Introduction

ADHD Diagnostic History

ADHD as a disorder was first noted in the Lancet in 1902 (1). George Still described 43 children in his clinical practice who were aggressive, defiant and resistant to discipline. Heightened emotionality and self-gratification were conjectured to be the most salient features of the disorder. He proposed a biological predisposition to this disorder that was, at least in some affected individuals, hereditary. This biological predisposition was thought to be either an inborn brain malformation or a mild head trauma (2).

Significant interest in this disorder in North America can be traced to the encephalitis epidemic of 1917-1918 at which time clinicians were presented with children who survived the brain infection but had significant behavioral and cognitive pathological conditions (3). These children were impaired in attention, activity regulation and impulsivity, as well as other cognitive functions. This disorder was known as Postencephalitic Behavior Disorder and was clearly due to brain damage. This linkage of brain trauma to behavioral syndromes prompted the study of other childhood sources of brain injury, including birth trauma, lead toxicity and epilepsy.

Between the 1940's and the 1960's a concept arose of the "brain-injured child" which was applied to a broad spectrum of disorders that included ADHD (or minimal brain dysfunction (MBD) as it was known then) but also included various learning disabilities, conduct disorders and various types of mental retardation. It was during these years that methylphenidate arose as a potential therapeutic for the disorder (4).

Over time, criticism arose over the term MBD and was supplanted by Hyperkinetic syndrome or Hyperactive Child Syndrome. This dissatisfaction stemmed from a critique of the broadness of the syndrome and the lack of clear and well-defined symptoms (5). Significant descriptive work was reported by Virginia Douglas (6) who proposed that deficits in sustained attention and difficulties with impulse control were the true hallmarks of this disorder. This view has dominated much of the thinking about ADHD as can be seen by its incorporation into the third edition of the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-III) (7) and the current focus on attention problems in ADHD affected individuals. During the 1970's stimulant medication became widely used as a therapy for ADHD.

In 1973, Dr. Benjamin Feingold, M.D. proposed that certain compounds called salicylates, food coloring and flavoring agents were the cause of ADHD (8). Due to the widespread health consciousness and as a possible backlash against childhood pharmaceutical treatment, this therapy and disease etiology became widespread. The proposed cure for the disorder was a radical change in the diet of the afflicted child, which would eliminate all of the compounds above and required 100% compliance. In several well-designed double blind studies, Wolraich and colleagues have shown that neither artificial sweeteners nor excessive sugar play a significant role in ADHD type behaviors (9-14).

The 1980's were a decade of tremendous improvements in assessment with diagnostic instruments such as the Child Behavior Checklist (CBCL) (15), which were more comprehensive and better-normed tests than those previously available. There was

also an increase in public awareness of the disorder due to the increase in the number of national networks and political action groups, which formed around ADHD (e.g. Children and Adults with ADD (CHADD), and the Attention Deficit Disorders Association). The public awareness was also tainted by a vicious campaign from the Church of Scientology funded Citizen's Commission on Human Rights (CCHR). CCHR sensationalized rare instances of stimulant overdose in methylphenidate (Ritalin) treated individuals and drew negative attention to the drug treatment of children. These campaigns would have a pervasive effect on the public perception of ADHD and its treatment by the medical profession. Ritalin was seen as a dangerous drug, overprescribed and controversial.

Research into the genetic basis of ADHD has been a focus since the early 1990's. For example, Biederman and colleagues (1995) (16), found that children with an ADHD diagnosed parent had a 57% offspring risk. Aggregation studies found that ADHD clusters in families. Further evidence for a genetic etiology was found that ADHD had a high heritability (17) and little contribution could be attributed to shared environments (18). More recent work has focused on the tests of association with a variety of polymorphisms in dopamine-related genes. These will be addressed more extensively in later sections.

The DSM of the American Psychiatric Association (19), the major diagnostic instrument of psychiatric disorders, has had several revisions of the ADHD diagnosis in the past two decades. Despite these revisions, there appears to be a highly significant overlap between the DSM-III, DSM-III-R and the DSM-IV diagnoses of ADHD. For

example, Biederman et al. (1997) found that 93% of individuals diagnosed with ADHD by DSM-III-R were also diagnosed with ADHD by DSM-IV (20). Furthermore, Morgan et al. (1996) found that the ADHD diagnosese are highly comparable. Thus there appears to be a significant amount of continuity between these diagnostic instruments. The most salient difference between the DSM prescribed diagnoses is the equal emphasis given to hyperactivity and inattention in DSM-III-R as opposed to DSM-III. In the beginning, hyperactivity was emphasized more prominently than inattention but currently, both inattention and hyperactivity-impulsivity are given equal footing in the diagnosis of ADHD.

ADHD disease characteristics

Attention Deficit Hyperactivity Disorder (ADHD) is the most common neuropsychiatric disorder of child onset (21), although the disorder does extend to adulthood in a subset of the affected population. ADHD is characterized by a persistent pattern of inattention and/or impulsivity-hyperactivity that is more extreme than is observed in individuals at the same developmental level or stage. The first criterion (Criterion A) for ADHD diagnosis is that the behaviors are persistent and non-transient. This is essential in order to rule out other causes for inattentive or hyperactive behaviors, which may act as phenocopies of ADHD. These phenocopies may be transiently present due to a variety of other causes including neurotoxicity (22). The second major criterion (Criterion B) requires that impairments of inattention (I) and/or hyperactivity-impulsivity (H-I) must have existed prior to the age of seven. The third major criterion (Criterion C) prescribes that the impairments from the symptoms of ADHD must be present in at least

two settings (often both school and home). The fourth criterion (Criterion D) requires that there must be a clear impairment in some social, academic or vocational functioning, which is appropriate to the age of the individual. Finally, Criterion E holds that the behaviors can't be accounted for by other mental and psychiatric disorders such as mood disorder, anxiety disorder, dissociative disorder or personality disorder. Further, the disturbing behaviors may not occur in the presence of pervasive developmental disorder, schizophrenia or other psychotic disorders (19). These criteria are summarized in Table 1.

ADHD Subtypes

As noted above, an ADHD diagnosis is determined along two major symptom dimensions, inattention and hyperactivity-impulsivity. A diagnosis of inattention is given if six or more of the following behaviors are shown to have persisted for 6 or more months in at a level which is inappropriate with developmental stage or is maladaptive: 1) failure in attention and/or making many careless mistakes in school, work or other environment, 2) difficulties in sustaining attention in activities, 3) inability in listening when spoken to, 4) inability to finish assignments or complete work activities (not due to misunderstanding or opposition to the chore), 5) organizational difficulties, 6) avoidance and reluctance in engaging in activities which require sustained attention, 7) often loses things which are required for activities, 8) easily distracted by extraneous stimuli, 9) unusually forgetful in everyday life. At least six of the above must be present to warrant a diagnosis of inattention and as noted all five of the above criteria need to be satisfied. The second symptom dimension is the dimension of hyperactivity-impulsivity. Like the

inattention dimension, hyperactivity-impulsivity is diagnosed if at least six of the following symptoms have persisted for at least six months: 1) excessive fidgeting or squirming in seat, 2) leaving seat when sitting is expected or required, 3) running around or climbing at inappropriate times, 4) difficulties in playing or doing things quietly, 5) appears to be "on the go," 6) excessive talking, 7) impulsively answers before question has been asked, 8) difficulties in waiting for turn at activity when in a group, 9) often interrupts in conversations. If both symptom dimensions are present then a combined diagnosis is made.

The percentage of children that fall into the three categories is not equal in typical clinical reports or in our sample collection. The combined subtype is the most common type, comprising 50% to 75% of all ADHD individuals. The inattentive subtype comprises 20% to 50% of ADHD and the hyperactive subtype comprises approximately 15 - 20% (23-25). ADHD affected individuals with a purely inattentive subtype comprise 40% of our sample, while those with the combined subtype comprise 54% of our sample. ADHD affected individuals with a purely hyperactive-impulsive subtype diagnosis are 6% of our sample. As seen in Table 2, our sample has considerable correlation of subtypes with other samples. However, the shortage of hyperactive individuals in our sample is a potential confound and may be related to various aspects of the sample collection process such as the geographic location or the focus on multiplex siblings.

Children diagnosed with ADHD are at a higher risk for many comorbid disorders. Oppositional defiant disorder (ODD), conduct disorder, anxiety disorder are the most

common comorbid disorders and will be discussed in greater detail in the section on the comorbid disorders of ADHD.

ADHD Prevalence and Disease course

ADHD is the most common neuropsychiatric disorder of school-aged children with prevalence officially listed as 2%-5% of school-aged children in the U.S. (19). A variety of studies have found considerably different prevalence statistics, however. For example, in Europe, where a diagnosis of hyperkinesis is made instead of ADHD (although in general they are thought to be the same), the prevalence ranges from 1%-2%. Often the prevalence differs even between studies within the United States depending on the population studied and the ascertainment instrument (26). For example, recent evidence indicates that the prevalence of ADHD has increased since the DSM-IV has been instituted as the standard diagnostic manual instead of the DSM-III-R (24).

While some parents may note and worry when their infant may move around excessively, there is a great difficulty in differentiating between a normal infant and one that may develop ADHD. Although diagnoses of ADHD are being made as early as three years old, most ADHD children are diagnosed in elementary school when higher levels of attention and restraint of impulses are required to finish assignments and maintain an orderly classroom. Children with the predominantly inattentive subtype tend to be diagnosed later in childhood while children with the hyperactive subtype are diagnosed fairly early in school-aged children (27). ADHD affected individuals with the predominantly inattentive subtype tend to have fewer emotional and behavioral problems but have more significant academic impairments than ADHD individuals with the

hyperactive subtype (28). Those with predominantly hyperactive subtype, however, show pronounced difficulties in behavior but often are not impaired academically (28).

ADHD symptoms are maintained throughout adolescence but tend to diminish by adulthood in most clinical cases. This is the generally accepted course of the disorder but there is a significant amount of debate surrounding the perception that by adulthood most people affected with ADHD are symptom free. In fact, some researchers have found that ADHD symptoms are fully maintained in many adults previously diagnosed with ADHD, while other previously diagnosed adults may have identified proper or improper coping mechanisms which mask ADHD symptoms (29-32).

ADHD Disease Impact

ADHD often poses a high burden both to the affected individual and to society as a whole. These burdens include increased risks for automobile and work accidents as well as lower performance in school and work (21). ADHD is characterized by behavioral symptoms of inattention and/or hyperactivity, which arise in early childhood and which manifest impairments in two or more settings, often school and home. In the school setting, ADHD affected individuals often display problems with reading and the completion of school assignments, which require sustained attention. Mannuzza and colleagues (1997) (33) utilized a prospective study design to investigate the educational outcomes of a group of children originally ascertained in the 1970's (34). These probands were followed prospectively and information about academic and occupational achievements was gathered and compared with a community control matched for age and SES status. The study found that probands initially diagnosed with ADHD had, as a

group, 2 years less schooling than the controls. Significantly, 25% of the probands did not graduate from high school compared with only 1% of controls. Only 15% of probands achieved a degree of bachelor's or higher compared with greater than half of controls.

The occupational level was also found to be lower in the probands than in controls (33) but only 8% of the probands were found to be unemployed. This finding indicates that while the occupational opportunities of ADHD affected individuals are more limited than that of the population at large, these individuals are typically gainfully employed.

An additional recognized burden is the high correlation of drug use and abuse amongst ADHD affected individuals, both during adolescence and in adulthood. This association has been investigated in a number of studies (35-38). In ADHD affected individuals the onset of substance abuse is typically earlier than in the general adolescent population.

Finally, ADHD appears to be a risk factor for criminality and delinquent behavior. There is some evidence from prospective studies, which indicates that attention deficits and hyperactivity contribute to risk of crime and incarceration in adolescence and adulthood. Satterfield and colleagues reported that the juvenile arrest rate for their cohort of ADHD individuals was 5 to 26 times higher than a control group, depending on the socioeconomic status. A potential shortcoming of this study was that after it was started, the ADHD diagnostic criteria were revised. Specifically, the authors refer to their subjects as "Hyperactive boys" which most correctly corresponds to ADHD individuals that are classified as having the hyperactive component with or without inattention. In a

follow up to the previous study, Satterfield and Schell (1997) found that hyperactive boys had over four times the arrest rates as adolescents than a matched control group. Furthermore, the same group had a 21% arrest rate as adults compared to only 1% of the controls (39). This updated study appears to be more reliable because all individuals (subjects and controls) passed through the normal adolescent risk period for juvenile offense. ADHD adolescents had a higher recidivism rate and a higher rate of violent crime. This is important because adolescent felony is highly predictive of adult criminality. It appears from current research that this risk can be attributed to conduct problems that are often found comorbid with ADHD (39, 40). While it may appear that ADHD is not a significant risk factor for criminality, an examination of the degree of comorbid overlap of ADHD with Conduct Disorder and Oppositional Defiant Disorder will reveal that a significant number of ADHD affected individuals fall into this unfortunate group.

Disorders Comorbid with ADHD

More than 60% of ADHD cases exhibit a co-morbid psychiatric disorder or learning disability. The most common co-morbid disorders include oppositional defiant-disorder (ODD), conduct disorder (CD), anxiety disorders, depressive disorders and learning disorders (LD) (41).

The co-occurrence of ADHD with one or more of the above listed disorders can have a devastating impact on not only the child but the family and society as a whole. ODD occurs in 40%-60% of ADHD affected individuals (42) and is exemplified by outbursts and actions of open hostility towards authority, negativism, and disobedience

without any sense of remorse (43). For example, Smalley et al (2000) found that 60% of ADHD individuals in our sample had ODD (40% without CD and 20% with CD) (44). While ODD is fairly common among ADHD individuals, CD is relatively rare. CD is a group of behaviors characterized by an inability to follow rules and behave in socially responsible ways. The CD child acts rudely and violently, without remorse or attempts to reform. CD is particularly interesting because it appears to "breed true" in families, indicating that it too may have a genetic component. However, family environment has been shown to play a role in the development of CD (45). ADHD, often in combination with conduct disorder, is significantly associated with a future risk for criminal behavior (46). The percentage of co-occurrence of comorbid disorders is summarized in Table 2.

Finally, learning disabilities are quite common among ADHD individuals and this has a severe impact on future academic and occupational success. Learning disability is a broad description of a large number of specific deficits in learning, including dyslexia, dyscalculia, or other deficits in reading, writing or comprehension. Learning deficits are complicated by the general difficulty in ADHD individuals of maintaining a focus on work and retaining desired information in short-term memory. For example, children with specific language impairment (SLI) comorbid with ADHD had an increased probability of having a first-degree relative with SLI. SLI is a developmental disorder in which, there is an inability to acquire normal expression or comprehension of language despite a normal auditory system (47).

ADHD pharmacological therapies

Psychostimulant medication is the most common pharmacological therapy for children and adults affected with ADHD. The most common of these, methylphenidate (Ritalin, Concerta, Metadate, etc.), is a specific blocker of the Dopamine Transporter (DAT1). DAT1 is responsible for removing dopamine from the synaptic cleft. By blocking the effectiveness of DAT-1, methylphenidate allows for the retention of a greater portion of dopamine released in the extracellular space (48). Many of the psychostimulants used in ADHD treatment are derivatives of methylphenidate (i.e. those having a slower release, longer action). Another, often used drug is amphetamine (Dexedrine, Adderall), which is a psychostimulant like methylphenidate. Dexedrine commonly induces more side effects, such as appetite suppression and insomnia, but less nausea, diarrhea and weight gain as with methylphenidate. A final psychostimulant prescribed for ADHD is pemoline (Cyclert). Methylphenidate and d-amphetamine are short acting compounds, which take effect 30 to 60 minutes after administration, peak 1 to 2 hours afterward, and last 2 to 5 hours. The amphetamine compounds and sustained release mixtures of methylphenidate and d-amphetamine are intermediate and last from 6 to 8 hours. A major thrust of pharmacological therapy has been in the effort to extend the duration of pharmacological effect and to limit undesirable peak effects such as headaches, moodiness and rebound. In addition, it would be desirable to allow for one dose to decrease symptoms throughout the day, allowing the ADHD individual to function without the hindrance of her disorder. The standard dose series is usually one dose in the morning and one in the afternoon. However, this usually contributes to a "rollercoaster" effect for the child's mental state. For these reasons, several extended

release preparations have been created which last from 8 to 12 hours and result in fewer side effects.

Psychostimulants work in all age groups and for approximately 70% of ADHD affected individuals. While there are occasional side effects of reduced appetite, insomnia, edginess and gastrointestinal upset, these are usually short term and rare (49). However, prolonged stimulant use remains controversial due to both side effects and concerns that introducing children to drug use will encourage illicit drug use in adolescence. Part of this concern may be due to the observation that ADHD affected individuals are more likely to use cigarettes, alcohol and drugs (38, 50), start using them earlier (51), and maintain their addictions for a longer time (52). While abuse of methylphenidate has been reported in case studies (53), there is little evidence that this behavior is widespread (54). In fact, there is considerable evidence that pharmacotherapy decreases risk for developing substance abuse disorders (23).

Clonidine and guanfacine are two agonists of the alpha2-adrenergic receptor, which are antihypertensives unlike the majority of ADHD treatment medications, which are psychostimulants. Often, a patient will be prescribed methylphenidate and if this is not an effective treatment, other medication will be tried. As it can be seen, those affected with ADHD comprise a diverse group of individuals with differences in disease manifestation and drug response.

ADHD etiology

While the clinical relevance of ADHD is clear, its etiology is not. There has been some evidence from limited studies, which have shown that specific brain regions differ

in the extent of brain symmetry, morphology and receptor localization in those affected with ADHD than in unaffected controls. These regions include the frontal lobes, the prefrontal cortex, the limbic system and the reticular activating system. These regions have been implicated in a variety of brain imaging modalities and are pointing to a role for these regions in the effects of this disorder. This evidence implicates biology as playing an important role. It is clear, however, that there are other possible components of ADHD susceptibility, such as negative family environments (55), or birth complications (56).

Due to this complexity there is some perception in the lay public that ADHD is over-diagnosed and Ritalin (the most widely used pharmacotharapy for ADHD) is over-prescribed. This has been examined in a number of studies, which have all found that there is not any evidence of a systematic practice of inappropriate diagnosis while isolated instances are present (57). A goal of understanding the causality of ADHD is to be able to have a more firm diagnosis by a genetic or biochemical test. This will help avoid criticisms of inappropriate diagnosis. Finally, an understanding of ADHD biology will aid in the design of pharmacological therapies through the understanding of the precise positions and pathways, which should serve as sensible drug targets.

Genetics is likely to play a significant part in the etiology of ADHD. This is clear from family (58, 59) and twin studies (60), which find that heritability for this trait ranges from 60-80% (reviewed in Smalley, 1997) (61). In addition, several case-control and family based studies have found significant associations of ADHD with specific alleles of dopamine genes as well as others. Initially, it was argued that a major gene could be

responsible for susceptibility to ADHD. It is clear, however, that a single gene of major effect is unlikely in this disorder due to the lack of significant linkage in a recently completed genome scan (62). This genome scan did not yield any significant linkage peaks. The scan did yield several suggestive peaks that are being examined, one of which has yielded significant evidence of linkage (63). Further, several segregation analyses have indicated that a single gene Mendelian inheritance mechanism is unlikely (64). The presence of several minor peaks indicates that while genetics plays a role in development of this disorder, the true cause is likely to be a complex tangle of gene-gene and gene-environment interactions. Further, each gene contributing to the trait will most likely be of small effect. Even before the completion of the genome survey, it was predicted that many genes of small effect would be instrumental in the etiology of ADHD (65). Therefore, the identification of susceptibility genes may require a synthesis of many approaches to identify all or most meaningful candidates. While the genome scan did not show major peaks, it did point to suggestive regions, which will need to be investigated further.

Genetic Associations in ADHD

Possibly due to these early indications of the modest gene effects of any single gene, many groups focused their studies on likely biological candidate genes in ADHD. It was noted that methylphenidate was an effective therapeutic for a majority of ADHD affected patients and thus formed a starting point for candidate gene studies. The primary target of methylphenidate is the dopamine transporter (DAT1), which normally binds to dopamine and removes it from the synaptic cleft. Cook and colleagues showed an

association of a DAT1 40bp VNTR polymorphisms and ADHD (66). The association of DAT1 with ADHD was replicated by two groups, (67), (68), but several groups , including our own, published negative findings regarding this candidate gene's association with ADHD (69, 70). The association of multiple dopamine genes prompted the widespread acceptance of a dopaminergic hypothesis regarding ADHD and the introduction of a whole series of candidate gene studies, which are still being examined in a variety of populations (68, 71, 72). The hypothesis argued that ADHD resulted from a mis-regulation in dopamine levels. The candidate genes include dopamine D4 receptor (DRD4), dopamine D2 receptor (DRD2)(68, 71, 73), dopamine D3 receptor (DRD3) (74-76), dopamine D5 receptor (DRD5) (72, 73, 75, 77, 78), and catecholamine 0-methyl transferase (COMT) (75, 77, 79-81). DRD4, in particular, has been shown by a number of groups, including our own, to be associated with ADHD. However, it appears that for almost all studies, which find an association with a particular candidate gene, a study soon follows that shows no such association in their study sample. There are several studies, which do not show an association between DAT1 and ADHD (Palmer et al., 1999; Asherson et al 1998), despite having an adequate sample size and power to detect susceptibility genes of moderate effect. Likewise, there are numerous studies, which were unable to confirm an association of the 7 allele of the 48 bp VNTR polymorphism of DRD4 (DRD4.7) and ADHD. While there are three studies, which have not confirmed this association, the positive findings predominate and in overall suggest more power due to their larger sample sizes and thus their ability to identify genes of modest effect (γ =

1.5 – 2). Our group has also found a 120 bp promoter polymorphism to be associated with ADHD.

In addition to the genes, which function directly within the dopaminergic system, a variety of candidate genes have been considered for their relevance to ADHD susceptibility. These include an androgen receptor (ADRA2C) (82, 83) and the synaptosomal-associated protein of molecular weight 25kDa (SNAP-25.) These genes have been implicated by other hypotheses of the central causes of ADHD and animal models that display ADHD-like behaviors. SNAP-25, for example, had been identified as a candidate gene for ADHD due to the finding that a mouse that is hemizygous for a 2 cM region containing the SNAP-25 gene exhibits many of the symptoms of ADHD affected individuals (84). Barr et al. (85) found an association of a haplotype, constructed from two closely spaced SNPs, and ADHD. We have found evidence for an association in paternal transmission of SNAP-25 alleles within our sample.

While all of the aforementioned genes may play a role in ADHD, none of these have been thoroughly examined in model systems or organisms. Some notable exceptions include *in vitro* binding studies of DRD4 variants of the 48-bp VNTR (86) and studies of the Coloboma mouse, which is hemizygous for SNAP-25 (84). The scientific literature examining ADHD and other complex traits is full of hints at susceptibility genes but it is quite rare to find genes with a large effect. Often, a polymorphism may increase risk but in a limited way and with little indication as to the biological relevance. It is clear that something has been lacking in genetic research, and

has foiled hundreds of investigators from achieving their desired goals of identifying true risk alleles for these complex traits.

Description of Dopamine pathway and associated genes.

Dopamine is a key neurotransmitter in the biology of attention, movement, emotional response and the ability to experience pleasure and pain. While dopamine exists throughout the nervous system, dopaminergic neurons are clustered in the substantia nigra and project to neurons of the neostriatum and the mesocorticolimbic pathway. These are composed of neurons of the ventral tegmental area and connect with those of the limbic cortex and other limbic structures. Dopamine exerts its biochemical effects by binding to two classes of dopamine receptors, D1-like or D2-like. These two types of receptors can be distinguished pharmacologically, biologically, and by their differential anatomical distribution (87, 88). Biologically, the two types of receptors couple to and activate two different types of G proteins. The D1 receptor type couples with Gs in order to activate adenylyl cyclase while the D2 receptor types couple to the Gi protein, which inhibits the production of cAMP. Pharmacologically, these two types of receptors bind different antagonists with high affinity. D1 and D5 receptors share similar pharmacological profiles and they are quite similar genetically. The D2 class of receptors are much more divergent with greater differences in both receptor biology (sequence identity) and pharmacology as well. For example, dopamine binds to the D3 receptor with an affinity 20 times higher than D2. Due to its localization in the substantia nigra, D3 is thought to be a presynaptic receptor.

Of special note is the DRD4 receptor, which has received a high amount of interest in ADHD research. This receptor is highly expressed in the frontal cortex, a region of the brain involved in executive functioning, working memory and attention. Dopamine is an agonist of the DRD4 receptor and thus DRD4 would be under robust stimulation by dopamine if DAT1 action of uptake were inhibited, as is accomplished by psychostimulant treatments of ADHD. DRD4 is located on chromosome 11 and there are a number of well-characterized polymorphisms within the gene. The most widely examined polymorphism in DRD4 is the 48 bp repeat polymorphism in exon 3, which has been shown to have a minor functional effect on binding of clozapine to the receptor (89).

DAT1 was the first dopamine candidate gene examined with regards to association with ADHD. This protein is responsible for the re-uptake of extracellular dopamine released from pre-synaptic neurons. A knockout mouse deficient in dopamine transporter was shown to exhibit hyper-locomotion (90).

A candidate gene study of several genes in the dopamine pathway was carried out to examine for association and linkage disequilibrium within our sample. The transmission disequilibrium test was employed to carry out this analysis, which is described in Chapter 4.

Description of other pathways and genes

The synapse is a crucial juncture in nerve signaling where an electrochemical impulse traveling down the axon is changed to a chemical signal, which must bridge a physical divide. This is achieved by the release of vesicles filled with neurotransmitter

messenger molecules across the synaptic cleft. As expected, this is a highly regulated event. Signaling at the synapse must take place in a calcium-dependent way such that depolarization of the post-synaptic cell occurs in response to synaptic vesicle release from pre-synaptic cells via the calcium regulated synaptic vesicle machinery. This machinery includes VAMP, Synaptobrevin, and SNAP-25, among others.

The synaptosomal-associated protein of molecular weight 25kDa (SNAP-25) was implicated by studies on the mouse mutant strain *coloboma* (*Cm/+*). This radiation induced mutant strain displays spontaneous hyperactivity that is suppressed by dextro-amphetamine and has thus been proposed as an animal model for ADHD (84). The *coloboma* strain is hemizygous for a 2-cM deletion encompassing multiple genes, including SNAP-25. Steffensen (91) observed that in *Cm/+* mice, the SNAP-25 mRNA and protein are expressed at half the wild type levels. Further, in vitro examination of their synaptosomes showed a failure to release dopamine in response to depolarization. Finally, transgenic insertion of SNAP-25 eliminated the hyperactivity (and the hyperactivity suppressing effect of dextro-amphetamine) but had no effect on other phenotypic abnormalities present in the strain (91).

Despite the suggestive evidence for a connection between hyperactivity and SNAP-25, an early small study found no linkage between ADHD in humans and seven microsatellite markers in the chromosome 20p11-12 region encompassing the gene.(92) In contrast, Barr (85) found significant evidence of linkage between ADHD and SNAP-25. They identified two novel single nucleotide polymorphisms (SNPs) located 3 bases apart in the 3' untranslated region of SNAP-25 and tested for biased transmission of these

SNP alleles and also the combined haplotype alleles, using the TDT on a sample of 97 nuclear families with 122 ADHD affected children. There was significant biased transmission of the TC haplotype allele. This suggests that variants of SNAP-25 play a role in the disorder, although it is possible that a gene tightly linked to SNAP-25 is responsible. The association study of SNAP-25 and ADHD will be described in detail in Chapter 3.

Reduced activity of serotonin has been proposed by implicated in the dysregulation of impulse control and in aggressive behaviors in model systems as well as in human subjects (93, 94). Evidence from a number of association studies points to a role for serotonin transporter polymorphisms in susceptibility to ADHD (95-100). However, there are several studies, which do not find this association (101, 102) and thus prompt researchers to carry out future work in this field.

ADHD Genome scan

An alternative strategy exists to the biological hypothesis driven research into putative candidate gene association studies. This alternative involves the examination of linkage of the disease trait and anonymous genetic markers throughout the genome. A genome scan was conducted to look for genetic loci, which influence ADHD in a large sample of affected sibling pairs and their parents. This genome scan used a marker spacing of approximately 10 cM and 126 affected sibling pairs to examine for sharing of both marker and the disease trait (62). A detailed description of linkage methodologies will be treated in later sections of the introduction. Briefly, the analysis utilized a

statistical genetic measure, which was uninfluenced by the particular inheritance model of the disease. This is important because in ADHD, this model is still undetermined.

The families utilized in this genome scan (and in the analyses reported in this dissertation) were identified through clinics, hospitals, schools, and community organizations in the greater Los Angeles area. Most families were ascertained through an advertisement for families with at least two children greater than four years old showing symptoms of ADHD. ADHD, as well as CD and ODD were diagnosed by DSM-IV criteria through the use of semi-structured interviews utilizing several separate assessment instruments. These included the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (KSADS-PL) (103), the SNAP-IV (104), the Child Behavior Checklist and the Teacher's Report Form (105). In addition, all information from the individual diagnostic instruments was combined by two senior psychiatrists in order to review all positive diagnoses. The inter-rater reliability (kappa) was 1.0 for the diagnosis of ADHD and was a bit lower for the diagnosis of some comorbid conditions. Full scale IQ measurements and academic achievement measures were gathered by the WISC-III (106) and the Peabody Individual Achievement Test-Revised (107). Children with IQ scores below 70 were excluded from the study and all further analyses.

The genome scan yielded no regions which had significant evidence for a major genetic locus conferring susceptibility to ADHD. However, several regions were highlighted with modest evidence that susceptibility loci may exist. Three regions, 10q26, 12q23, and 16p13 yielded multipoint LOD scores greater than 1.5. The

conclusions of the paper are sobering to those who expected a single major gene to be the cause of ADHD susceptibility. For example, some 96% of the genome could be excluded from containing a gene with a sibling relative risk (λ_s) of only 3. This indicates that the heritability and familial nature of ADHD is probably accounted for by multiple genetic loci of small and/or moderate effect. While the possibility exists that ADHD may be inherited in a mendelian fashion, as in some Alzhiemer's families, due to mutations in the presenelin 1 gene (108), ADHD in the population is most likely due to multiple genes possibly interacting with a negative environment. Another interesting outcome of the genome scan is the exclusion of many genes whose polymorphisms are often tested in association studies. Among the excluded genes are DRD4, DRD2 and DAT1. However, these candidate genes were excluded at a sibling relative risk of 2 or higher. The genotype relative risk may be considerably higher than this and thus may explain a significant portion of the liability.

The genome scan provided a starting point for fine-mapping studies of regions that showed some linkage (one of which is described in Chapter 5). This study also motivated further collection of families in order to increase the probability of uncovering genetic susceptibility loci of moderate effect.

Complex Traits

While research into the biology and genetics of ADHD has been ongoing for approximately 15 years, no single susceptibility gene has been established definitively. ADHD is a prototype of a complex trait whose genetics will need to be examined with all the tools that modern human genetics has to offer. In the following sections the

challenges and potential solutions to the problem of complex diseases will be addressed. Behavioral genetic disorders pose an additional challenge on top of the existing and significant challenge of the genetics of complex traits. Some of these challenges will be further discussed.

In the years leading up to the sequencing of the Human Genome, many researchers began to understand that the vast majority of diseases, which pose a public health burden upon Western countries were of a complex nature. While it could be acknowledged that many of these diseases "ran in families," the inheritance pattern could rarely be considered as Mendelian. Such diseases include cancer, heart diseases, schizophrenia and ADHD. These diseases are thought to be influenced by one or more genetic factors in interaction with the environmental. Therefore, while a single gene variant may account for a small percentage of diseases susceptibility, the identification of these factors would be important to uncovering the molecular pathways through which the disease unfolds. Genetic epidemiology has played an important role in the gathering and analysis of evidence for the study of these diseases. As with ADHD, initial insights into the genetic influences on behavioral disorders were drawn from family, twin and adoption studies from which heritability and sibling relative risk statistics can be estimated. Heritability refers to the ratio of additive genetic variance to the total phenotypic variance and can vary significantly from 0.26 for male longevity (109) to 0.66 – 0.74 identified for stroke (110). A disease may be further refined by analyses of transgenic and knockout mice and the identification of brain expressed candidate genes. Further, the identification of both functional and non-functional alleles in the candidate

genes is important for testing potential associations between the disease in question and any DNA variant. Toward this goal, several groups, including our own, had uncovered single nucleotide polymorphisms throughout the genome in order to facilitate genetic analysis by providing a denser set of markers than were available. This effort is described in Chapter 1.

A watershed publication by Risch and Merikangas (111) proposed that the ability of researchers to identify a disease –associated gene variant is determined by the amount of the gene's effect on the diseases. A gene variant of small effect will require large samples in order to detect its influence. Therefore, while the heritability of a disease may be moderate, the ability to detect individual genes of influence on this disease may be hamstrung if the effect from each individual gene is small. This work outlined a calculation for the number of families required, in order to reasonably expect to identify a gene variant of a given effect. In Figure 1, a power curve is displayed which relates the sample size necessary to identify a given genotype relative risk (GRR) in the transmission disequilibrium test methods which were employed in most of the studies that follow. Finally, it was argued that family-based association studies were more reasonable than linkage methods for the elucidation of complex traits due to the decreased power of even non-parametric methods to identify gene variants, which account for less than 5-10% of variance in the disease.

Since the early days of genetic analysis, the standard linkage method has been a powerful tool for the identification of genetic loci contributing to a genetic trait. With Mendelian traits, the process is fairly straightforward (although cumbersome). The

central assumption of the classic linkage approach is that a trait of interest will be co-inherited with an anonymous genetic marker and thus the portion of the genome that contributes to the trait of interest will be defined by the DNA markers with which the trait is co-inherited. A statistic, the LOD score, was created that would represent the degree of evidence or likelihood that a particular DNA was "linked" to the trait. Furthermore, a distribution could be plotted of the LOD score at successively increasing values of distance separating the marker and the trait. The maximum LOD score determines the putative distance, which separates the marker and the trait genes and could be used for localization of the physical gene. The problem with this approach is that it is generally dependent on a precise specification of the genetic model of inheritance. Potential areas of misspecification include the mode of transmission (autosomal recessive, autosomal dominant, etc.), the degree of penetrance, and the phenocopy probability. Misspecification in these areas is often unpredictable and thus a precise understanding of inheritance is required for the classical LOD score method to work. Given this requirement for precise specification of parameters, an approach was developed based on the assumption that if a trait is linked to a marker, the marker will be shared, identical-by-descent, more often than expected in pairs of relatives concordant for the trait and less often in relatives discordant for the trait (112, 113). Importantly, this type of association should be detectable even when the precise model is not understood or specified. Typically, affected sibling pairs are collected and, for each family a count is made of the number of marker alleles shared. If no linkage exists, zero alleles should be shared 25% of the time, one allele should be shared 50% of the time and two alleles should be shared

25% of the time. The empirical counts of shared alleles are compared to the expected number of counts and a chi-square statistic is used to assign a probability score for the sharing of the marker alleles. A significant advantage in the use of affected sibling pair (ASP) families is their relative ease of collection, compared with large extended families with multiple affected individuals. Chapter 5 details a fine mapping study of ADHD utilizing the ASP approach.

Association studies represent an alternative and complementary approach for the identification of genes, which may play a role in a given disease. In most cases, the genes utilized in these types of studies are already characterized and polymorphisms are already identified. Association studies were designed such that specific variants in genes that are thought to play a role in the disease were examined for potential association with the disease state. Initially, these types of studies used unrelated individuals with the disease (cases) and unrelated, unaffected individuals (controls). If a particular marker is found at significantly increased frequency in those with the disease when compared to unaffected individuals, a relationship is implied between the gene and the disease.

Despite standard epidemiological practices of matching cases and controls on a variety of potentially confounding variables such as age and ethnicity, an association can exist for a number of both genetic and non-genetic factors. Particular gene variants may be associated with disease due to the actual functional role of the gene variant on protein function or expression. Another genetic cause for the association is the extremely close proximity of the examined polymorphism to an unexamined polymorphism. This close proximity on the same chromosome results in a statistical dependence of marker alleles at

the population level and is known as linkage disequilibrium, gametic disequilibrium or allelic association. Linkage disequilibrium occurs when a novel allele occurs by mutation on a chromosomal background and is transmitted as a block. This block is steadily cut away by the steady action of recombination such that over generations, the degree of linkage disequilibrium will be reduced between two markers. However, because this reduction is occurring on a population-wide basis, the process is slow and thus the detection of association may be done by the analysis of seemingly unrelated individuals as in case-control studies. As noted, non-genetic factors exist for the association of genetic variants and disease. Population stratification, admixture and small sample fluctuations are significant confounding variables when case-control studies are used. Population stratification refers to situations when, in a multi-ethnic population, random mating does not exist between the ethnic groups and if a disease is found at higher frequency in some ethnic groups, an association may be identified for genetic markers which are not genetically linked to the disease locus. This type of confound befell some early studies into the genetics of alcoholism (114, 115).

Falk and Rubinstein (116) developed the haplotype relative risk (HRR) method to avoid the confounding from non-genetic factors and avoid the pitfalls of early association studies. The researchers hypothesized that alleles, which were not transmitted to the diseased individuals could be considered as controls. Further, if there was a skewing in the frequency of a particular gene variant in the cases and the alleles, which were not passed on from the parents, a functional role could be inferred. The counts of the transmitted and non-transmitted alleles could be compared via a Pearson chi-square

contingency statistic and a cross product odds ratio. While being a significant advance, the HRR method had other problems, which limited the types of studies that could be done in the genetics of complex traits. A problematic aspect of the HRR method was the null hypothesis, which did not differentiate between the absence of linkage and the absence of association (i.e. linkage disequilibrium).

The transmission disequilibrium test (TDT) was developed to test for association in the presence of linkage and represents a more robust method of determining linkage disequilibrium (LD). This method considers the alleles for each pair and counts the transmitted and non-transmitted alleles passed from a heterozygous parent to an affected child. If there is no LD, the transmission of alleles should be unbiased but a bias should exist if there is LD. A McNemar chi-square test statistic with 1 degree of freedom (df) is used to determine if the bias in allele transmissions is significantly deviant from expectation. One benefit of the TDT is that it does not require the specification of a genetic model, as evidenced by its nearly ubiquitous use in candidate gene studies of complex traits. Also, because the TDT is conditioned on parental genotypes, non-genetic associations such as assertive mating, population stratification and allele frequency skewing are removed from consideration. This method has been utilized in analyses of putative association with ADHD and two polymorphisms in SNAP-25, a synaptosomal vesicle protein (Chapter 3) and polymorphisms in four candidate genes of the Dopamine signaling pathway (Chapter 4).

Summary

ADHD is a complex genetics disorder, which has required the utilization of high throughput genomic technologies and statistical genetic methods to uncover genes conferring susceptibility. This effort has uncovered the first genetic locus contributing to ADHD susceptibility from a genome-wide analysis. Furthermore, a large data set of predominantly nuclear families has been used to examine the association of ADHD and several dopamine candidate genes.

References

1. Still GF. Some abnormal psychical conditions in children. Lancet 1902;1:1008-1012, 1077-1082, 1163-1168.

2. Tredgold AF. Mental deficiency (amentia). New York, NY: W.Wood; 1908.

3. Ebaugh FG. Neuropsychiatric sequelae of acute epidemic encephalitis in children. American Journal of Diseases of Children 1923(25):89-97.

4. Bradley W. The behavior of children receiving benzedrine. Am J Psychiatry 1937(94):577-585.

5. Rutter M. Brain damage syndromes in childhood: concepts and findings. J Child Psychol Psychiatry 1977;18(1):1-21.

6. Douglas VI. Stop, look, and listen: the problem of sustained attention and impulse control in hyperactive and normal children. Canadian Journal of Behavioural Science 1972;4:259-282.

7. Association AP. Diagnostic and statistical manual of mental disorders. 3rd ed. Washington, D.C.: Author; 1980.

8. Feingold BF. Hyperkinesis and learning disabilities linked to artificial food flavors and colors. Am J Nurs 1975;75(5):797-803.

9. Wolraich M, Milich R, Stumbo P, Schultz F. Effects of sucrose ingestion on the behavior of hyperactive boys. J Pediatr 1985;106(4):675-82.

10. Wolraich ML, Stumbo PJ, Milich R, Chenard C, Schultz F, Milch R. Dietary characteristics of hyperactive and control boys. J Am Diet Assoc 1986;86(4):500-4.

11. Wolraich ML. Sugar intolerance: is there evidence for its effects on behavior in children. Ann Allergy 1988;61(6 Pt 2):58-62.

12. White JW, Wolraich M. Effect of sugar on behavior and mental performance. Am J Clin Nutr 1995;62(1 Suppl):242S-247S; discussion 247S-249S.

13. Wolraich ML, Lindgren SD, Stumbo PJ, Stegink LD, Appelbaum MI, Kiritsy MC. Effects of diets high in sucrose or aspartame on the behavior and cognitive performance of children. N Engl J Med 1994;330(5):301-7.

14. Wolraich ML, Wilson DB, White JW. The effect of sugar on behavior or cognition in children. A meta-analysis. Jama 1995;274(20):1617-21.

15. Achenbach TM, Edelbrock CS. Manual for the Child Behavior Profile and Child Behavior Checklist. Burlington, VT: Author; 1983.

16. Biederman J, Faraone SV, Mick E, Spencer T, Wilens T, Kiely K, et al. High risk for attention deficit hyperactivity disorder among children of parents with childhood onset of the disorder: a pilot study. Am J Psychiatry 1995;152(3):431-5.

17. Gilger JW, Pennington BF, DeFries JC. A twin study of the etiology of comorbidity: attention-deficit hyperactivity disorder and dyslexia. J Am Acad Child Adolesc Psychiatry 1992;31(2):343-8.

18. Edelbrock C, Rende R, Plomin R, Thompson LA. A twin study of competence and problem behavior in childhood and early adolescence. J Child Psychol Psychiatry 1995;36(5):775-85.

19. Association AP. *Diagnostic and Statistical Manuel of Mental Disorders*. In. 4th ed. Washington DC: APA; 1994.

20. Biederman J, Faraone SV, Weber W, Russell RL, Rater M, Park KS. Correspondence between DSM-III-R and DSM-IV attention- deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 1997;36(12):1682-7.

21. Wolraich ML, Hannah JN, Baumgaertel A, Feurer ID. Examination of DSM-IV criteria for attention deficit/hyperactivity disorder in a county-wide sample. J Dev Behav Pediatr 1998;19(3):162-8.

22. Hagino OR, Weller EB, Weller RA, Washing D, Fristad MA, Kontras SB. Untoward effects of lithium treatment in children aged four through six years. J Am Acad Child Adolesc Psychiatry 1995;34(12):1584-90.

23. Wilens TE, Biederman J, Spencer TJ. Attention deficit/hyperactivity disorder across the lifespan. Annu Rev Med 2002;53:113-31.

24. Baumgaertel A, Wolraich ML, Dietrich M. Comparison of diagnostic criteria for attention deficit disorders in a German elementary school sample. J Am Acad Child Adolesc Psychiatry 1995;34(5):629-38.

25. Wolraich ML, Hannah JN, Pinnock TY, Baumgaertel A, Brown J. Comparison of diagnostic criteria for attention-deficit hyperactivity disorder in a county-wide sample. J Am Acad Child Adolesc Psychiatry 1996;35(3):319-24.

26. Foreman DM, Foreman D, Prendergast M, Minty B. Is clinic prevalence of ICD-10 hyperkinesis underestimated? Impact of increasing awareness by a questionnaire screen in an UK clinic. Eur Child Adolesc Psychiatry 2001;10(2):130-4.

27. Applegate B, Lahey BB, Hart EL, Biederman J, Hynd GW, Barkley RA, et al. Validity of the age-of-onset criterion for ADHD: a report from the DSM- IV field trials. J Am Acad Child Adolesc Psychiatry 1997;36(9):1211-21.

28. Marshall RM, Hynd GW, Handwerk MJ, Hall J. Academic underachievement in ADHD subtypes. J Learn Disabil 1997;30(6):635-42.

29. Biederman J. Attention-deficit/hyperactivity disorder: a life-span perspective. J Clin Psychiatry 1998;59(Suppl 7):4-16.

30. Biederman J. A 55-year-old man with attention-deficit/hyperactivity disorder. Jama 1998;280(12):1086-92.

31. Faraone SV, Biederman J, Spencer T, Wilens T, Seidman LJ, Mick E, et al. Attention-deficit/hyperactivity disorder in adults: an overview. Biol Psychiatry 2000;48(1):9-20.

32. Wender PH, Wolf LE, Wasserstein J. Adults with ADHD. An overview. Ann N Y Acad Sci 2001;931:1-16.

33. Mannuzza S, Klein RG, Bessler A, Malloy P, Hynes ME. Educational and occupational outcome of hyperactive boys grown up. J Am Acad Child Adolesc Psychiatry 1997;36(9):1222-7.

34. Gittelman-Klein R, Klein DF, Katz S, Saraf K, Pollack E. Comparative effects of methylphenidate and thioridazine in hyperkinetic children. I. Clinical results. Arch Gen Psychiatry 1976;33(10):1217-31.

35. Mannuzza S, Klein RG, Bessler A, Malloy P, LaPadula M. Adult outcome of hyperactive boys. Educational achievement, occupational rank, and psychiatric status. Arch Gen Psychiatry 1993;50(7):565-76.

36. Sullivan MA, Rudnik-Levin F. Attention deficit/hyperactivity disorder and substance abuse. Diagnostic and therapeutic considerations. Ann N Y Acad Sci 2001;931:251-70.

37. Horner BR, Scheibe KE. Prevalence and implications of attention-deficit hyperactivity disorder among adolescents in treatment for substance abuse. J Am Acad Child Adolesc Psychiatry 1997;36(1):30-6.

38. Biederman J, Wilens TE, Mick E, Faraone SV, Spencer T. Does attention-deficit hyperactivity disorder impact the developmental course of drug and alcohol abuse and dependence? Biol Psychiatry 1998;44(4):269-73.

39. Satterfield JH, Schell A. A prospective study of hyperactive boys with conduct problems and normal boys: adolescent and adult criminality. J Am Acad Child Adolesc Psychiatry 1997;36(12):1726-35.

40. MacDonald VM, Achenbach TM. Attention problems versus conduct problems as 6-year predictors of signs of disturbance in a national sample. J Am Acad Child Adolesc Psychiatry 1999;38(10):1254-61.

41. Jensen PS, Martin D, Cantwell DP. Comorbidity in ADHD: implications for research, practice, and DSM-V. J Am Acad Child Adolesc Psychiatry 1997;36(8):1065-79.

42. Biederman J, Faraone SV, Lpey K. Comorbidity of diagnosis in attention-deficit hyperactivity disorder. In: Weiss G, editor. Child and adolescent psychiatry clinics in North America: Attention-deficit hyperactivity disorder. Philadelphia: W.B. Saunders; 1992. p. 335-360.

43. Lavigne JV, Cicchetti C, Gibbons RD, Binns HJ, Larsen L, DeVito C. Oppositional defiant disorder with onset in preschool years: longitudinal stability and pathways to other disorders. J Am Acad Child Adolesc Psychiatry 2001;40(12):1393-400.

44. Smalley SL, McGough JJ, Del'Homme M, NewDelman J, Gordon E, Kim T, et al. Familial clustering of symptoms and disruptive behaviors in multiplex families with attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 2000;39(9):1135-43.

45. Burt SA, Krueger RF, McGue M, Iacono WG. Sources of covariation among attention-deficit/hyperactivity disorder, oppositional defiant disorder, and conduct disorder: the importance of shared environment. J Abnorm Psychol 2001;110(4):516-25.

46. Babinski LM, Hartsough CS, Lambert NM. Childhood conduct problems, hyperactivity-impulsivity, and inattention as predictors of adult criminal activity. J Child Psychol Psychiatry 1999;40(3):347-55.

47. Kovac I, Garabedian B, Du Souich C, Palmour RM. Attention deficity/hyperactivity in SLI children increases risk of speech/language disorders in first-degree relatives: a preliminary report. J Commun Disord 2001;34(4):339-54.

48. Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L, et al. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci 2001;21(2):RC121.

49. Varley CK. Effects of methylphenidate in adolescents with attention deficit disorder. J Am Acad Child Psychiatry 1983;22(4):351-4.

50. Milberger S, Biederman J, Faraone SV, Chen L, Jones J. ADHD is associated with early initiation of cigarette smoking in children and adolescents. J Am Acad Child Adolesc Psychiatry 1997;36(1):37-44.

51. Wilens TE, Biederman J, Mick E, Faraone SV, Spencer T. Attention deficit hyperactivity disorder (ADHD) is associated with early onset substance use disorders. J Nerv Ment Dis 1997;185(8):475-82.

52. Wilens TE, Biederman J, Mick E. Does ADHD affect the course of substance abuse? Findings from a sample of adults with and without ADHD. Am J Addict 1998;7(2):156-63.

53. Jaffe SL. Intranasal abuse of prescribed methylphenidate by an alcohol and drug abusing adolescent with ADHD. J Am Acad Child Adolesc Psychiatry 1991;30(5):773-5.

54. Biederman J, Wilens T, Mick E, Spencer T, Faraone SV. Pharmacotherapy of attention-deficit/hyperactivity disorder reduces risk for substance use disorder. Pediatrics 1999;104(2):e20.

55. Biederman J, Milberger S, Faraone SV, Kiely K, Guite J, Mick E, et al. Impact of adversity on functioning and comorbidity in children with attention-

deficit hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 1995;34(11):1495-503.

56. Milberger S, Biederman J, Faraone SV, Guite J, Tsuang MT. Pregnancy, delivery and infancy complications and attention deficit hyperactivity disorder: issues of gene-environment interaction. Biol Psychiatry 1997;41(1):65-75.

57. Goldman LS, Genel M, Bezman RJ, Slanetz PJ. Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents. Council on Scientific Affairs, American Medical Association. Jama 1998;279(14):1100-7.

58. Biederman J, Faraone SV, Keenan K, Knee D, Tsuang MT. Family-genetic and psychosocial risk factors in DSM-III attention deficit disorder. J Am Acad Child Adolesc Psychiatry 1990;29(4):526-33.

59. Biederman J, Faraone SV, Keenan K, Benjamin J, Krifcher B, Moore C, et al. Further evidence for family-genetic risk factors in attention deficit hyperactivity disorder. Patterns of comorbidity in probands and relatives psychiatrically and pediatrically referred samples. Arch Gen Psychiatry 1992;49(9):728-38.

60. Eaves LJ, Silberg JL, Meyer JM, Maes HH, Simonoff E, Pickles A, et al. Genetics and developmental psychopathology: 2. The main effects of genes and environment on behavioral problems in the Virginia Twin Study of Adolescent Behavioral Development. *J Child Psychol Psychiatry* 1997;38(8):965-80.

61. Smalley SL. Genetic influences in childhood-onset psychiatric disorders: Autism and Attention-deficit/hyperactivity disorder. American Journal of Human Genetics 1997;60(6):1276-82.

62. Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, et al. A genomewide scan for loci involved in attention-deficit/hyperactivity disorder. Am J Hum Genet 2002;70(5):1183-96.

63. Smalley SL, Kustanovich V, Minassian SL, Stone JL, Ogdie MN, McGough JJ, et al. Genetic linkage of attention-deficit/hyperactivity disorder on chromosome 16p13, in a region implicated in autism. Am J Hum Genet 2002;71(4):959-63.

64. Maher BS, Marazita ML, Moss HB, Vanyukov MM. Segregation analysis of attention deficit hyperactivity disorder. Am J Med Genet 1999;88(1):71-8.

65. Faraone SV, Biederman J. Is attention deficit hyperactivity disorder familial? Harv Rev Psychiatry 1994;1(5):271-87.

66. Cook EH, Jr., Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE, et al. Association of attention-deficit disorder and the dopamine transporter gene. *Am J Hum Genet* 1995;56(4):993-8.

67. Gill M, Daly G, Heron S, Hawi Z, Fitzgerald M. Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. Mol Psychiatry 1997;2(4):311-3.

68. Rowe DC, den Oord EJ, Stever C, Giedinghagen LN, Gard JM, Cleveland HH, et al. The DRD2 TaqI polymorphism and symptoms of attention deficit hyperactivity disorder. Mol Psychiatry 1999;4(6):580-6.

69. Palmer CG, Bailey JN, Ramsey C, Cantwell D, Sinsheimer JS, Del'Homme M, et al. No evidence of linkage or linkage disequilibrium between DAT1 and

attention deficit hyperactivity disorder in a large sample. Psychiatr Genet 1999;9(3):157-60.

70. Todd RD, Jong YJ, Lobos EA, Reich W, Heath AC, Neuman RJ. No association of the dopamine transporter gene 3' VNTR polymorphism with ADHD subtypes in a population sample of twins. Am J Med Genet 2001;105(8):745-8.

71. Comings DE, Wu S, Chiu C, Ring RH, Gade R, Ahn C, et al. Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: the additive and subtractive effect of the three dopaminergic genes--DRD2, D beta H, and DAT1. Am J Med Genet 1996;67(3):264-88.

72. Daly G, Hawi Z, Fitzgerald M, Gill M. Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. Mol Psychiatry 1999;4(2):192-6.

73. Comings DE. Clinical and molecular genetics of ADHD and Tourette syndrome. Two related polygenic disorders. Ann N Y Acad Sci 2001;931:50-83.

74. Muglia P, Jain U, Kennedy JL. A transmission disequilibrium test of the Ser9/Gly dopamine D3 receptor gene polymorphism in adult attention-deficit hyperactivity disorder. Behav Brain Res 2002;130(1-2):91-5.

75. Payton A, Holmes J, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, et al. Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: a family- based study. Am J Med Genet 2001;105(5):464-70.

76. Barr CL, Wigg KG, Wu J, Zai C, Bloom S, Tannock R, et al. Linkage study of two polymorphisms at the dopamine D3 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 2000;96(1):114-7.

77. Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry 2000;5(4):396-404.

78. Barr CL, Wigg KG, Feng Y, Zai G, Malone M, Roberts W, et al. Attention-deficit hyperactivity disorder and the gene for the dopamine D5 receptor. Mol Psychiatry 2000;5(5):548-51.

79. Eisenberg J, Mei-Tal G, Steinberg A, Tartakovsky E, Zohar A, Gritsenko I, et al. Haplotype relative risk study of catechol-O-methyltransferase (COMT) and attention deficit hyperactivity disorder (ADHD): association of the high-enzyme activity Val allele with ADHD impulsive-hyperactive phenotype. Am J Med Genet 1999;88(5):497-502.

80. Hawi Z, Millar N, Daly G, Fitzgerald M, Gill M. No association between catechol-O-methyltransferase (COMT) gene polymorphism and attention deficit hyperactivity disorder (ADHD) in an Irish sample. Am J Med Genet 2000;96(3):282-4.

81. Barr CL, Wigg K, Malone M, Schachar R, Tannock R, Roberts W, et al. Linkage study of catechol-O-methyltransferase and attention-deficit hyperactivity disorder. Am J Med Genet 1999;88(6):710-3.

82. Comings DE, Gade-Andavolu R, Gonzalez N, Blake H, Wu S, MacMurray JP. Additive effect of three noradrenergic genes (ADRA2a, ADRA2C, DBH) on attention-deficit hyperactivity disorder and learning disabilities in Tourette syndrome subjects. Clin Genet 1999;55(3):160-72.

83. Barr CL, Wigg K, Zai G, Roberts W, Malone M, Schachar R, et al. Attention-deficit hyperactivity disorder and the adrenergic receptors alpha 1C and alpha 2C. Mol Psychiatry 2001;6(3):334-7.

84. Hess EJ, Jinnah, H.A., Kozak, C.A., Wilson, M.C. Spontaneous locomotor hyperactivity in a mouse mutant with a deletion including the *Snap* gene on Chromosome 2. J Neurosci 1992;12(7):2865-2874.

85. Barr CL, Feng Y, Wigg K, Bloom S, Roberts W, Malone M, et al. Identification of DNA variants in the SNAP-25 gene and linkage study of these polymorphisms and attention-deficit hyperactivity disorder. *Mol Psychiatry* 2000;5(4):405-9.

86. Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochem 1995;65(3):1157-65.

87. Palacios JM, Camps M, Cortes R, Probst A. Mapping dopamine receptors in the human brain. J Neural Transm Suppl 1988;27:227-35.

88. Reader TA, Molina-Holgado E, Dewar KM. Comparative biochemical pharmacology of central nervous system dopamine D1 and D2 receptors. Mol Neurobiol 1992;6(4):425-50.

89. Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, et al. Multiple dopamine D4 receptor variants in the human population. Nature 1992;358(6382):149-52.

90. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 1996;379(6566):606-12.

91. Steffensen SC, Henriksen, S.J., Wilson, M.C. Transgenic rescue of SNAP-25 restores dopamine-modulated synaptic transmission in the coloboma mutant. Brain Research 1999;847:186-195.

92. Hess EJ, Rogan, P.K., Domoto, M., Tinker, D.E., Ladda, R.L., Ramer, J.C.,. Absence of linkage of apparently single gene mediated ADHD with the human syntenic region of the mouse mutant *Coloboma*. Am J Med Genet 1995;60:573-579.

93. Lucki I. The spectrum of behaviors influenced by serotonin. Biol Psychiatry 1998;44(3):151-62.

94. Halperin JM, Newcorn JH, Schwartz ST, Sharma V, Siever LJ, Koda VH, et al. Age-related changes in the association between serotonergic function and aggression in boys with ADHD. Biol Psychiatry 1997;41(6):682-9.

95. Zoroglu SS, Erdal ME, Alasehirli B, Erdal N, Sivasli E, Tutkun H, et al. Significance of serotonin transporter gene 5-HTTLPR and variable number of tandem repeat polymorphism in attention deficit hyperactivity disorder. Neuropsychobiology 2002;45(4):176-81.

96. Seeger G, Schloss P, Schmidt MH. Functional polymorphism within the promotor of the serotonin transporter gene is associated with severe hyperkinetic disorders. Mol Psychiatry 2001;6(2):235-8.

97. Quist JF, Barr CL, Schachar R, Roberts W, Malone M, Tannock R, et al. Evidence for the serotonin HTR2A receptor gene as a susceptibility factor in attention deficit hyperactivity disorder (ADHD). Mol Psychiatry 2000;5(5):537-41.

98. Levitan RD, Masellis M, Basile VS, Lam RW, Jain U, Kaplan AS, et al. Polymorphism of the serotonin-2A receptor gene (HTR2A) associated with childhood attention deficit hyperactivity disorder (ADHD) in adult women with seasonal affective disorder. J Affect Disord 2002;71(1-3):229-33.

99. Kent L, Doerry U, Hardy E, Parmar R, Gingell K, Hawi Z, et al. Evidence that variation at the serotonin transporter gene influences susceptibility to attention deficit hyperactivity disorder (ADHD): analysis and pooled analysis. Mol Psychiatry 2002;7(8):908-12.

100. Hawi Z, Dring M, Kirley A, Foley D, Kent L, Craddock N, et al. Serotonergic system and attention deficit hyperactivity disorder (ADHD): a potential susceptibility locus at the 5-HT(1B) receptor gene in 273 nuclear families from a multi-centre sample. Mol Psychiatry 2002;7(7):718-25.

101. Zoroglu SS, Erdal ME, Erdal N, Ozen S, Alasehirli B, Sivasli E. No Evidence for an Association between the T102C and 1438 G/A Polymorphisms of the Serotonin 2A Receptor Gene in Attention Deficit/Hyperactivity Disorder in a Turkish Population. Neuropsychobiology 2003;47(1):17-20.

102. Hawi Z, Myakishev MV, Straub RE, O'Neill A, Kendler KS, Walsh D, et al. No association or linkage between the 5-HT2a/T102C polymorphism and schizophrenia in Irish families. Am J Med Genet 1997;74(4):370-3.

103. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 1997;36(7):980-8.

104. Swanson J. SNAP-IV Scale. Irvine: University of California Child Development Center; 1995.

105. Achenbach T. Empirically Based Taxonomy: How to Use Syndromes and Profile Types Derived From the CBCL From 4 to 18, TRF, and WSR. Burlington: University of Vermont Department of Psychiatry; 1993.

106. Wechsler D. Examiner's Manual: Wechsler Intelligence Scale For Children - Third Edition. 3 ed: Psychological Corporation; 1991.

107. Markwardt F. Examiner's Manual: Peabody Individual Achievement Test - Revised: American Guidance Service; 1989.

108. The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. Alzheimer's Disease Collaborative Group. Nat Genet 1995;11(2):219-22.

109. Herskind AM, McGue M, Holm NV, Sorensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870-1900. Hum Genet 1996;97(3):319-23.

110. Duggirala R, Gonzalez Villalpando C, O'Leary DH, Stern MP, Blangero J. Genetic basis of variation in carotid artery wall thickness. Stroke 1996;27(5):833-7.

111. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996;273(5281):1516-7.

112. Haseman JK, Elston RC. The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 1972;2(1):3-19.

113. Penrose LS. The detection of autosomal linkage in data which consists of pairs of brothers and sisters of unspecified parentage. Annals of Eugenics 1937;6:133-138.

114. Parsian A, Todd RD, Devor EJ, O'Malley KL, Suarez BK, Reich T, et al. Alcoholism and alleles of the human D2 dopamine receptor locus. Studies of association and linkage. Arch Gen Psychiatry 1991;48(7):655-63.

115. Gelernter J, Goldman D, Risch N. The A1 allele at the D2 dopamine receptor gene and alcoholism. A reappraisal. Jama 1993;269(13):1673-7.

116. Falk CT, Rubinstein P. Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. Ann Hum Genet 1987;51 (Pt 3):227-33.

Table I-1 Description of ADHD diagnostic criteria

ADHD Criteria	**Description**
Criterion A	Persistent and non-transient behaviors
Criterion B	Age-of-onset predates7th birthday
Criterion C	Presence of ADHD symptoms in at least two settings
Criterion D	The impairment due to behaviors must be clear
Criterion E	The behavior can't be accounted for by other mental conditions

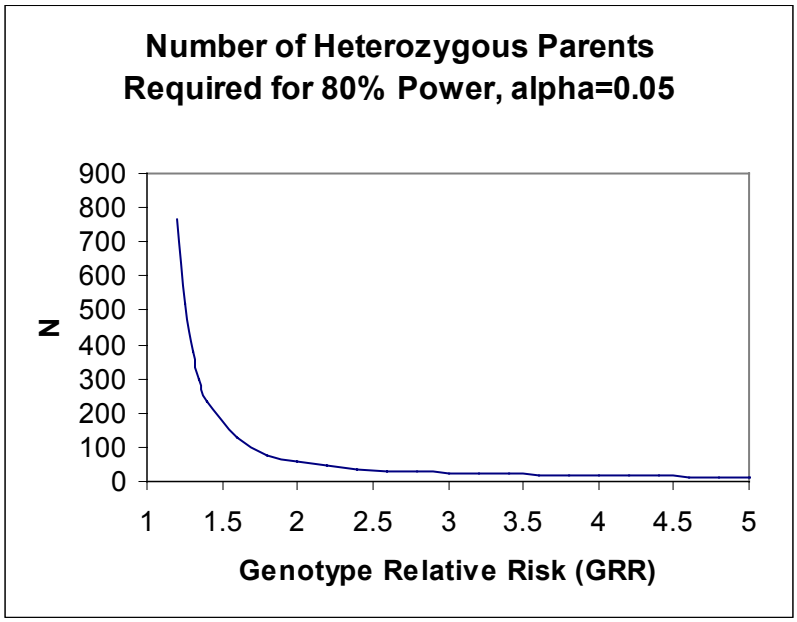
Table I-2. ADHD Subtype in ADHD affected children

ADHD subtype	Number of Affected	% in UCLA study	% in populaton (25)
Inattentive	314	49.2%	47%
Combined	275	43.1%	31%
Hyperactive	49	7.6%	21%

Table I-3 Presence of comorbid disorders in the ADHD set.

Disorder	**# in ADHD**	**% in ADHD**
Conduct Disorder	122	19.1%
Oppositional Defiant Disorder	352	55.2%
Anxiety Disorder	52	8.2%

Figure I-1 – Number of heterozygous parents required for 80% power, alpha=0.05



Chapter 1

Genome-wide analysis of single nucleotide polymorphisms in human expressed sequences.