual twins registered in the VTSABD on previous 3-month history of CD as assessed with the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al., 2000) and reported by maternal, paternal or child self-report using a symptom-or rule at any wave of data collection. Under the symptom-or rule, a symptom was rated as being present when either parent or child endorsed the item.

Results

Table 6.1 details the variable importance statistics for the life events variables considered to be most important in the classification of CD diagnosis. The 4 items determined to be most important highlighted the recognition of individual achievement. The following 6 items reflected a loss or change of some feature of the environment such as death or a close friend moving away. Items with the largest mean decrease Gini represent variables whose splits result in the largest improvements in node impurity. Further, the mean decrease accuracy provides an estimate of how much an item contributes to the prediction accuracy of CD diagnosis. None of the values for mean decrease accuracy are large, suggesting that no single item is good at classifying CD.

Table 6.1 Variable Importance Statistics for Life Events Items

Item	Mean Decrease Accuracy	Mean Decrease Gini
Made an athletic team, band or other special group	-0.02	23.45
Received a special prize or recognition for doing well in an activity	-0.02	22.64
Joined a new club	-0.02	19.86
Received special recognition for good grades	-0.02	20.25
Death of a grandparent, uncle or aunt	-0.01	18.2
Death of a pet	-0.01	16.1
Breaking up with someone you have been regularly dating	-0.01	14.53
Changing to a new school	-0.01	17.24
A close friend moved a far distance away	-0.01	17.12
Gender	-0.01	11.68
Regularly dated a new person	-0.01	13.84

Table 6.2 details the variable importance statistics for the parental psychopathology diagnoses considered to be most important in the classification of CD diagnosis. The number of parental diagnoses was more important than the actual diagnoses. Further, of the disorders, simple phobia and major depression in both parents were most important.

Table 6.2 Variable Importance Statistics for Measures of Parental Psychopathology

Item	Mean Decrease Accuracy	Mean Decrease Gini
Number of paternal diagnoses	-0.04	12.81
Number of maternal diagnoses	-0.06	11.91
Gender	0.01	7.57
Paternal simple phobia	0	5.81
Paternal major depression	-0.02	4.44
Maternal simple phobia	-0.02	3.97
Maternal major depression	-0.04	4.03
Maternal social phobia	0	3.55
Paternal alcoholism	-0.01	3.47
Maternal generalized anxiety disorder	-0.01	3.17
Paternal social phobia	-0.01	2.85
Paternal generalized anxiety disorder	-0.01	2.67
Maternal alcoholism	0	2.29
Maternal panic disorder	0	2.27
Maternal agoraphobia	0	1.89
Paternal antisocial personality disorder	0	1.87

Table 6.3 details the variable importance statistics for the peer group items. The most striking aspect of these items is that peer group size rather than the activities that the individuals engage in was considered most important for the diagnosis of CD. Additionally, the number of boys either in close or wide peer groups was more important that the number of girls.

Table 6.3 Variable Importance Statistics for Peer Group Items

Item	Mean Decrease Accuracy	Mean Decrease Gini
Size of peer group	-0.02	32.3
Number of girls in wide peer group	-0.01	30.08
Number of boys in wide peer group	-0.02	32.27
Number of girls in close peer group	-0.01	20.22
Number of boys in close peer group	0	22.07
Peer group sex composition	-0.01	15.88

Table 6.4 details the variable importance statistics for the census variables. Educational attainment was very important in classifying CD. In particular, two items identified low levels of neighborhood educational attainment ranging from elementary- to associate degree- studies. Additionally, two items identified occupation for the classification of CD. Low levels of socioeconomic status appear to classify CD diagnosis.

Table 6.4 Variable Importance Statistics for Census Measures of the Neighborhood

Item	Mean Decrease Accuracy	Mean Decrease Gini
Proportion of individuals in block group with 9-12th grade education but no high school diploma	0.03	44.63
Proportion of individuals in block group with secondary expenditures consisting of alcoholic beverages	0.02	32.99
Proportion of individuals in block group with an associates degree	0.02	30.93
Proportion of individuals in block group working as handlers, equipment cleaners, helpers, and laborers	0.02	27.08
Paternal Occupation	0.02	19.26

Table 6.5 details the variable importance statistics for the pre- and peri-natal environments. Birth weight, gestational age and birth complication were considered to be the most important classifiers of CD diagnosis. Maternal prescription drug use during pregnancy was also considered to be an important variable, although the mean decrease in accuracy was 0 and suggests that it is a weak classifier

Table 6.5 Variable Importance Statistics for Pre- and Peri-natal Environments

Item	Mean Decrease Accuracy	Mean Decrease Gini
Birth weight	-0.01	31.86
Gestational age	-0.02	24.88
Birth complications	-0.02	11.48
Gender	0	6.55
Maternal use of prescribed medication during pregnancy	0	6.09

Table 6.6 summarizes the variable importance statistics for family social environment. Three general groups of items appeared to be most important, including demonstration of parental affection, parental awareness of the child's friends and parental discipline.

Table 6.6 Variable Importance Statistics for Family Social Environment

Item	Mean Decrease Accuracy	Mean Decrease Gini
Frequency of parental drinking	0	22.65
Father hugs and kisses a lot	0.01	13.67
Mother hugs and kisses a lot	0	13.12
Gender	0	9.52
Number of friends mother knows	0	10.79
Number of friends father knows	0	11.50
Parents enjoy being together	0	10.39
Father breaks promises	0	7.32
Child has a curfew on school nights	0	7.36
Child has worried about a family member in the past 5 years	0	9.09
Child receives discipline from mother	0	7.65
Child has a curfew on weekend nights	0	8.21

Table 6.7 summarizes the misclassification errors using the OOB estimate for each measurement of environmental exposure. The full OOB estimate details misclassification error using all items for each measurement. The reduced OOB estimate reflects the misclassification error for the items determined to be most important by random forest as summarized in tables 6.1-6.6. In general, the reduced error estimates were slightly higher than the full measures, reflecting increased misclassification due to fewer items. In the case of parental psychopathology, the reduced OOB estimate was better than the full OOB estimate, which suggests that the full complement of parental disorders is not necessary. However, parental psychopathology had OOB estimates of more than 50%, which suggests that parental psychopathology was not a good predictor of CD diagnosis. This weak ability to classify CD risk is also demonstrated in figures 6.2-6.7, which illustrate item

dissimilarity. These items do not differentiate CD and non-CD groups into separate clusters and are thus weak predictors of CD.

Table 6.7 Out-Of-Bag Estimates for Full and Reduced Environmental Measures

Environmental Measure	Full OOB Estimate (%)	Reduced OOB Estimate (%)
Life Events	9.63	12.65
Parental Psychopathology	59.63	56.77
Pre-/Perinatal Environment	20.27	23.20
Peers	11.52	15.81
Census	11.05	11.60
Family Environment	10.64	11.73

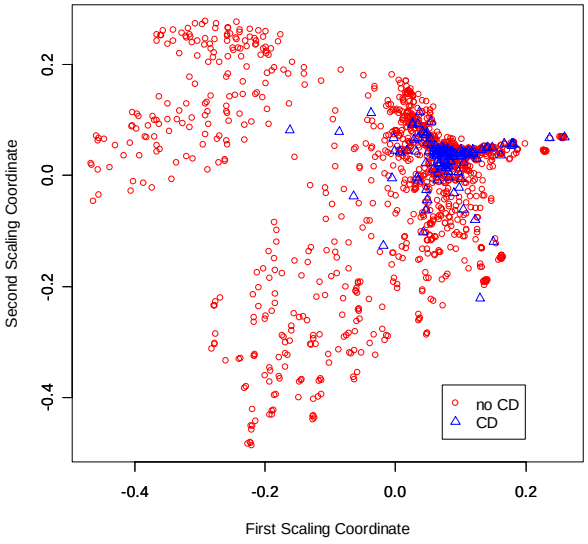

Figure 6.2 Multidimensional Scaling Plot of Life Events Items

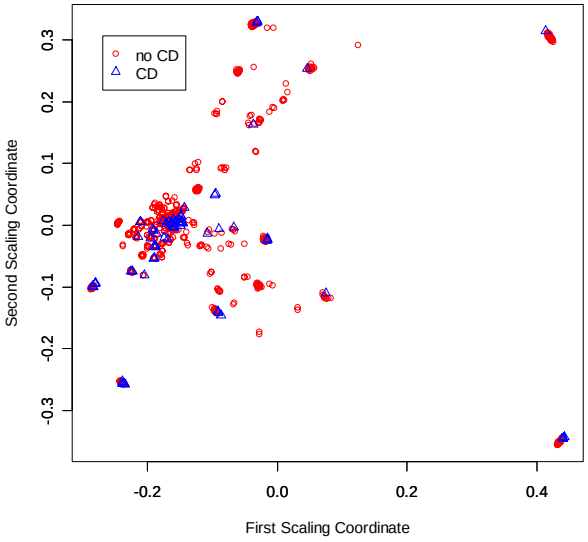

Figure 6.3 Multidimensional Scaling Plot of Parental Psychopathology

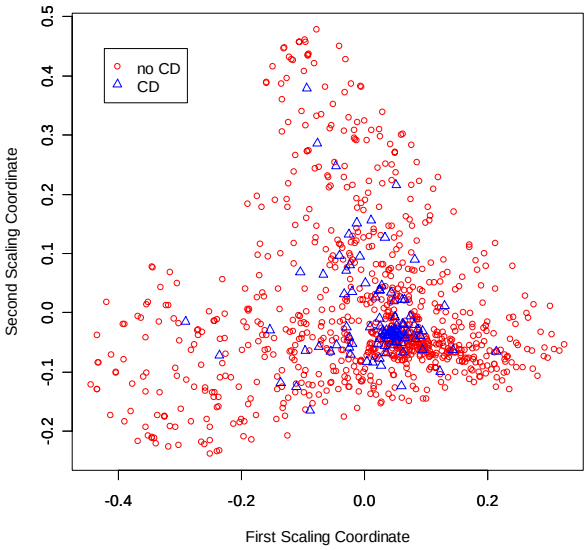

Figure 6.4 Multidimensional Scaling Plot of Peer Groups

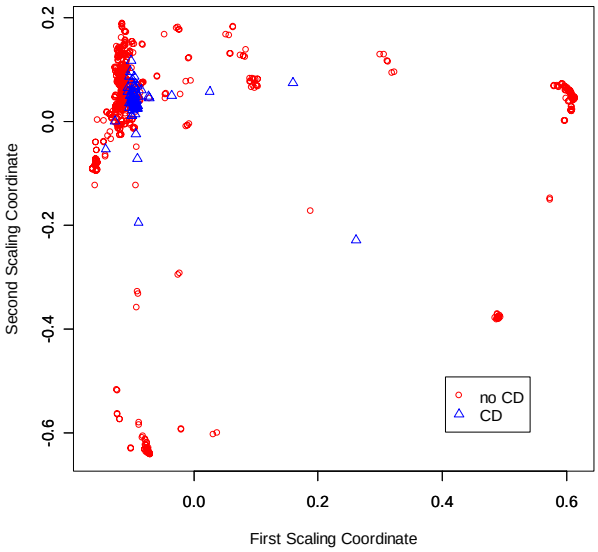

Figure 6.5 Multidimensional Scaling Plot of Census Measures

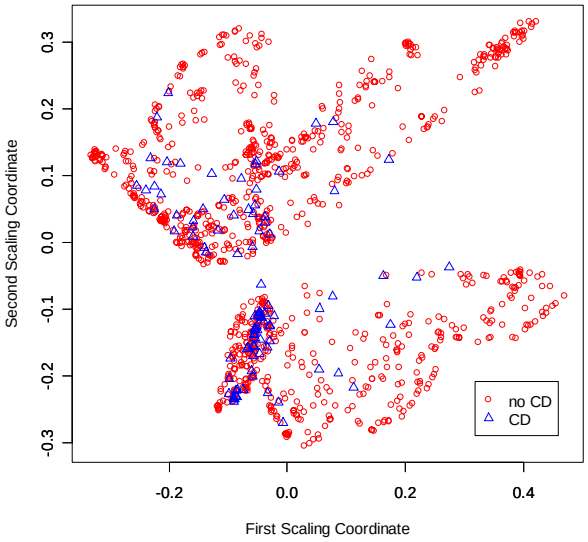

Figure 6.6 Multidimensional Scaling Plot of Pre-and Perinatal Environments

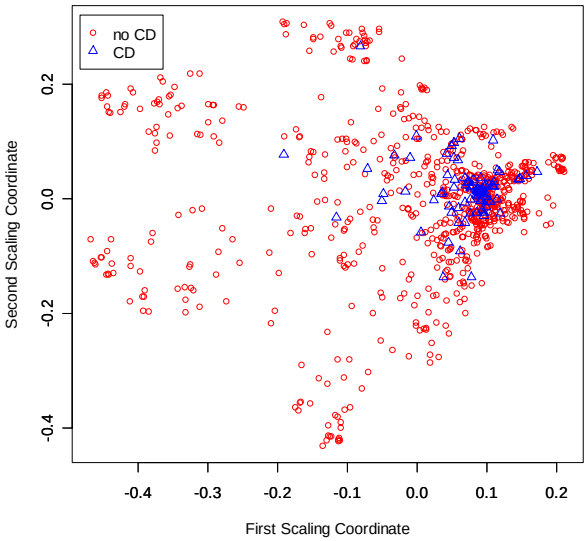

Figure 6.7 Multidimensional Scaling Plot of Family Social Environment

Discussion

Recent reports of GxE in the development of CD have defined environmental risk as exposure to childhood adversity, particularly household neglect and abuse. However, several other risk factors for CD have been separately identified across varying levels of exposure. In an effort to replicate the initial report of GxE, many studies chose variables of environmental exposure that were similar in content to the items used in the original study. The use of single items in recent studies of GxE suggests that certain features of the environment, measured by a few items might appropriately estimate environmental risk. Therefore, the identification of environmental exposure as measured by a series of single items offers an opportunity to improve the definition of the environment and further understanding of GxE.

Large-scale twin studies of genetic and environmental risk factors such as the VTSABD have measured several different environments at various levels of risk, many of which have often been associated with CD in the literature. The use of single items to reflect a specific environmental exposure for GxE has been successfully implemented in previous studies, encouraging the systematic review of the environment to identify items within larger measurement scales that are related to CD classification. However, when multiple measures of environmental exposure are available, the task of environment identification using conventional approaches is prohibitively large. A supervised learning approach using the random forest algorithm was used to rank variables measuring environmental exposure according to their importance in classifying CD diagnosis. Out-of-bag estimates and visualization of CD affected and

non-affected clusters using MDS assessed how well these variables classified the two groups.

Life Events

Three classes of items were most important in the classification of CD, recognition of achievements, loss and dating relationships. Recognition of achievements has been reported as a protective factor against CD (Bassarath, 2001). Conversely, academic underachievement has been reported as a risk factor for CD(Mandel & Mandel, 1995; Mandel, Marcus, & Dean, 1995). However in these analyses, "having failed a class" was not considered highly important. This suggests that the acknowledgement of success by others may encourage behavior that is protective against CD. Interpersonal relationships such as those that occur in dating others have been suggested as an environment that is specific to female antisocial behavior (Moffitt et al., 2001a). Additionally, females with CD often associate with antisocial partners (Robins, Tripp, & Pryzbeck, 1991), which may also result in an environment that promotes CD-related behaviors. It would be interesting to look at these two dating variables and determine whether any association with CD would differ by gender.

Parental Psychopathology

While the number of parental disorders was considered most important for CD classification, simple phobias, depression and alcoholism were also highlighted. CD

diagnosis in the child has been previously associated with each of these disorders in at least one parent for this sample (Foley et al., 2001). In a study of psychiatric disorders in parents and children, maternal alcoholism was associated with an increase in conduct disorder symptoms for males and females. Paternal alcoholism was also associated with a significant increase in CD symptoms in males and females, with males having a larger increase in symptoms than females. Further, maternal depression and parental alcoholism were reported to increase CD symptoms for both males and females, with the increase in males being larger than in females. Maternal and paternal alcoholism were found to increase the number of CD symptoms in males and females, with paternal alcoholism resulting in a greater increase in symptoms among males. Paternal simple phobias were shown to increase the number of oppositional defiant disorder (ODD) symptoms in males only. ODD is defined as antisocial behaviors reported during childhood and is often a precursor to CD in adolescence.

Peer Influences

The number of individuals in a peer group was identified as an important classifier of CD diagnosis. Further, the number of boys in a peer group was more important than the number of girls. It has been noted in research of deviant peers that deviant behavior is concentrated in certain adolescent groups and if one member of a group engages in problem behavior, it is likely that the other members in the group will follow suit (Cairns, Cairns, Neckerman, Gest, & Gariepy, 1988; Dishion & Andrews, 2007). Therefore, the identification of peer group size may reflect large peers groups

and the increased risk of including a deviant peer in a group as a peer group increases. Alternatively, this variable might be identifying small peer group size and peer rejection, since peer rejection has been reported to be associated with CD (Burke et al., 2002). Further, chronically maltreated children are more likely to be aggressive and to be rejected by peers (Bolger & Patterson, 2001), which may fit in future assessments of the environment with the commonly used measures of childhood adversity.

Neighborhood Environment/Socioeconomic Status

Two classes of the environment were identified, education and occupation. Low levels of education were strong classifiers for CD, which is an indicator of socioeconomic status that is often associated with financial resources available and with CD (McLoyd, 1998). Additionally, "secondary expenditures on alcoholic beverages" was also important. This particular item is intriguing since it highlights a feature of what the community does with its resources as well as what may be available for purchase in the community. It has often been noted anecdotally that businesses such as convenience stores which sell alcohol and check-cashing establishments are often located in or near disadvantaged neighborhoods. However, the identification of this variable over a more commonly used variable of neighborhood such as per capita income may identify a theme other than the economic disadvantage of a neighborhood since individuals from "poor neighborhoods" do not have higher rates of CD (Bassarath, 2001).

Pre-/Perinatal Environment

Environments surrounding birth such as birth weight, gestational age and birth complications have been frequently highlighted in the literature. Many studies have reported that babies who suffer birth complications are more likely to develop CD and commit impulsive crime and violence in adulthood. Further, birth complications significantly interacted with maternal rejection of the child in predicting violent offending at age 18 (Raine, 2002). Low birth weight has also been associated with ADHD in children (Breslau, Brown, & DelDotto, 1996), which is a precursor of and comorbid with CD in adolescents. The relationship between low birth weight and CD may be mediated by intellectual and the neuro-motor delays that are frequently associated with premature birth (Nadeau, Boivin, Tessier, Lefebvre, & Robacy, 2001).

Household Environment

Two general classes were identified as important for CD classification, parental monitoring and parental affection (either with one another or with the child). These two items are frequently highlighted in the literature although the reported associations usually reflect the lack of monitoring and affection and CD (Burke et al., 2002). This may reflect one of two ideas. First, these items produced by the random forest reflect classification that the presence of positive parenting is more important for the non-affected CD group. However, items regarding negative parenting were included and were not identified as strong predictors. Alternatively, it is possible that the absence of positive parenting is more important than the presence of negative parenting. Parenting

behaviors that involve low emotional warmth and minimal involvement have received as much attention as negative parenting behaviors with respect to CD risk (Bassarath, 2001). These items are most proximal to the child and will require a more thorough approach before including in models of GxE. Proximal variable such as these are anticipated to meet criteria for environmental pathogen status, which includes an expectation of being part of a biologically plausible hypothesis and ultimately GxE (Moffitt et al., 2005). Parental drinking was also identified as a strong classifier, which may be an indicator of the child's observation of parental alcoholism.

The random forest approach has identified several environments which have also been determined to be risk factors in the literature. In the careful consideration of each of these items in risk for CD, it will also be necessary to include how these variables differ by gender, since gender was consistently identified as an important variable.

It is important to note that many of the other variables ranked "less important" can and should be studied if a particular interest exists. For example, poor parenting or negative parental interactions were not included as strong classifiers of CD, despite its pervasiveness throughout the literature. It may be necessary to include some of these popular risk factors to compare them against the identified variables. Additionally, variables determined to be "important" to CD classification require careful examination prior to inclusion of models of GxE since no items were found to be strong predictors of CD. However, the lack of strong predictive ability for these items is not surprising since "environment" functions simultaneously at various levels in a variety of ways.

Small effects for any particular risk factor are sensible since clear risk for antisocial behavior has been reported to accrue only when a person accumulates a large number of risks (Rutter et al., 1998), for which each may have a small effect (Daniels et al., 1985). Further, this study assessed the effects of environments alone. Once included in an appropriate model, the effect of the environment conditional on genotype may increase the effect of the environment (Moffitt, 2005). Furthermore, these items could be used along with variables from other measures in a manner that treats environmental risk as a constellation of risk at various levels rather than as a series of specific environments to capture the accruement of risk that is absent when assessing environments as individual constellations. Ultimately, by identifying several "very important" variables from a larger group of risk factors with known associations to CD, it is anticipated that environment identification could be performed in a manner that is efficient for the emerging study of GxE.

The most serious limitation of the use of supervised learning and data reduction is the lack of interpretability of the results. While many variables important to CD classification have been identified, assessments of how these relate to risk for CD cannot be assessed from this approach. Thus this analysis has only served to reduce the data and encourage further review. Second, despite low overall prediction as estimated by the OOB error, there is a fair degree of misclassification for CD affected status. Therefore, these environments are anticipated to do a better job in classifying non-affected CD status than affected status. However, this result would provide insight into the nature of CD prevention.

Third, the random forest approach has recently received criticism as it is anticipated that the algorithm is sensitive to issues of scale when a variety of different data types are used simultaneously (Strobl, Boulesteix, Zeileis, & Hothorn, 2007). Therefore, some items which have been identified as most important may be the result of they way they were measured rather than how they truly compare with other items (ie: number of parental disorders versus the actual disorders). However, some environments such as life events had the same types of responses and could be appropriately compared. Future work on the identification of environmental variables should either use different sub-samples of items or use an unbiased variable selection approach with the random forest algorithm (Strobl et al., 2007).

CHAPTER 7 Discussion

This final chapter summarizes and discusses results from the research described in chapters 2-6. It is divided into three sections, the detection of GxE, the study of alternate candidate genes for future use in the study of GxE and the identification of alternative environments for future use in the study of GxE. The chapter is concluded with some ideas for future research.

The Detection of GxE

The detection of GxE depends on several factors including (1) the identification of an appropriate genotype that is informative in a variety of populations, (2) the use of an appropriate measurement of the phenotype, (3) the use of an appropriate measure of the environment, and (4) the use of appropriate analyses that handle the shortcomings in the measurement of phenotype and/or environment.

Gender Differences in Risk for CD

Despite previous apprehension to include females in the study of GxE using *MAOA* genotype due to the uncertainty regarding X-inactivation, the genotypic information was incorporated to produce informative results. By defining both the homozygous (additive) and heterozygous (dominance) effects of *MAOA*, we have demonstrated that the inclusion of females heterozygous for the low and high activity *MAOA* alleles is reasonable and yields meaningful results in spite of the ambiguity around the issue of X-inactivation. Additionally, the risk associated with the

heterozygous *MAOA* genotype is intermediate to that of the homozygous groups. This result resembles a previous genetic neuroimaging study of aggression which reported similar trends in *MAOA* contribution in risk for CD (Meyer-Lindenberg et al., 2006).

Among females, a main genetic effect of the low/low *MAOA* genotype remained after controlling for all other risk factors and suggested that *MAOA* confers greater risk for CD at all observed levels of adversity. However, when GxE was detected, its effects were modest, and were removed with a transformation of the environmental measures. In contrast, no significant effect of the low activity *MAOA* allele was detected in males although significant GxE was detected and remained after transformation of the environmental measure. Further, the sign associated with the interaction for males was opposite that of females and identified the genetic sensitivity to childhood adversity as differing by gender. This result has also been recently reported in another study, with the direction of the female interaction being opposite of what has been reported in males (Sjoberg, Nilsson, Wargelius et al., 2007). However, these results did not confirm the presence of any significant main effects of *MAOA*.

The initial results of the direction of GxE differing by gender highlighted the need to address whether GxE was due to gender differences in sensitivity (also known as gene-sex interaction) to the environment or rather a result of X-linked effects. Results from chapter 3 indicated that X-linked effects functioning in a manner resembling that of the *MAOA* genotype did not explain the sex differences for CD. It seems more likely that the gender difference in GxE is due to a genotype-sex interaction

involving *MAOA* since an X-linked locus is at least three times more likely to be involved in sexual development than genes on an autosomal chromosome and sex-linked genes often exert their phenotypic effects by regulating the expression of autosomal genes (Fairbairn et al., 2006). Therefore, an X-linked gene may mediate the expression of other genes by gender or moderate genotypic sensitivity to environmental exposure.

Meyer-Lindenberg and colleagues (2006) also reported a significant genotype-by-sex interaction for orbito-frontal cortex (OFC) structure and function, where there was reduced reactivity in males and females with the low *MAOA* genotype. Males also had significantly reduced OFC connectivity with the amygdala when compared with females. The OFC and OFC-amygdala interaction has been implicated in the pathway of stimulus-reinforcement association learning and is important in assigning reward value to behavioral reinforcers (Meyer-Lindenberg et al., 2006). The current results along with those of the neuroscience literature suggest that although males and females generally are similar in their exposure to genetic and environmental risk factors, these risk factors may be processed differently by gender as a function of genotype. Ultimately, the study of GxE is applicable to both males and females and may offer some insight into the cause of gender differences in CD through the identification of gene-sex interaction in the development of risk for CD.

Detection of GxE is Dependent on the Measurement of Phenotype

The detection of GxE has been subject to criticism as a function of measurement scale of both the phenotype and the environment. This study has demonstrated the need for caution when reporting significant GxE. Chapter 2 reported significant GxE in females that disappeared after collapsing the measure of environmental exposure to reflect fewer levels of exposure. When GxE was assessed again using twin correlations across two levels (0/1 or more) of environmental exposure, no significant interaction was detected. Further, there were too few twin pairs exposed to more than one childhood adversity event to attempt detection of GxE. Since GxE was detected using a measure of 0/1/2 or more exposures to childhood adversity, it is possible that GxE would have been detected if enough pairs were exposed to higher levels of childhood adversity. Additionally, the twin study of GxE assessed CD as a continuous trait measured by the polychoric correlations of CD. It has been suggested that GxE will often be detected when measuring psychopathology as a binary diagnosis and analyzing in logistic regression and rarely detected using continuous measures of a trait (Eaves, 2006).

In an effort to address the issue of false detection of GxE as a result of CD measurement, chapter 4 tested for the presence of GxE using an MCMC approach that included a genetic IRT model to evaluate CD as a trait with continuous liability. While among females, models that included GxE in the presence of the main effects of *MAOA*, childhood adversity and age were most appropriate in predicting risk for CD using both maternal and child ratings of CD, there was little improvement in model fit by including

the effects of GxE. In comparison, males models of risk based on child reports of CD indicated that the inclusion of GxE as a predictor of risk for CD is moderately justified, while maternal reports do not. Therefore, the detection of GxE measuring CD as a continuous latent trait is weak at best. It is expected that the inclusion of environmental exposure also measured as a continuous trait would produce similar results. Therefore, although GxE was detected in the first instance (Chapter 2), the results of the following chapters (3 and 4) suggest that detection is highly dependent on appropriate measurements. Further, when GxE has been detected, it has been associated with small effect sizes. Consequently, the effect of GxE between *MAOA* and childhood adversity on CD is not expected to be large.

Detection of GxE is Dependent on Informant

Chapter 4 demonstrated inconsistency in the detection and interpretation of GxE in males resulting from rater differences in the measurement of CD. This discrepancy between raters in the detection of GxE did not occur in females and their point estimates were similar to those obtained using a maximum likelihood approach (see Chapter 2). These results highlight the issue of rater heterogeneity inherent in behavior genetic studies using twin samples. Generally, parental ratings provide higher estimates of additive genetic effects and child ratings have higher estimates of the shared environment, which includes the contribution of GxE (Eaves et al., 1997). The use of a CD diagnosis based on the symptom-or algorithm (Chapter 2) to detect GxE was initially reasonable since such a measure relies on the aggregate responses of both

parents and children. However, in order to address the issue of CD measurement as discussed in Chapter 4, it may be better to use the symptoms for both raters rather than assigning a diagnosis. This could be addressed using the genetic IRT approach used in Chapter 4.

The identification of GxE is important in the development of public health prevention efforts. If a program is developed with a particular interaction in mind, it may have limited success since current models of GxE are very focused for a single environment of risk and a single genotype (Caspi et al., 2002; Foley et al., 2004; Haberstick et al., 2005; Reif, Rosler, Freitag et al., 2007; Widom & Brzustowicz, 2006; Young et al., 2006). Since the contribution of main and interaction effects are relatively weak, a model including childhood adversity and MAOA genotype risk for CD would not be appropriate in building a focused intervention for childhood adversity. However, it might be an appropriate paradigm by which to ascertain children and their families for therapy who may be at high risk for antisocial behaviors.

Studying Alternate Candidate Genes for Use in Studies of GxE for CD

By addressing alternate genes whose products function with one another in the serotonin and dopamine neurotransmitter systems, genotypes from other candidate genes were tested for interaction with *MAOA* to significantly affect risk for CD. No significant epistatic effects were detected. Main genetic effects for multiple systems and comorbidity were detected.

Tests of Candidate Gene Associations

Among females, the presence of a main effect of *MAOA* after controlling for *5HTTLPR* genotype as well as the main effect of the low activity genotype approaching significance after controlling for *DAT1* genotype was detected and suggests the importance of *MAOA* within the serotonin and dopamine systems for CD diagnosis.

In males, a significant main effect of the 9/9 *DAT1* genotype for both ADHD and CD was detected. No significant main effects were detected for *MAOA* or *5HTTLPR*. It is possible that these genes are not as important to the etiology of CD in males compared to females, or that we simply do not have the power to detect significant main effects given the current sample size and their relative weak effects as evidenced by the female results.

Test of Genetic Differences in Comorbidity for CD and ADHD

The 9/9 *DAT1* genotype was a risk factor for CD and ADHD in males. However, after controlling for comorbidity, the 9-repeat *DAT1* allele was only a significant risk factor for CD diagnosis. Additionally, after controlling for ADHD the main effect of *MAOA* on CD diagnosis in females was no longer significant in either serotonin or dopamine systems.

These results indicate that comorbid illness may be genetically different from ADHD or CD alone. *DAT1* in males and *MAOA* in females could highlight externalizing behaviors common to both disorders, since externalizing behaviors are

often measured as symptoms similar to CD (Young et al., 2002). Alternatively, CD diagnosis is a confounder in the association of *DAT1* and ADHD, and should be controlled for in association studies of ADHD and *DAT1*. Another possibility is that the distinction between an ADHD+CD subtype is necessary to identify "pure" ADHD in association studies. Both issues have been identified (Thapar et al., 2001; Waldman et al., 1998b) but have not been addressed in previous association studies of candidate genes for ADHD.

This study highlighted the advantage of using multiple candidate genes known to function together in a system to improve understanding of the genetic contributions towards comorbidity. While common genetic effects have been addressed using anonymous genetic effects, few studies have addressed this issue using measured genotypes. Additionally, through this approach, *DAT1* could be used in future investigations of GxE using childhood adversity in males.

Studying Alternate Environments for Use in Studies of GxE for CD

The systematic assessment of the environment through the use of random forests produced several interesting environments that will require more thoughtful consideration before incorporation into a model testing GxE. Since several environments have been identified for proximal and distal risk factors, these environments might eventually be used to test a model of cumulative environmental risk (Jaffee, Caspi, Moffitt, Polo-Thomas, & Taylor, 2007) or one of environment-environment interaction. Ultimately, the identification of these environments is simply a

gateway for a more thorough analysis of the environment and how it functions to affect risk for CD.

Future Studies

The study of GxE for CD will continue to demand separate, focused research of both genetic and environmental factors as well as alternate models of risk utilizing the two. For example, further analysis of the pattern of correlations between twins and their parents is required to resolve the role of childhood adversity in the correlation between parental ASP and CD and to disentangle the pathways between rGE, GxE and assortative mating. Such an approach has recently been addressed by modeling the effects of genetic and social transmission of information from parents to children, and the environmental effects of parents may be mediated through measured features of the home environment (Eaves, Prom, & Silberg, 2007). This study has reported significant genetic and environmental effects for antisocial behaviors measured as CD-related symptoms in adolescence and in antisocial personality symptoms in adulthood. Additionally, all significant environmental effects of parental ASP were mediated through the measure of adversity and the effects of passive rGE measured as parental ASP was very small. The twin and parent approach could be modified to include the effects of measured genotypes such as *MAOA* to determine the specific effects of genotype on CD. Additionally, since antisocial behavior appears to manifest differently by gender and males have higher rates of CD than females, there may still be reason to study the transmission of *MAOA* or other genes on the X-chromosome in the presence

of childhood adversity and parental ASP to assess their contributions to the gender difference in CD. Similarly, the twin and parent approach may provide further insight into the study genotype-sex interaction. The genetic and social transmission of information from parents to children of opposite-sex pairs might be compared with that of same-sex twin pairs to assess how males and females might respond to their environments as a result of genetic mediation for a measured genotype and environmental exposure.

Since the detection of GxE is dependent on the definition of the phenotype, it is necessary to address the measurement of CD and antisocial behavior in general. Different genes were associated with comorbid and non-comorbid forms of CD and ADHD (Chapter 5). Further, the distinction between antisocial behaviors associated with childhood disorders such as ADHD versus antisocial behavior alone has been detailed in identifying taxonomy relative to the development of antisocial behaviors (Rutter et al., 1998). Likewise, developmental heterogeneity defined as occurring either throughout the lifetime of the individual (lifetime-persistent) or only during adolescence (adolescent-limited) has also been addressed to understand the role of genetic effects in the development of antisocial behavior (Moffitt, 1993). Future work in the detection of GxE for antisocial behavior in general must consider the role of developmental heterogeneity to determine if and how its effects on the phenotype differ across stages of development. Similarly, as our understanding of how gene products of neurotransmitter systems function to increase risk of CD, we must also consider how to

best assess risk for a disorder or comorbid disorders using single as well as multiple candidate genes.

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