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Attention-Deficit/Hyperactivity Disorder and Disruptive Behavior Disorders in adolescence related to levels of platelet MAO-B and polymorphisms in two candidate genes

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To my mother and father

ABSTRACT

Background: Attention-Deficit/Hyperactivity Disorder (ADHD) and Disruptive Behavior Disorders (DBD) (including Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD)), are common developmental and behaviour diagnoses among adolescents. Several of their symptoms, have been linked to genotypes (e.g. monoamine oxidase A Variable Number of Tandem Repeats (MAO-A VNTR) and the 5–HydroxyTryptamine Transporter gene-Linked Polymorphic Region (5-HTT LPR)) as well as to platelet Monoamine oxidase B (MAO-B) activity.

Aim: 1) To study the symptoms of ADHD and DBD phenotypes in adolescents and their association with MAO-B activity in platelets, MAO-A VNTR and 5-HTT LPR genotypes separately and in combination. 2) To describe the complexity of the phenotypes in relation to various psychiatric problems and risk behaviours. 3) To investigate psychiatric and behavioural symptoms separately, rather than the complete diagnosis and their association with individual and combinations of candidate genes.

Methods: A population-based sample of twins including 177 girls and 135 boys was interviewed using the Swedish version of Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL). Diagnoses of ADHD and DBD were compiled based on the Diagnostic and Statistical Manual of Mental Disorders Text Revision (DSM-IV-TR) diagnostic criteria. Blood was drawn from the subjects and analysed for platelet MAO-B activity and polymorphisms in the MAO-A VNTR and 5-HTT LPR genotypes.

Results: 1) For both boys and girls the heterozygote 5-HTT LPR genotype was found to be related to symptoms of CD. Girls exhibited an association between low platelet MAO-B activity and symptoms of ODD and DBD. An association was found in boys between the short MAO-A VNTR allele and symptoms of DBD, as well as between ADHD-like problems and the presence of a short 5-HTT LPR or short MAO-A VNTR allele, in combination with high levels of platelet MAO-B enzyme activity. 2) In girls, subthreshold diagnoses of ADHD and DBD coexisted with symptoms of depression, mania, panic attacks, phobias, anorexia nervosa, motor tics and post-traumatic stress disorder (PTSD) while in boys with symptoms of depression and PTSD. In both boys and girls, smoking and high alcohol consumption contributed to a high risk of having these phenotypes. 3) The combination of low MAO-B activity in platelets and polymorphism in the 5-HTT LPR genotype was associated with several psychiatric symptoms.

Conclusions: Polymorphisms in the 5-HTT LPR and MAO-A VNTR as well as MAO-B activity platelet separately and in combination are related to both ADHD and DBD. The complexity of the ADHD and DBD phenotypes was shown by the association with several psychiatric and behavioural problems. A broad clinical assessment is needed for adolescents with such preliminary diagnoses and the serotonergic system should be further investigated when studying genetic influences on the complex ADHD and DBD phenotypes.

Keywords: MAO-A, MAO-B, 5-HTT, ADHD, ODD, CD, subthreshold diagnosis

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CONTENTS

LIST OF ABBREVIATIONS

ADHD	Attention-Deficit/Hyperactivity Disorder
ASP	Anti Social Personality
ASD	Autism Spectrum Disorder
CD	Conduct Disorder
CGAS	Children Global Assessment Scale
CNS	Central Nervous System
DA	Dopamine
DAT	Dopamine Transporter
DBD	Disruptive Behavior Disorders
DRD	Dopamine Receptor
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th edition Text Revision
GAD	Generalized anxiety disorder
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-HydroxyTryptamine, serotonin
5-HTT	5-HydroxyTryptamine Transporter
K-SADS-PL	Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version
LPR	gene-Linked Polymorphic Region
MZ	Monozygote
MAO	Monoamine Oxidase
MDD	Major Depressive Disorder
NA	Noradrenaline
OR	Odds Ratio
ODD	Oppositional Defiant Disorder
VNTR	Variable Number of Tandem Repeats

1 PROLOGUE

I graduated from medical school in 1984 and when I was working to get a medical licence, I came in contact with child and adolescent psychiatry, which aroused my interest in this area. After obtaining my medical licence in 1988, I began my education as a child and adolescent psychiatrist and became a specialist 1993. Early in my training I developed an interest in helping children and adolescents with neuropsychiatric disorders and I have an extensive clinical experience of assessment and treatment of children in this area. During my clinical work I encountered many children with neuropsychiatric disorders. To learn more about the cause of these disabilities, I began to study scientific reports, which made me even more interested and inspired me to start my own project. This thesis has given me the opportunity to explore issue concerning children with neuropsychiatric symptoms and behavioural problems as well as the association of these conditions to some biological markers.

2 INTRODUCTION

The overall aim was to explore biological markers associated with Attention-Deficit/ Hyperactivity Disorder (ADHD) and Disruptive Behavior Disorders (DBD). In discussion with Professor Oreland, Uppsala university, with a lot of experience of behavioural research on the genetic level, it was decided that we should investigate biological markers with regard to the serotonergic system, since many of the symptoms, which are included in the diagnoses, can be linked to activity of the serotonergic system. The decision became that Monoamine Oxidase B (MAO-B) enzyme activity in platelets and functional genotypes of the 5-HydroxyTryptamine Transporter gene-Linked Polymorphic Region (5-HTT LPR) and Monoamine Oxidase A Variable Number of Tandem Repeats (MAO-A VNTR) were analysed, since there are a lot of scientific reports that deviant behaviours, as well as indirect or direct effects on Central Nervous System (CNS) serotonergic activity, have been demonstrated for these biological factors (Deckert, Catalano et al. 1999; Gorwood, Batel et al. 2000; Oreland, Damberg et al. 2002; Lawson, Turic et al. 2003; Oreland 2004; Oreland, Hallman et al. 2004; Paaver, Nordquist et al. 2007).

In psychiatric disorders the correspondence between genotype and phenotype is not yet fully understood, and in this thesis the focus is on the association between these complex phenotypes of ADHD and DBD as well as various psychiatric symptoms and genotypes with regard to the serotonergic system. ADHD and DBD are commonly reported disorder and there are several studies focusing on their respectively etiology, while here they are studied together.

The diagnoses of psychiatric disorder phenotypes are based on assessment of symptoms and their clinical manifestation according to manuals for example Diagnostic and Statistical Manual of Mental Disorders 4th edition Text Revision (DSM-IV-TR), (American Psychiatric Association. and American Psychiatric Association. Task Force on DSM-IV. 2000). The assessment of the complex phenotypes and various psychiatric/psychological symptoms was based on a structured clinical interview with the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL).The K-SADS-PL is a widely used semi structured diagnostic interview designed to assess current and past episodes

of psychopathology in children and adolescent according to DSM-IV-TR. K-SADS-PL has been shown to be a reliable and valid diagnostic instrument for child and adolescent psychiatric diagnoses (Kaufman, Birmaher et al. 2000).

In Paper I, there was an association between symptoms of deviant behaviour with respect to these separate biological markers. Paper II analysed a combination of these biological markers in relation to the phenotypes, and a more conclusively picture of ADHD and DBD emerged. Paper III found that the phenotypes of ADHD and DBD are not isolated conditions, however, they coexist with various psychiatric and behavioural problems as well as risk factors for addiction. Paper IV, found that these biological markers separately and in interaction are related to various psychiatric and behavioural problems.

3 BACKGROUND

ADHD is one of the most common developmental disorders; it has two separate underlying dimensions; an inattentive dimension, associated difficulties in sustained attention, distractibility, disorganization and lack of task persistence, and a hyperactive-impulsive dimension, including excessive activity and impulsive responding. Table 1 presents the symptoms of ADHD, to fulfil the criteria 6/9 symptoms are required for ADHD inattentive, 6/9 symptoms are required to fulfil the criteria for ADHD hyperactive, ADHD combined type includes the criteria for both inattentive type and the hyperactive/impulsivity type according to DSM-IV-TR. DBD is a behavioural disorder and includes two dimensions Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD). ODD characterized by a sustained pattern of chronic argumentativeness, and anger associated with compromised poor social relations with parents and peers. Table 2 presents the symptoms of ODD of which 4/8 are required for diagnosis according to DSM-IV-TR. CD characterized by a variety of persistent antisocial behaviours including acts of aggression, destruction of property, deceitfulness, theft and violation of commonly adhered to social norms. Table 3 presents the symptoms of CD, of which 3/15 are required for diagnosis according to DSM-IV-TR. Consensus estimates suggest that ADHD affects 3-10%, ODD 1-5% and CD 1-5% of school aged children (Spencer, Biederman et al. 2002; Kutcher, Aman et al. 2004; Biederman 2005). ADHD and DBD are disabling and associated with high costs, both for society and in terms of individual suffering and often associated with various psychiatric/psychological problems and environmental factors (Faraone, Wilens et al. 2007; Freitag, Rohde et al. 2010; Froehlich, Anixt et al. 2011; Malmberg, Edbom et al. 2011). Aggressive behaviour and temperament is often associated with ADHD and DBD and impulsivity is including in the criteria for ADHD and aggression in CD (Schmeck and Poustka 2001; Hirshfeld-Becker, Biederman et al. 2002; Rettew, Copeland et al. 2004).

Table 1. Symptoms of ADHD in accordance with DSM-IV-TR

A. Six (or more) of the following symptoms of inattention and/or six(or more) of hyperactive/impulsivity have been present for at least 6 months to a degree that is maladaptive and inconsistent with developmental level:

Inattention

1. often fails to give close attention to details or makes careless mistakes in schoolwork, work, or other activities
2. often has difficulty sustaining attention in tasks or play activities
3. often does not seem to listen when spoken to directly
4. often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behavior or failure to understand instructions)
5. often have difficulty organizing tasks and activities
6. often avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework)
7. often loses things necessary for tasks or activities (eg, toys, school assignments, pencils, books, or tools)
8. is often easily distracted by extraneous stimuli
9. is often forgetful in daily activities

Hyperactivity

10. often fidgets with hands or feet or squirms in seat
11. often leaves seat in classroom or in other situations in which remaining seated is expected
12. often runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, may be limited to subjective feelings of restlessness)
13. often has difficulty playing or engaging in leisure activities quietly
14. is often "on the go" or often acts as if "driven by a motor"
15. often talks excessively

Impulsivity

16. often blurts out answers before questions have been completed
17. often has difficulty awaiting turn
18. often interrupts or intrudes on others (eg, butts into conversations or games)

B. Some hyperactive-impulsive or inattentive symptoms that caused impairment were present before age 7 years.

C. Some impairment from the symptoms is present in two or more settings (e.g. at school or work and at home).

D. There must be clear evidence of clinically significant impairment in social, academic or occupational functioning.

E. The symptoms do not occur exclusively during the course of a Pervasive Developmental Disorder, Schizophrenia, or other Psychotic Disorder and are not better accounted for by another mental disorder (e.g. Mood Disorder, Anxiety Disorder, Dissociative Disorder, or a Personality Disorder.

Table 2. Symptoms of ODD in accordance with DSM-IV-TR

A. A pattern of negativistic, hostile, and defiant behavior lasting at least 6 months, during which four (or more) of the following are present:

1. often loses temper
2. often argues with adults
3. often actively defies or refuses to comply with adults' requests or rules
4. often deliberately annoys people
5. often blames others for his or her mistakes or misbehavior
6. is often touchy or easily annoyed by others
7. is often angry and resentful
8. is often spiteful or vindictive

Note: Consider a criterion met only if the behavior occurs more frequently than is typically observed in individuals of comparable age and developmental level.

B. The disturbance in behavior causes clinically significant impairment in social, academic, or occupational functioning.
C. The behaviors do not occur exclusively during the course of a Psychotic or Mood Disorder.
D. Criteria are not met for Conduct Disorder, and, if the individual is age 18 years or older, criteria are not met for Antisocial Personality Disorder.

Table 3. Symptoms of CD in accordance with DSM-IV-TR

A. A repetitive and persistent pattern of behavior in which the basic rights of others or major age-appropriate societal norms or rules are violated, as manifested by the presence of three (or more) of the following criteria in the past 12 months, with at least one criterion present in the past 6 months.

Aggression to people and animals

1. often bullies, threatens, or intimidates others
2. often initiates physical fights
3. has used a weapon that can cause serious physical harm to others (e.g. , a bat, brick, broken bottle, knife, gun)
4. has been physically cruel to people
5. has been physically cruel to animals
6. has stolen while confronting a victim (e.g., mugging, purse snatching, extortion, armed robbery)
7. has forced someone into sexual activity

Destruction of property

8. has deliberately engaged in fire setting with the intention of causing serious damage
9. has deliberately destroyed others' property (other than by fire setting)

Deceitfulness or theft

10. has broken into someone else's house, building, or car
11. often lies to obtain goods or favors or to avoid obligations (i.e., "cons" others)
12. has stolen items of nontrivial value without confronting a victim (e.g., shoplifting, but without breaking and entering; forgery)

Serious violations of rules

13. often stays out at night despite parental prohibitions, beginning before age 13 years
14. has run away from home overnight at least twice while living in a parental or parental surrogate home (or once without returning for a lengthy period)
15. is often truant from school, beginning before age of 13 years

B. The disturbance in behavior causes clinically significant impairment in social, academic or occupational functioning.

C. If the individual is aged 18 years or older, criteria are not met for Antisocial Personality Disorder.

3.1 SUBTHRESHOLD DIAGNOSIS AND DIMENSIONAL SCALE OF SYMPTOMS

In child and adolescent psychiatry there has been increasing interest in the distinction between subthreshold and full syndrome disorders, and specifically whether subthreshold conditions develop into or predict the onset of full syndrome disorders over time. Diagnosing ADHD in adolescents could be difficult if the clinician cannot establish the onsets prior to the DSM-IV-TR criterion of age seven years or if the number of symptoms is below the DSM-IV-TR threshold for diagnosis. In a series of papers the validity of atypical groups has been discussed: patients who meet all the criteria for ADHD except for age and onset and patient who exhibit impairing ADHD and DBD symptoms that do not exceeded the DSM-IV-TR threshold criteria (Faraone, Biederman et al. 2006; Faraone, Biederman et al. 2006).

The symptoms in the DSM-IV-TR are categorical diagnoses of ADHD and DBD. In a 15-year longitudinal study of subthreshold psychiatric conditions conducted with young adults Shankman et al. (Shankman, Lewinsohn et al. 2009) reported that subthreshold major depression, bipolar disorder, anxiety disorders, alcohol use, substance use, CD and/or ADHD were precursors of the corresponding full syndrome disorder and that subthreshold conditions were precursors for other full syndrome; and persisted after adjusting for comorbidity. Thus, many subthreshold conditions have predictive validity as they may represent precursors of full syndrome disorders and are therefore good targets for preventive interventions.

Another way of describing diagnoses is to use a dimensional scale of symptoms, i.e. the summary scores of symptoms for developmental or psychiatric diagnoses. Many clinicians believe that a dimensional scale of symptoms is a better way to describe psychopathology (Rubio-Stipec, Walker et al. 2002; Thapar, Langley et al. 2006).

It is important to have broader conceptualizations of dimensions of symptoms as well as categorical and subthreshold diagnoses of psychiatric disorders, such as studying a spectrum of externalizing psychopathology when identifying susceptibility genes and understanding the pathways through which genetic factors affect vulnerability to a variety of poor outcomes.

3.2 ADHD, DBD AND COMORBIDITY

Individuals with ADHD are at greater risk of poor educational attainment, lower income, underemployment, problems with the law, and impaired social relationships (Barkley 2002; Ek, Westerlund et al. 2011). Children with ADHD have a high risk of coexisting conditions such as DBD (Kutcher, Aman et al. 2004). ODD is a significant precursor of adolescent CD in children with ADHD, irrespective of ADHD severity (Barkley 2004; Whittinger, Langley et al. 2007; Taylor 2010). There is a negative prognosis for children with ADHD and comorbid CD, in addition to increased risk of criminality and drug abuse in adolescence and adulthood (Hirshfeld-Becker, Biederman et al. 2002; Hazell 2010; Heffner, Johnson et al. 2010). A high degree of overlap has been found between CD and ODD, both in terms of epidemiology and shared risk factors, such as low socioeconomic status, parental antisocial personality (ASP) disorder, and deficient parenting.

In a reviewed article by Freitag et al (Freitag, Rohde et al. 2010), up to 90% of the children with ADHD suffered from at least one comorbid disorder. The most prevalent comorbid disorders reported were ODD (40–65%), CD (27–47%), major depressive disorder (MDD; 0–24%) and generalized anxiety disorder (GAD; 13–21%). This is in agreement with findings from other studies showing that patients with ADHD had a higher risk than the control group of developing ODD, CD, tic disorder, mood disorders, anxiety disorders, specific phobias as well as ever tried and regular use of nicotine (Biederman, Newcorn et al. 1991; Barkley 2004; Faraone, Wilens et al. 2007).

ADHD and DBD have been described as risk factors for addictive behaviour such as smoking, high consumption of alcohol and other drugs (Faraone, Wilens et al. 2007; Heffner, Johnson et al. 2010). Moss et al. (Moss and Lynch 2001) demonstrated that symptoms of CD were associated with alcohol use disorder symptoms in adolescents. ASP disorder symptoms predict deterioration in global adaptive functioning, while the number of arrests, early-onset severe externalizing pathology and the quality of peer relationships (Biederman, Petty et al. 2010) are symptoms of DBD with onset in childhood/adolescence. The comorbid conditions that coexist with childhood ADHD often continue into adulthood (Shankman, Lewinsohn et al. 2009; Biederman, Petty et al. 2010).

3.3 ADHD, DBD AND HERITABILITY

Evidence from family, twin, and adoption studies suggest that genetic factors substantially contribute to the development of ADHD and several reports have indicated genetic factors add to the etiology of the disorder and that the susceptibility of the phenotype occurs in interaction between genotype and environmental risk factors in complex ways (Larsson, Larsson et al. 2004; Larsson, Lichtenstein et al. 2006; Coghill and Banaschewski 2009; Freitag, Rohde et al. 2010; Anokhin, Golosheykin et al. 2011; Froehlich, Anixt et al. 2011). A strong genetic component in the pathogenesis of ADHD with an estimated heritability of 70-80% has been reported (Biederman 2005). Evidence from behavioural genetics supports the conclusion that a significant amount of the variance in ASP and behaviour is due to genetic contributions (Biederman 2005; Ferguson 2010).

3.4 GENETICS

It is now generally agreed that many different genes are involved in personality. If a gene can explain 2% of a disorder that is a rather big effect and it is now holding that probably ever gene has very small effect for example 0.1% in explaining a disorder and it is often a combination of genes that are responsible for vulnerability for disorders. Biological and behavioural research is linked to genetics and the past three decades have witness the most dramatic changes in behavioural studies. Acceptance of the genetic influence in behavioural research has been due to an increasing number of scientific papers and popular books. There are different techniques of analysing genes. Genetic influence does not mean that the environment is unimportant. The corresponding between the environmental factors and the genetics are shown in Fig. 1.

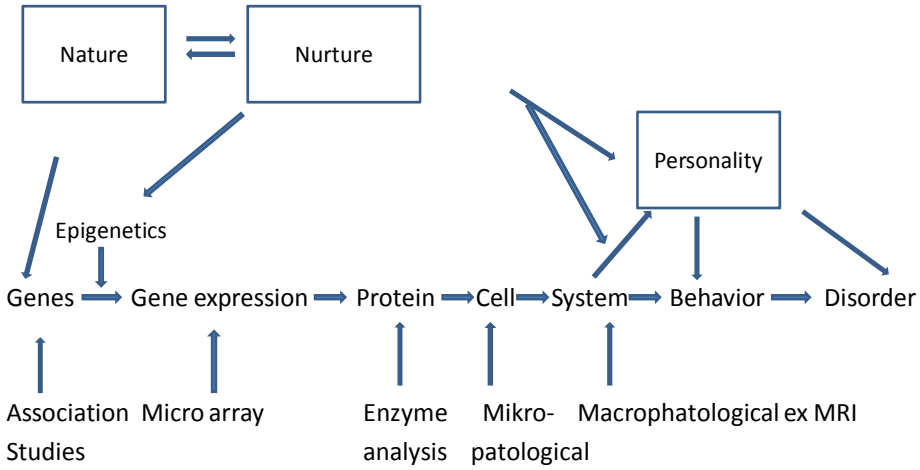

Fig. 1 Genes and environmental factors influence, and are dependent on and independent of each other, and how different genetic markers can be analysed.

3.4.1 Candidate genes and neurotransmitter

Examples of risk factors are specific candidate genes, genes that control the transmitter substance in CNS. A candidate gene is any gene considered likely to cause a disease. It may be a candidate because it is located in a particular chromosome region suspected of being involved in the disease or its protein product may suggest that it could be the disease gene in question. A candidate gene can also be identified by association with the phenotype and by linkage analysis to a region of the genome. A neurotransmitter is a chemical that is released from neurons in order for signals to be passed from one neuron to another in the CNS. Lack of communication between neurons can lead to a number of physical and mental disorders.

3.4.2 Dopaminergic system

Dopamine (DA) is the neurotransmitter that regulates the system that plays an important function in learning, motivation, goals, drives, reward and emotion all of which are crucial to survival. There are five known types of DA receptors (DRD) —D_1, D_2, D_3, D_4, and D_5 —and their variants. DA is produced in several areas of the brain, including the substantia nigra and the ventral tegmental area. DA is also a

neurohormone released by the hypothalamus and its main function as a hormone is to inhibit the release of prolactin from the anterior lobe of the pituitary.

Converging evidence has implicated abnormalities of DA neurotransmission to the pathology of ADHD and there is increasing evidence of reward-motivation deficits in this disorder. The inattention associated ADHD could stem in part from understimulation of reward and motivation centers in the brain. A reduction in DA synaptic markers associated with symptoms of inattention was shown in the dopamine reward pathway of participants with ADHD (Levy 1991; Volkow, Wang et al. 2009).

3.4.3 Noradrenergic system

Noradrenaline (NA) is the neurotransmitter involved in the detection of stimuli that are important for the alertness and readiness necessary to process such stimuli. The noradrenergic system originates in brain stem structures, such as the locus ceruleus, and in the area behind the locus ceruleus called the medulla. One part of this called the dorsal ascending system, which projects throughout the forebrain, including the neocortex and the hippocampus but the striatum. It is really turned on by novelty. Excess stimulation, including too much novelty, may cause stress that can be linked to anxiety.

3.4.4 Serotonergic system

Serotonin or 5-hydroxyTryptamine (5-HT) was first described as a vasoconstrictor compound in serum and later identified as a neurotransmitter (Rapport, Green et al. 1948; Gaddum 1953; Twarog and Page 1953). Serotonin is primarily found in the gastrointestinal tract, platelets, and in the CNS of animals including humans. Central serotonergic neurons originate from the raphe nuclei in the brainstem and innervate major brain regions, such as the hypothalamus, the limbic system, the striatum, and the neocortex. The serotonin system is involved in several vital functions like attention, memory, motivation, emotions, behaviour, appetite, and sleep. Serotonin is mainly metabolized to 5-Hydroxyindoleacetic acid (5-HIAA). 5-HT is a monoamine neurotransmitter biochemically derived from tryptophan. Metabolism involves first oxidation by MAO to the corresponding aldehyde followed by oxidation by aldehyde dehydrogenase to 5-HIAA which is a marker for serotonin turnover (Placidi, Oquendo et al. 2001). Serotonin is stored in vesicles until release into synaptic cleft

where it binds to receptors and is reuptaken into the cell via serotonin transporter gene 5-HydroxyTryptamine Transporter (5-HTT).

Two key genes expressing proteins of major importance for serotonergic activity are the genes encoding the 5-HTT and the MAO-A enzyme. Both of these genes have functional promoter polymorphisms that have been shown to be associated with behaviour: the 5-HTT LPR and the MAO-A VNTR (Lesch, Bengel et al. 1996; Deckert, Catalano et al. 1999).

3.4.4.1 Serotonin transporter gene (SLC6A4; 5-HTT)

The serotonin transporter gene 5-HTT is located on the long arm of chromosome 17. It codes for a solute carrier protein responsible for the reuptake of serotonin from the synaptic cleft back into the presynaptic neuron and thus represents a primary mechanism for the regulation of serotonergic activity in the brain. 5-HTT is expressed in brain regions such as the amygdala, hippocampus, thalamus, putamen, and anterior cingulate cortex. It represents a functional candidate for ADHD, which is further supported by studies suggesting its involvement in the etiology of impulsivity and in modulating stimulant response in alleviating hyperactivity (Seeger, Schloss et al. 2001; Zoroglu, Erdal et al. 2002; Faraone, Perlis et al. 2005).

A polymorphism in the 5-HTT LPR with the short allele (*s*) having lower *in vitro* transcriptional activity than the long allele (*l*), has been associated with personality traits (Canli and Lesch 2007). This polymorphism in the promoter region of the gene 5-HTT LPR is one of the most frequently studied genetic markers in psychiatric genetic research and has been extensively tested for association with depressive and anxiety disorders and related traits. The 5-HTT LPR is a 44-bp insertion/deletion yielding long and short alleles. The long variant is associated with more rapid serotonin reuptake resulting in lower levels of active serotonin, whereas the short variant appears to be associated with reduced serotonin reuptake resulting in higher levels of active serotonin (Heils, Teufel et al. 1995; Lesch, Bengel et al. 1996).

3.4.5 Monoamine Oxidase

MAO is the best understood enzyme associated with central monoamine transmitter systems. MAO proteins exist in two forms, MAO-A and MAO-B, which metabolises monoamines, DA, NA, and serotonin. Both enzymes are expressed in human tissues

however, only MAO-B is expressed inhuman platelets and therefore, in contrast to MAO-A, its activity is readily accessible for estimation in venous blood samples. MAO-A plays a major role in the degradation of the monoamine transmitters NA and serotonin in the human brain, while MAO-B plays a greater role in the degradation of DA (Oreland 1991).

3.4.5.1 MAO-A

The MAO-A gene is located on the X-chromosome (Xp11.23–11.4) and codes for an enzyme (MAO-A; EC 1.4.3.4), which is involved in the metabolism of biogenic amines including DA, NA and serotonin. The X-linked MAO-A gene harbours a variable number of tandem repeats (VNTR). Short alleles (≤3 repeats) show less MAO-A activity than long alleles (>3 repeats) (Deckert, Catalano et al. 1999). MAO-A VNTR has been linked to personality traits and deviant behaviour (Manuck, Flory et al. 2000; Meyer-Lindenberg, Buckholtz et al. 2006).

More specific support for MAO-A as a candidate gene for ADHD comes from a linkage study conducted in a large Dutch family demonstrating a relation between MAO-A and impulsive, aggressive behaviour (Brunner, Nelen et al. 1993). Studies have mainly focused largely on a functional 30-bp VNTR 1.2 kb upstream of the gene that has been associated with impulsivity and aggression (Manuck, Flory et al. 2000; Caspi, McClay et al. 2002). This polymorphism consists of alleles of 2, 3, 3.5, 4 and 5 copies with evidence suggesting the 2 and 3 repeat alleles to be less efficiently transcribed than the longer ones (Deckert, Catalano et al. 1999). There is some evidence to suggest that the 5-repeat allele also results in less efficient transcription (Sabol, Hu et al. 1998; Meyer-Lindenberg, Buckholtz et al. 2006). Nevertheless, the majority of studies testing for association between this polymorphism and ADHD have relied on the classification system described by in which the 2- and 3-repeat alleles are considered ‘low-activity’ alleles and the 3.5-, 4- and 5-repeat alleles are considered ‘high-activity’ (Deckert, Catalano et al. 1999).

3.4.5.2 MAO-B

MAO-B is present in platelets where its activity has been suggested to be a marker for the monoaminergic capacity in the brain (Oreland 2004; Oreland, Hallman et al. 2004). Low platelet MAO-B activity correlates with personality traits such as sensation

seeking, impulsiveness and monotony avoidance, and has also been associated with deviant behaviour such as type II alcoholism. Low MAO-B activity has been linked to ADHD (Shekim, Bylund et al. 1986) and temperament (Oreland 2004). The relationship between novelty seeking and MAO-B and externalizing psychopathology is described in the literature (Ruchkin, Koposov et al. 2005). Platelet MAO-B activity is highly heritable (>76%) and stable over time (Pedersen, Oreland et al. 1993). Low platelet MAO-B activity does not directly predispose individuals to psychopathology but is related to specific personality traits, and has had a great impact on the understanding of the nature of constitutional factors that render individuals vulnerable. Smoking inhibits MAO-activity in platelets, and is a confounding factor that was not controlled for in previous studies (Oreland, Damberg et al. 2002).

3.4.6 ADHD, DBD and candidate genes

ADHD can be considered a disorder with particular focus on the neurotransmitters DA and NA (Faraone, Perlis et al. 2005; Wohl, Purper-Ouakil et al. 2005). With regard to the serotonergic system, there are studies reporting involvement of serotonin in the etiology of ADHD (Halperin, Newcorn et al. 1997; Lucki 1998; Seeger, Schloss et al. 2001; Hawi Z, Dring M et al. 2002; Heiser, Dempfle et al. 2007). Genetic, developmental and neuro-biological factors are known to play an important role in the emergence of aggressive behaviour, substance abuse, as well as family and marital discord (Farrington and Loeber 2000). Several neurotransmitter systems such as serotonin, DA and NA were suspected of being involved in aggression (Dolan, Anderson et al. 2001; Nelson and Chiavegatto 2001). Serotonergic components are involved in several behavioural traits such as aggression and impulsiveness, which are frequently associated with ADHD and DBD (Brunner, Nelen et al. 1993; Halperin, Newcorn et al. 1997; Mitsis, Halperin et al. 2000; Seeger, Schloss et al. 2001; Hawi Z, Dring M et al. 2002; Lawson, Turic et al. 2003; Zammit, Jones et al. 2004; Wohl, Purper-Ouakil et al. 2005; Heiser, Dempfle et al. 2007; Grigorenko, De Young et al. 2010).

A range of candidate genes, such as 5-HTT, MAO-A Dopamine Receptor 1-4 (DRD1-DRD4) has been implicated in psychiatric comorbidity (Cerda, Sagdeo et al. 2009). Polymorphisms in three DA loci (DRD4, DRD5, Dopamine Transporter (DAT)) are the most frequently replicated molecular correlates of ADHD (Faraone, Perlis et al. 2005). Reports are inconsistent with regard to associations between ADHD-like

behaviours and the 5-HTT LPR or MAO-A VNTR, (Manor, Tyano et al. 2002; Lawson, Turic et al. 2003; Domschke, Sheehan et al. 2005; Lung, Yang et al. 2006; Heiser, Dempfle et al. 2007; Qian, Liu et al. 2009; Baker, Prevatt et al. 2011).

In the present study 5-HTT LPR was studied as well as MAO-A genes and MAO-B activity in platelets in addition to ADHD, DBD and psychiatric symptoms. These two key genes express proteins of major importance for serotonergic activity. Both have functional promoter polymorphism 5-HTT LPR and MAO-A VNTR, which has been found to be associated with behaviour such as anxiety and associated with the risk for substance use disorders (Sabol, Hu et al. 1998; Deckert, Catalano et al. 1999; Lesch 2005). The low activity variant in both 5-HTT LPR and MAO-A VNTR genes confers vulnerability to deviant behaviour. Studies on humans indicate that the short, and thus presumably less active, variant of this MAO-A VNTR polymorphism seems to interact with environmental factors such as childhood maltreatment in explaining male adolescent delinquency (Caspi, McClay et al. 2002; Foley, Eaves et al. 2004; Nilsson, Sjoberg et al. 2005). Lower serotonergic activity responsively assessed in childhood predicted the development of ASP disorder (Flory, Newcorn et al. 2007). Research has suggested that trait-impulsivity and aggressiveness in ADHD could be potential moderators of serotonin-dependent aggressive behaviour in children and adolescents (Zepf 2010).

4 AIMS

The overall aim was to study symptoms of ADHD and DBD, including ODD and CD in adolescence and their relationships with candidate genes 5-HTT and MAO-A as well as MAO-B activity in platelets.

4.1 PAPER I

To study whether subthreshold diagnosis and dimensional symptoms of ADHD and DBD phenotypes are associated with MAO-B activity in platelets and polymorphism in MAO-A VNTR and 5-HTT LPR genotypes.

4.2 PAPER II

To study the association between ADHD and DBD phenotypes with regard to the combination of platelet MAO-B activity and MAO-A VNTR or 5-HTT LPR genotype.

4.3 PAPER III

The aim was to study how subthreshold diagnoses of ADHD_comb and DBD are related to other symptoms of child and adolescent psychiatric disorders and risk behaviours related to smoking, alcohol and various drugs.

Specific aims:

1. The prevalence of possible or certain symptoms in boys and girls revealed by the screening questions in the Kiddie-SADS-Present interview.
2. The relationship between subthreshold diagnoses of ADHD/DBD and the screening questions for other psychiatric problems in the K-SADS-PL using odd ratios.
3. The relationship between subthreshold diagnoses of ADHD/DBD and smoking, alcohol and drug use.

4.4 PAPER IV

Instead of using the DSM-IV-TR classification based on categorical diagnoses the purpose was in the present study to study how separate psychiatric and behavioural symptoms based on the K-SADS-PL interviews were related to platelet MAO-B activity and polymorphism in the 5-HTT LPR genotype. The aim was to study the association between symptoms and the individual biological markers and combinations of these markers.

5 METHODS

5.1 STUDY GROUP

The participants were part of the population-based Swedish Twin study of CHild and Adolescent Development (TCHAD) (Lichtenstein, Tuvblad et al. 2007), a longitudinal study of all 1481 twin pairs born in Sweden between May 1985 and December 1986. They were followed in four waves of measurements targeting the whole population and by a clinical investigation of a sub-sample from childhood (8 years) into adulthood (21–22 years) (Larsson, Larsson et al. 2004; Edbom, Malmberg et al. 2010). Papers I-IV is part of the TCHAD study, although twin methodology was not used. The clinical investigation was conducted on the twin pairs from the TCHAD study who resided in the Stockholm area in 2001-2002. Out of the 271 twin pairs originally invited to participate in the study, 156, i.e. 312 individuals (135 boys and 177 girls), and one or both parents took part in the interview. Blood was drawn from 247 individuals and genetic association was analysed from 197 individuals. The mean age was 16 years, ranging from 14.6 to 16.7 years. The 115 twin pairs, i.e. 230 individuals who did not participate, either declined (67 twin pairs, i.e. 134 individuals) or failed to answer the telephone call or a letter (48 twin pairs, i.e. 96 individuals). The most common reason for declining was lack of time on the part of the parents and in a few cases the reason stated was that one of the twins was disabled. A sample selection procedure is presented in Fig. 2.

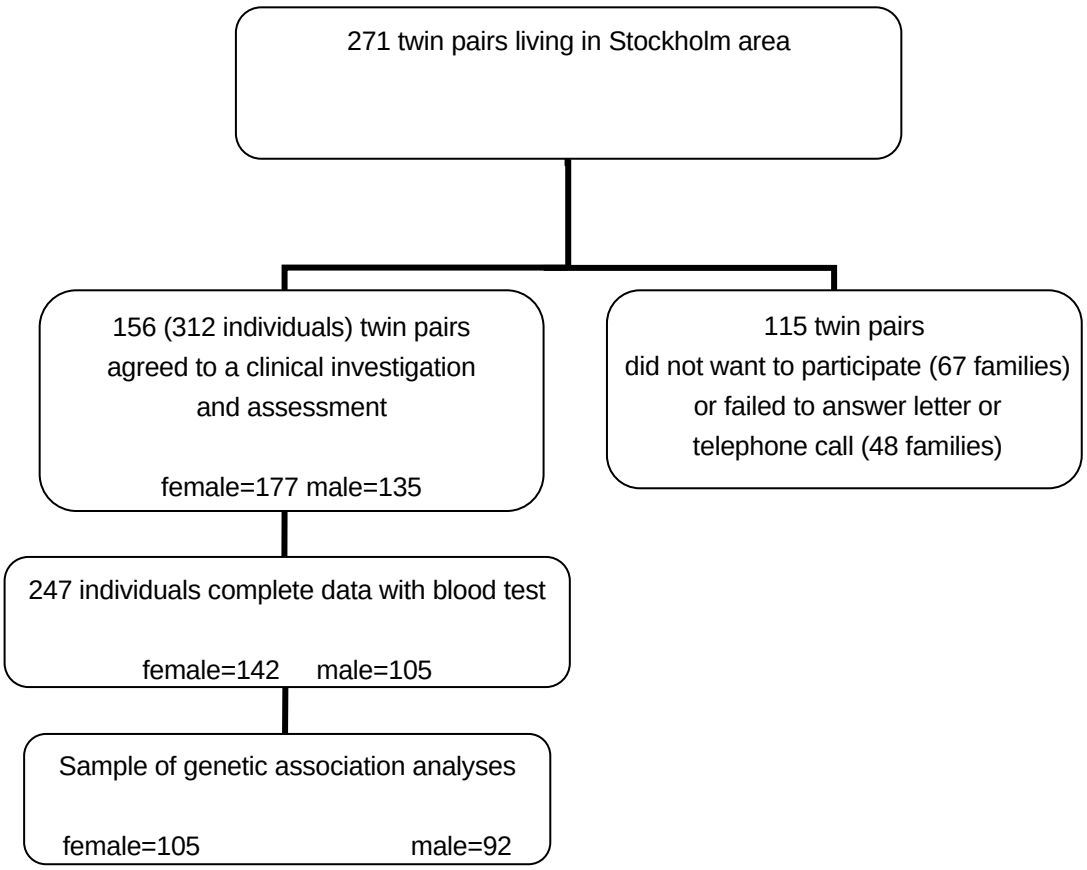

Fig. 2 Sample selection procedures

Of the 312 individuals, 65% lived with two parents, 34% with one parent and 1% in foster care. Academic qualifications were reported for 55% of the mothers and 49% of the fathers. 80% of the individuals had parents of Swedish origin, 14% had one parent who was an immigrant and, in the case of 6%, both parents were immigrants. The participants in the study were born in Sweden, as requirements for inclusion in the Swedish twin registry are born in Sweden and both twins being alive.

5.2 CLINICAL INTERVIEW

The K-SADS-PL is a widely used semi-structured diagnostic interview designed to assess current and past episodes of psychopathology in children and adolescents in accordance with the DSM-IV-TR criteria. K-SADS-PL has been shown to be a reliable and valid diagnostic instrument for child and adolescent psychiatric diagnoses and is divided into a screening interview and five diagnostic supplements (Kaufman, Birmaher et al. 2000).

The twin pairs and their parents were invited to an assessment including a structured interview based on the K-SADS-PL (Kaufman 1996). In this study, the Swedish K-SADS-PL present version (Ivarsson 2010) was used to assess the symptoms in accordance with DSM-IV-TR. Each individual was interviewed separately. The interviewer assessed the information from one of the parents and the children and classified the symptoms as *"not present" (0), "possible" (1)* or *"certain" (2)* and trauma, drugs, alcohol and smoking as*" yes"* or *"no"*. The interview with parents in most cases involved the children's mother.

For the purpose of the study, the symptoms included in the criteria for ADHD subtypes, ODD and CD were assessed according to K-SADS-PL and compiled in three ways:

1. Dimensional scales of the symptoms i.e., the summary scores of symptoms included in the criteria for the following diagnoses (scaled 0-2) were calculated: ADHD inattentive type, ADHD hyperactive type, ADHD combined type, ODD, CD and a combined scale for CD and ODD.

2. DSM-IV diagnostic criteria were applied using the information from K-SADS-PL. Each symptom was counted if the item was assessed as "certain" by the interviewer leading to the following diagnoses: ADHD inattentive type, ADHD hyperactive type, ADHD combined type (ADHD_comb), ODD, CD and CD or ODD combined (Table 4).

3. The symptoms of the diagnoses described in 1 and 2 were also dichotomized. DSM-IV diagnostic criteria were applied as follows; the individual child was regarded as having "subthreshold diagnosis" if the symptom was assessed as "possible" or "certain" using the same diagnostic criteria as in 2 above (Table 4).

Table 4. ADHD and DBD derived from K-SADS -PL interviews in a population-based sample of adolescents

Diagnosis	Girls		Boys		Total	
	n/N*	(%)	n/N*	(%)	n/N*	(%)
Threshold						
ADHD inattentive	4/174	2.3	4/134	3.0	8/308	2.6
ADHD hyperactive	2/173	1.2	7/134	5.2	9/307	2.9
ADHD combined	0/173	0.0	1/134	0.8	1/307	0.3
ODD	1/173	0.6	2/133	1.5	3/306	1.0
CD	1/174	0.6	1/133	0.8	2/307	0.7
CD or ODD	2/173	1.2	2/133	1.5	4/306	1.3
Subthreshold **						
ADHD inattentive	29/174	16.7	42/134	31.3	71/308	23.1
ADHD hyperactive	21/173	12.1	25/134	18.7	46/307	15,0
ADHD combined	13/173	7.5	16/134	11.9	29/307	9.5
ODD	23/173	13.3	19/133	14.3	42/306	13.7
CD	9/174	5.2	15/133	11.3	24/307	7.8
CD or ODD	25/173	14.5	25/133	18.8	50/306	16.3

*Number of children fulfilling criteria for diagnosis or subthreshold diagnosis/total number of children
**Subthreshold diagnosis based on possible and certain symptoms

5.2.1 Children Global Assessment Scale

To study the validity of subthreshold diagnoses were used by the Children Global Assessment Scale (CGAS) a scale for children very similar with the Global Assessment of Functioning in DSM-IV (Shaffer, Gould et al. 1983).

The CGAS scores were significantly lower (p-values from 0.01 to 0.049) in the children with each subtype of subthreshold diagnosis of ADHD phenotype (inattentive, hyperactivity and combined types) and DBD (ODD, CD and ODD or CD) compared to children without diagnosis.

5.3 GENOTYPING AND MAO-B ACTIVITY MEASUREMENT

Blood samples were obtained from 247 individuals (123 twin pairs and one individual), 106 boys and 141 girls, and genomic DNA was isolated by standard methods. For MAO-B activity measurement, platelet rich plasma was prepared by low-speed centrifugation, 200 x g for 10 minutes. Platelet concentration of the plasma samples

were estimated electronically and the plasma was stored at -80°C. PCR-based genotyping was performed as described for MAO-A VNTR (Nilsson, Sjoberg et al. 2007) and 5-HTT LPR (Collier, Stober et al. 1996). The MAO-A and 5-HTT PCR products were analyzed by electrophoresis on 2 % agarose gels and visualized under UV light by ethidium bromide staining. Genotypes were based on two separate readings.

Because the MAO-A gene is X linked, only boys were included in the MAO-A VNTR analysis. Having two X chromosomes, girls can be heterozygous and cannot be functionally characterized with certainty because it is impossible to know which of the two alleles is inactivated.

Catalytic activity of platelet MAO-B was analyzed with C^{14}-labelled 2-phenylethylamine (β-PEA) as substrate. Before analysis, the samples of platelet rich plasma were thawed and sonicated at 0°C for 5x10 seconds with intervals of 5 seconds for lysis of the platelets. 50 µl of the plasma was added to 50 µl of 0.1 mM 14C-β-PEA (0.5 µCi/ml) in 0.1 M sodium phosphate buffer. The reaction mixture was incubated at 37°C for 4 minutes, and the reaction terminated by the addition of 30 µl 1 M HCL. Thereafter, the radioactive aldehyde product formed was extracted by shaking for 30 seconds into 750 µl toluene: ethylacetate (1:1). The samples were then centrifuged at room temperature for 5 minutes at 1000 x g. The organic phase (500 µl), containing the aldehyde product was pipetted into vials with 8 ml scintillation fluid and the amount of radioactive aldehyde product subsequently quantified by scintillation analysis. Enzyme activity is expressed as nmol of substrate oxidized per 10^{10} platelets per minute. All samples were analyzed blindly and in duplicate.

5.4 SMOKING

Compounds in cigarette smoke exert inhibitory effect on MAO activity, but are only reported to be significant in quantities exceeding 300 cigarettes per month (Snell, Glanz et al. 2002). Information about cigarette smoking was obtained by asking the subjects at the time of the blood sampling whether they had smoked in the past 24 hours (10% boys, 11% girls). This information was used as a covariate in the statistical analyses of the relationships between behaviour and platelet MAO-B activity. The K-SADS-PL inventory also contains questions about smoking habits as well. Smoking more than

two cigarettes per day were used as a cut off (11% boys, 12% girls). In order to further explore the possible effects of smoking on our results, subjects with positive smoking information from K-SADS-PL were, for the statistical analyses, combined with the group who had answered the question that they had smoked in the 24 hours prior to the blood test.

5.5 STATISTICAL ANALYSES

The main behavioural symptoms ADHD, ODD and CD, included either as categorical variables (subthreshold diagnosis) or dimensional scales, were analyzed with respect to their association with MAO-B activity in platelets and genotype of the MAO-A VNTR and the 5-HTT LPR. Separate analyses were performed for boys and girls. For these analyses the generalized linear model (GLM) in the Stata statistical software package was used (StataCorp 2005). Standard errors were adjusted for clustering within twin pairs by increasing the estimated standard errors, thus providing robust estimates of for example p-values. The method is based on the sandwich or Huber/White variance estimator a method available in Stata 9.0 and 11.0. The descriptive analysis of the data presented in the tables includes the entire sample.

However, in the genetic association analyses one of the children in each pair of monozygotic (MZ) twins was randomly excluded from the statistical analyses (n=51), as MZ twins share all genetic risk factors which makes it somewhat uncertain whether the adjustment of standard errors, as described above, is correct for these subjects.

The dimensional measures of ADHD, ODD and CD problems had skewed distributions. Therefore, non-parametric bootstrap tests were performed using the GLM model in Stata. In the GLM analyses, when calculating the relationships between behavioural problems and the 5-HTT genotype, the nominal scale included three variants (long-long, long-short and short-short alleles). This nominal scale was transformed into three index variables and employed in the GLM analyses using one of the variants as the reference variable (omitting this variable) in Paper I and II. In each of the GLM analyses of platelet MAO-B activity, the information about cigarette smoking at the time for the blood sampling (whether they had smoked within the past 24 hours), and from the K-SADS-PL was included as a covariate; smoking (yes/no). In the case of dimensional ADHD/DBD scales the association between sex and polymorphisms in the 5-HTT genotypes was analysed using GLM. In the same way the

relation between sex and platelet MAO-B activity was studied. Children with missing values for two or more symptoms included in the criteria for each of the diagnoses in this study (ADHD subtypes, ODD and CD respectively) were excluded from the analyses. If there was missing values for only one of the symptoms in each of the diagnoses it was recorded as "no symptom."

In Paper IV where β-coefficient and Confidence Interval (CI) calculated using logistic regression, and a p-value <0.05 was considered statistically significant. Standard errors were adjusted for clustering within twin pairs. The study subjects were calculated with MAO-B and 5-HTT LPR genotype separately and in combination. The 5-HTT LPR genotypes were analysed using the specific 5-HTT polymorphism and compared with the rest of the sample with the other 5-HTT polymorphism (*ll* compared to *ls+ss, ls* compared to *ll+ss, ss* compared to *ll+ls).* A multiplicative interaction model was used to analyse the combination of MAO-B as a continuous variable and the specific 5-HTT polymorphism.

The study was approved by the ethics committee at Karolinska Hospital, Stockholm, Sweden, and in all cases the participating parent and the teenagers gave their written informed consent.

6 SUMMARY OF RESULTS

6.1 PAPER I

With regard to the 5-HTT LPR genotype, 41% carried two copies of the long allele, 16% two copies of the short, and 43% were heterozygous. The genotype distribution of our sample did not deviate significantly from the Hardy-Weinberg equilibrium.

The platelet MAO-B activity range was 4.0-19.2 nmol/min/10^{10} in boys and 6.3-23.7 nmol/min/10^{10} in girls. Boys had significantly lower mean MAO-B activity than girls (p<0.001).

Girls

There were an association between low platelet MAO-B activity and symptoms of ODD and ODD or CD (Table 5). The heterozygote long/short 5-HTT LPR genotype and symptoms of CD for dimensional scale. There were no associations between the 5-HTT LPR genotypes and the subthreshold diagnoses in girls.

Table 5**.** Subtreshold diagnoses of ADHD/DBD and activity of MAO-B in platelets in girls

Subthreshold diagnosis	With diagnosis	Without diagnosis	n/N*	p†	p‡
	M(sd)	M(sd)			
ADHD inattentive	12.57(2.40)	13.31(3.53)	22/140	0.371	0.485
ADHD hyperactive	11.99(2.32)	13.32(3.47)	15/139	0.164	0.095
ADHD combined	11.97(1.95)	13.25(3.45)	8/139	0.424	0.176
ODD	11.50(2.18)	13.44(3.46)	18/140	**0.020**	**0.004**
CD	11.63(1.48)	13.26(3.42)	6/140	0.075	0.142
ODD or CD	11.52(2.07)	13.47(3.48)	20/140	**0.008**	**0.008**

*Number of children with subthreshold diagnosis/total number of children
† Analyses performed using subthreshold diagnosis
‡ Analyses performed using dimensional scales

Boys

The heterozygote 5-HTT LPR genotype in boys was found to be related to symptoms of CD which were true for both dimensional scale of symptoms and subthreshold diagnosis Hemizygosity for the short MAO-A VNTR allele was associated with symptoms of DBD (Table 6 and 7).

Table 6. Dimensional symptom scales and subthreshold diagnosis of ADHD and DBD related to the 5-HTT LPR genotype in boys

Dimensional symptom scale	**SS short/short** [*] **n=12**	**LS long/short n=47**	**LL long/long n=45**	**p**[†]	**p**[‡]
	Mean (S.D.)				
ADHD inattentive	4.17(3.10)	6.08(4.25)	4.09(3.73)	0.867	0.122
ADHD hyperactive	3.17(3.43)	4.71(4.00)	3.02(2.97)	0.990	0.089
ADHD combined	7.33(6.05)	10.79(7.04)	7.11(6.43)	0.928	0.064
ODD	1.00(1.48)	2.17(2.82)	1.24(2.05)	0.817	0.079
CD	1.08(1.68)	1.49(2.47)	0.42(1.08)	0.328	**0.006**
ODD or CD	2.08(2.64)	3.66(4.90)	1.67(2.86)	0.651	**0.018**
Subthreshold diagnosis***	**SS short/short**	**LS long/short**	**LL long/long**	**p**[†]	**p**[‡]
	n/N[**]				
ADHD inattentive	3/12	17/48	12/45	0.905	0.333
ADHD hyperactive	2/12	10/48	5/45	0.621	0.252
ADHD combined	2/12	6/48	5/45	0.621	0.853
ODD	1/12	11/47	5/45	0.790	0.150
CD	1/12	9/47	1/45	0.350	**0.035**
ODD or CD	2/12	14/47	5/45	0.630	**0.049**

[*]Total number with the specific genetic marker

[**]Number of children fulfilling diagnostic criteria/total number with the specific genetic marker

***Subthreshold diagnosis according to possible and certain symptoms

[†]SS and LL compared

[‡]LS and LL compared

Table 7. Subthreshold diagnoses of ADHD/DBD and the MAO-A VNTR genotype in boys

Subthreshold diagnosis*	Long 4 repeat	Short 3 repeat	p[†]	p[‡]
ADHD inattentive	21/71	12/35	0.641	0.348
ADHD hyperactive	9/70	8/35	0.241	0.185
ADHD combined	7/70	6/35	0.353	0.202
ODD	8/70	9/35	0.082	0.288
CD	5/70	6/35	0.119	0.292
ODD or CD	9/70	12/35	**0.021**	0.249

[*]Number of boys with high subthreshold diagnosis/total number with the specific genetic marker

**Subthreshold diagnosis according to possible and certain symptoms

[†]Analyses performed using subthreshold diagnosis

[‡]Analyses performed using dimensional scale

6.2 PAPER II

In this paper a combination of platelet MAO-B activity and MAO-A VNTR or 5-HTT LPR genotypes were associated with subthreshold ADHD and DBD. Boys with the short allele of 5-HTT LPR or MAO-A in combination with high MAO-B activity in platelets above the median, scored higher on ADHD related traits.

Within the group with high MAO-B activity, boys carrying the 5-HTT LPR_*s* allele scored significantly higher on all ADHD-related phenotypes tested than subjects carrying the 5-HTT LPR_*l* allele (ADHD_comb (p<0.001), ODD (p=0.007), CD (p=0.004) and DBD (p<0.001). No similar effect was found in the female subjects. Among boys carrying the MAO-A VNTR_*s* allele, the group with high platelet MAO-B activity scored higher on the ADHD combined type (p=0.002). In the group with high MAO-B activity, boys carrying the 5-HTT LPR_*s* allele scored significantly higher on all ADHD-related phenotypes tested than subjects carrying the 5-HTT LPR-*l* allele ADHD combined (p<0.001), ODD (p=0.007), CD (p=0.004) and DBD (p<0.001). No similar effect was found in the female subjects. Among boys carrying the MAO-A VNTR-*s* allele, the group with high platelet MAO-B activity scored higher on the ADHD combined type (p=0.002) (Fig. 3).

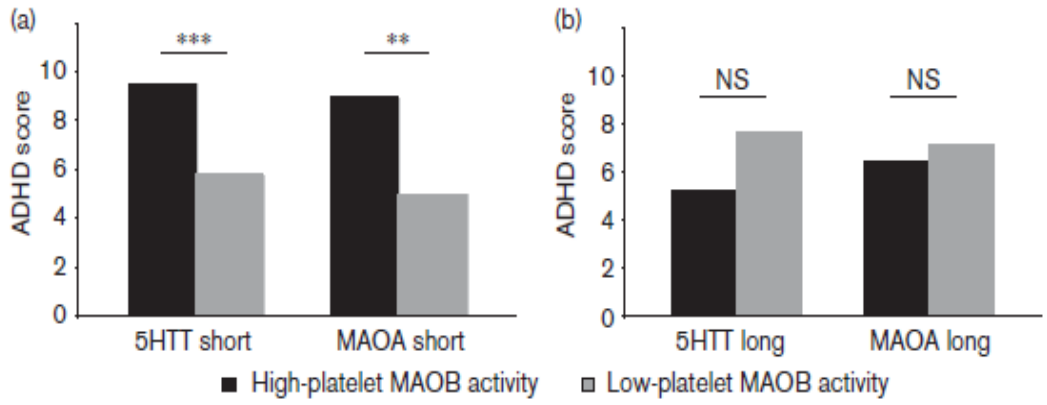

Fig. 3 ADHD symptoms and association to short 5-HTT LPR or short MAO-A VNTR genotype in combination with high platelet MAO-B activity

a) Individuals with short 5-HTT LPR or short MAO-A VNTR genotype scored higher on ADHD symptoms if exhibiting high platelet MAO-B activity. **p<0.01, ***p<0.001
b) Individuals with long 5-HTT LPR or long MAO-A VNTR genotype exhibited no differences in ADHD scores associated to platelet MAO-B activity. NS=Not significant

6.3 PAPER III

The comorbidity between the subthreshold diagnoses for ADHD_comb and DBD was as follows; the study group consisted of 312 participants; 252 individuals did not fulfil the criteria for any of the diagnoses, 10 had subthreshold ADHD_comb alone, 31 had subthreshold DBD alone and 19 individuals fulfilled the criteria for both subthreshold ADHD_comb and subthreshold DBD.

6.3.1 K-SADS-PL screening interview

There were several psychiatric symptoms from the K-SADS-PL interview that are frequently reported and some of which differed significantly between genders (Fig.4).

Commonly reported symptoms from the K-SADS-PL are as follows for depressive disorder in the item ”Irritability” (boys 13.3%, girls 18.6%), for mania; in the items “Increased goal directed activity” (boys 8.2%, girls 13.6%) and “Racing thoughts” (boys 7.4%, girls 13.0%) as well as separation anxiety disorder symptom in the “Fears harm befalling attachment figure”, which was commonly reported by girls (boys 11.1%, girls 17.0%). In the social phobia avoidant disorder, the items reported were, “Shrinks from contact” (boys 25.2%, girls 24.9%), “Fears social situations” (boys 28.2%, girls 32.8%) and “Avoidance” (boys 21.5%, girls 25.4%). Among the symptoms of ODD; “Loses temper” was one of the most commonly reported items (boys 19.3%, girls 22.6%). With regard to PTSD among the most frequently mentioned items was; “Ever confronted with traumatic news” (boys 31.9%, girls 41.8%). Cigarette/tobacco use, regular use of alcohol and substance use did not differ significantly between the genders, as the items evoked the following responses; ever tried smoking (boys 51.9%, girls 54.5%), regular use of alcohol (boys 18.5%, girls 13.0%) and ever tried cannabis (boys 9.6%, girls 4.5%).

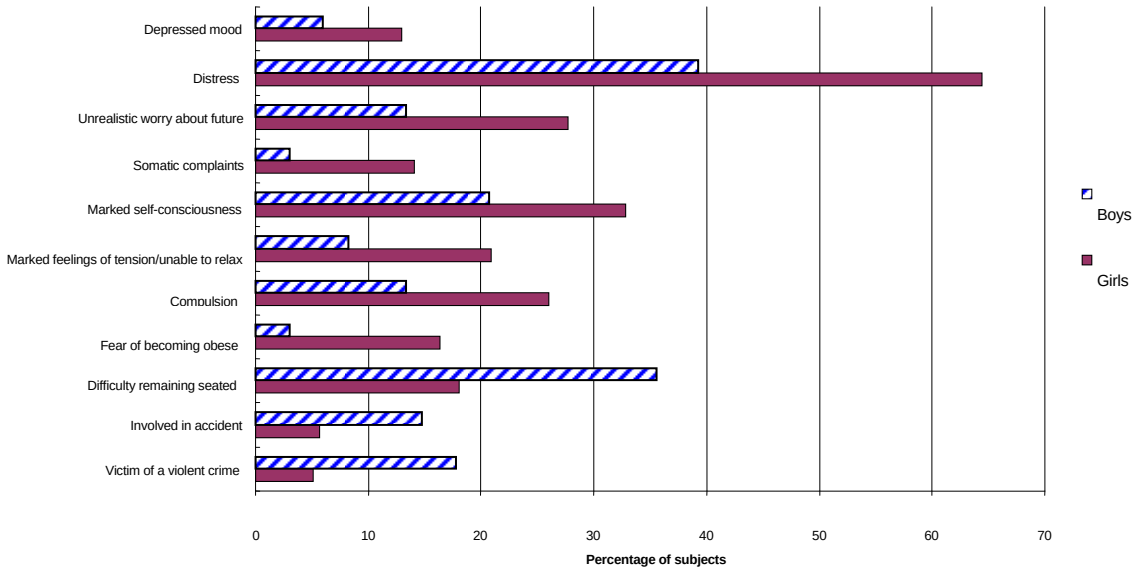

Fig. 4 Statistically significantly gender differences for items in the K-SADS-PL.

6.3.2 Subthreshold diagnoses of ADHD/DBD and coexisting psychiatric symptoms

Subthreshold diagnoses of ADHD and DBD coexisted with the K-SADS-PL screening questions on depression, mania, panic attacks, phobias, anorexia nervosa, motor tics and posttraumatic stress disorder (PTSD) in girls. In boys, these subthreshold diagnoses coexisted with symptoms of depression and PTSD.

Statistically significantly results with regard to the K-SADS-PL screening questions and subthreshold diagnoses of ADHD and DBD for boys and girls (total population) are presented in Fig. 5 and 6.

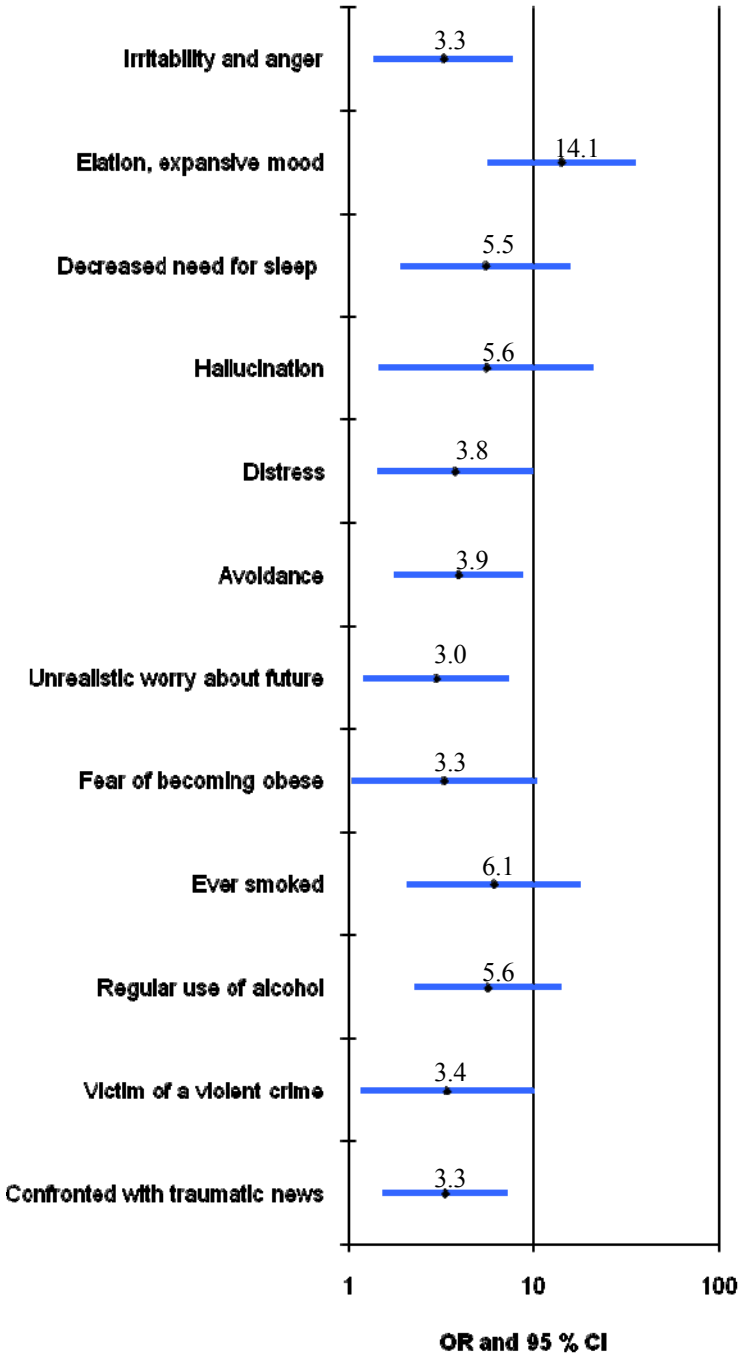

Fig. 5 The items with a significant OR in relation to subthreshold diagnoses of ADHD_comb and coexisting problems in the total population based on the K-SADS-PL screening interview

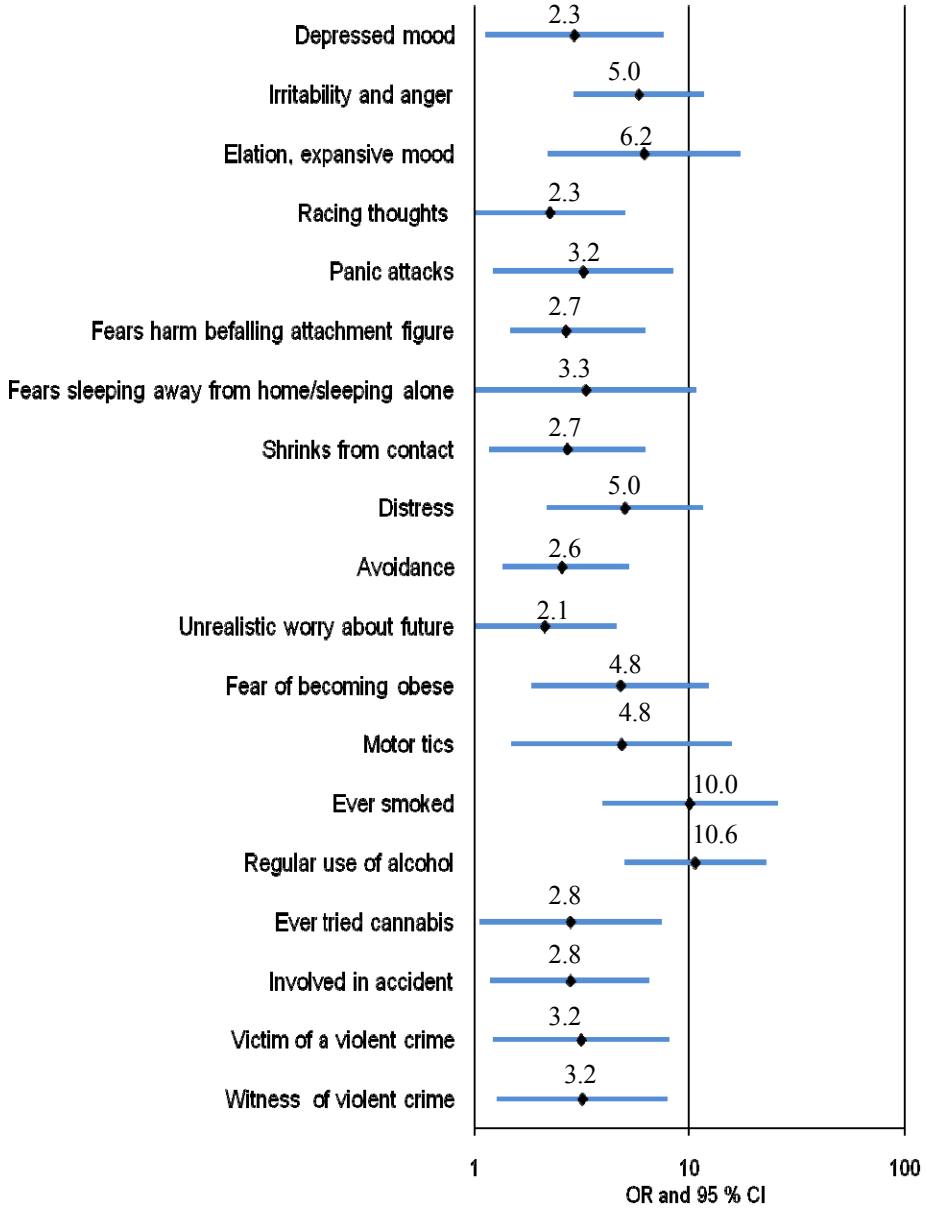

Fig. 6 The items with significant OR in relation to subthreshold diagnoses of DBD and coexisting problems for the total population based on the K-SADS-PL screening interview

6.3.3 Subthreshold diagnoses of ADHD/DBD and high consumption of smoking, alcohol and use of various drugs

In both boys and girls, smoking and high consumption of alcohol contributed to a high OR with regard to ADHD and DBD.

The in-depth interview in the K-SADS-PL for alcohol abuse in the item; "Quantity" was related to both ADHD_comb and DBD in boys, while in girls it was associated with DBD. In boys in the items; "Frequency drinking" was related to DBD and "Concern from others about drinking" was linked to ADHD_comb. In both boys and girls, the item smoking more than two cigarettes/day was related to ADHD_comb and DBD .For boys and girls together the results are presented in Table 8.

Table 8. The relationships between the subthreshold diagnoses of ADHD_comb/DBD based on the in-depth interview in the K-SADS-PL in relation to symptoms of alcohol and smoking in the total population (n=312)

Diagnostic class and items	**ADHD_comb OR (95%CI)**	**DBD OR (95%CI)**
Alcohol Abuse		
Quantity	**4.25** (1.66–10.90)**	**10.73*** (4.90-23.50)**
Frequency drinking	2.55 (0.23-28.00)	**14.59* (1.45-146.17)**
Concern from Others about Drinking	2.20 (0.55-8.85)	**13.62*** (3.82-48.61)**
Smoking		
> 2 cigarettes/day	**7.70*** (3.52-16.84)**	**11.77*** (5.11-21.13)**

*p<0.05 ,** p<0.01, ***p<0,001

6.4 PAPER IV

In this paper MAO-B activity in platelets and 5-HTT LPR polymorphism and association with various psychiatric symptoms based on the K-SADS-PL screening questionnaire was studied.

6.4.1 MAO-B and 5-HTT LPR associated to psychiatric symptoms

Low MAO-B activity in platelets was associated with symptoms of depressive disorder in the item "Irritability and anger", symptoms of separation anxiety disorder in the item "Fears harm befalling attachment figure" and symptoms of ODD in the item "Disobeys rules a lot" (Table 9).

Homozygosity of the long allele in 5-HTT was related to symptom of depressive disorder in the items "Irritability and anger" and "Anhedoni lack of interest", symptoms of separation anxiety disorder in the item "Fears being alone at home" and substance abuse in the item "Ever tried cannabis" (Table 9).

Heterozygosity for 5-HTT genotype was associated with symptom of ADHD in the item "Impulsiveness" (Table 9).

Homozygote of the short 5-HTT LPR allele did not show any statistically significance in relation to any items in the K-SADS-PL questionnaire alone (Table 9).

The percentage of individuals reporting significant items (symptoms) related to the K-SADS-PL and 5-HTT LPR genotype compared to those reporting the items without the specific phenotype are presented in Table 10.

Table 9. MAO-B activity in platelets and 5-HTT LPR genotype in relation to psychiatric symptoms according to K-SADS-PL questionnaire

Diagnostic class and item	**MAO-B[1] β-coeff (95% CI)**	**5-HTT-*ll*[2] β-coeff (95% CI)**	**5-HTT-*ls*[2] β-coeff (95% CI)**	**5-HTT-*ss*[2] β-coeff (95% CI)**
Depressive Disorders				
Depressed mood				
Irritability and anger	**-0.17 (-0.30; –0.04)***	**0.92 (0.22;1.63)***		
Anhedoni, lack of interest		**1.51 (0.04; 2.98)***		
Separation anxiety disorder				
Fears harm befalling attachment figure	**-0.09 (-0.18; -.001)***			
Fears being alone at home		**1.51 (0.08; 2.94)***		
Obsessive Compulsive Disorder				
Obsession				
Attention Deficit Hyperactivity Disorder				
Impulsiveness			**0.96 (0.34;1.59)****	
Oppositional Defiant Disorder				
Disobeys rules a lot	**-0.14 (-0.28; -0.001)***			
Tic Disorder				
Phonic tics				
Substance abuse				
Ever tried Cannabis		**1.03 (0.01; 2.05)***		

[1]MAO-B activity in platelets as a continuous variable
[2]The specific 5-HTT polymorphism compared with the sample without the specific 5-HTT polymorphism
*p<0.05, **p<0.01, ***p<0.001

Table 10. Percentage of the individuals having the items according to K-SADS-PL questionnaires and 5-HTT LPR genotype

Diagnostic class and item	**5-HTT-*ll*[1]**		**5-HTT-*ls*[1]**		**5-HTT-*ss*[1]**	
	No	Yes	No	Yes	No	Yes
Depressive Disorders						
Irritability and anger	**11.1***	**23,2**				
Anhedoni, lack of interest	**2.6**	**9.8**				
Separation anxiety disorder						
Fears being alone at home	**2.6**	**9.8**				
Attention Deficit Hyperactivity Disorder						
Impulsiveness			**30.6**	**53.5**		
Substance abuse						
Ever tried Cannabis	**5.1**	**13.4**				

[1]The specific 5-HTT polymorphism compared with the sample without the specific 5-HTT polymorphism

* Each row shows the percentage having the item (symptom) according to K-SADS-PL. Individuals with the specific phenotype compared with the sample without the specific phenotype. The items are presented with the statistically significant items according to Table 9

6.4.2 The interaction between MAO-B and 5-HTT LPR associated to psychiatric symptoms

The interaction between 5-HTT LPR genotype and MAO-B activity in platelets was analysed. Interaction between homozygosity for the long 5-HTT allele and MAO-B activity in platelets was associated with symptoms of separation anxiety disorder in the item “Fear of being alone home”. Interaction between heterozygosity for 5-HTT and MAO-B activity in platelets was associated with symptoms of separation anxiety disorder in the items “Fears harm befalling attachment figure”, with symptoms of CD in the item ”Nonaggressive stealing” and with symptoms of tic disorder in the item “Phonic tics”. Interaction between homozygosity for the short 5-HTT allele and MAO-B activity in platelets was associated with symptoms of depressive disorder in the items “Depressed mood” and “Irritability and anger”, with symptoms of separation anxiety disorder in the items “Fears harm befalling attachment figure”, with symptoms of OCD in the item “Obsession” and with symptoms of tic disorder in the item “Phonic tics“ (Table 11).

Table 11. Interactions between MAO-B activity in platelets and 5-HTT LPR genotype based on the K-SADS-PL questionnaire

Diagnostic class and item	**MAO-B/5-HTT-*ll*[1] β-coeff (95% CI)**	**MAO-B/5-HTT-*ls*[1] β-coeff (95% CI)**	**MAO-B/5-HTT-*ss*[1] β-coeff (95% CI)**
Depressive Disorders			
Depressed mood			**8.79 (8.01; 9.57)*** **
Anhedoni, lack of interest			**8.54 (7.77; 9.31)*** **
Separation anxiety disorder			
Fears harm befalling attachment figure		**-0.24 (-0.47; -0.01)***	**-0.09(-0.18; -.001)***
Fears being alone at home	**-0.41 (-0.79; -0.02)***		
Obsessive Compulsive Disorder			
Obsession			**-0.50 (-0.75; -0.25)*** **
Attention Deficit Hyperactivity Disorder			
Impulsiveness			
Oppositional Defiant Disorder			
Disobeys rules a lot			
Conduct Disorder			
Nonaggressive stealing		**-0.69 (-1.36; -0.27)***	
Tic Disorder			
Phonic tics		**0.26 (0.01: 0.52)***	**-0.28 (-0.56; -0.01)***
Substance abuse			
Ever tried Cannabis			

[1] The interaction between MAO-B activity in platelets as a continuous variable and the specific 5-HTT polymorphism compared with the sample without the specific 5-HTT polymorphism.
*p<0.05, **p<0.01, ***p<0.001

In order to further show the combination between MAO-B activity in platelets and 5-HTT LPR genotype and the relation with specific symptom the sample were divided into four groups, according to high/low level of MAO-B and the specific 5-HTT polymorphism yes/no. The results for the group with low MAO-B activity in platelets and 5-HTT LPR-*ll+ls* for the item "Iirritability and anger" (Fig. 7).

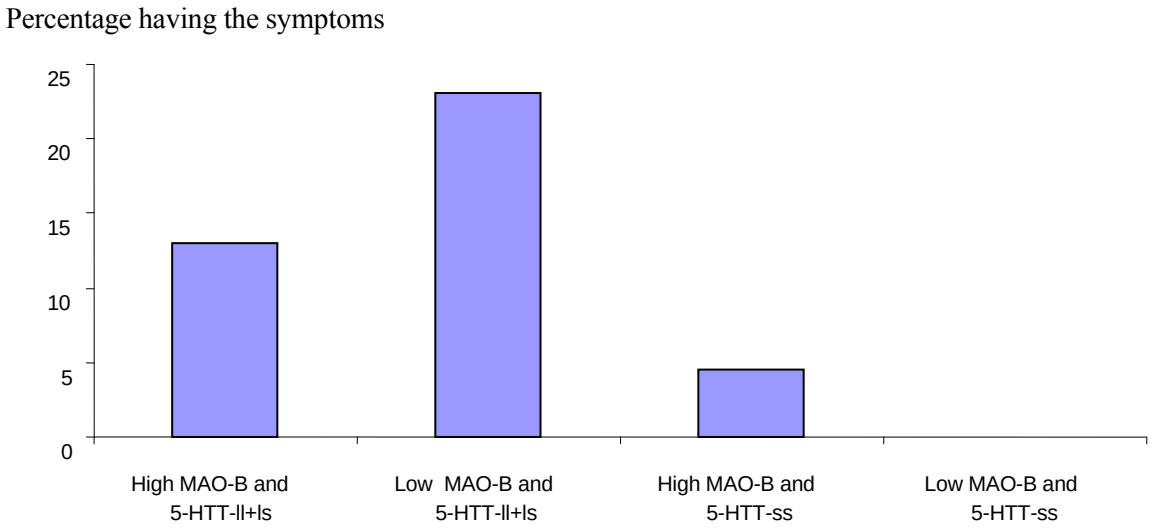

Fig. 7 The item irritability and anger

7 DISCUSSION

The main findings of this population-based study in adolescents are that ADHD and DBD phenotypes were associated with MAO-B activity in platelets as well as with the 5-HTT LPR and with the MAO-A VNTR genotypes. The ADHD and DBD phenotypes were based on criteria in the DSM-IV-TR. A number of reports have found that deviant behaviour, correlates with these candidate genes and MAO-B activity in platelets.

In Paper I we investigated the association between phenotypes and candidate genes/MAO-B activity in platelets separately. The findings were that MAO-B activity in platelets as well as the 5-HTT LPR and the MAO-A genotypes were associated with DBD.

In Paper II we continued to study how these phenotypes were associated with the combination of candidate genes and MAO-B activity in platelets. The combination of the 5-HTT LPR and MAO VNTR genotypes with low and high platelet MAO-B activity were analysed. A stronger correlation with ADHD-like problems evolved when these markers were combined.

In Paper III we studied the complexity of these phenotypes in association with various coexisting psychiatric problems. The results revealed that subthreshold diagnosis of ADHD_comb and DBD coexist with several psychiatric problems such as depression, mania, panic attacks, phobias, anorexia nervosa, motor tics, PTSD as well as with smoking and high alcohol consumption.

Paper IV we further explored the results in Papers I-III and studied how separate psychiatric symptoms based on the K-SADS-PL interview were associated with MAO-B activity in platelets and the 5-HTT LPR genotype. These studies revealed that psychiatric symptoms were associated with the 5-HTT LPR genotype and with platelet MAO-B activity separately and in interaction.

A discussion of the results from Papers I-IV will now continue by highlighting a few key questions.

Are ADHD and DBD phenotypes associated with MAO-B activity in platelets and polymorphism in the MAO-A VNTR and 5-HTT LPR genotypes separately and in combination?

Paper I: The main finding of this study was the association between low platelet MAO-B activity and high scores for symptoms for ODD and DBD in girls. Hemizygosity of the short MAO-A VNTR allele was associated with DBD symptoms in boys. In both girls and boys the heterozygote 5-HTT LPR genotype was associated with CD, while in boys it was also associated with DBD.

MAO-B is present in platelets and its activity has been suggested to be a marker for the monoamine capacity of the brain. Low platelet MAO-B activity correlates with deviant behaviour and has also been linked to ADHD as well as temperament (Shekim, Bylund et al. 1986; Klinteberg, Levander et al. 1987; Furmark, Tillfors et al. 2004; Oreland 2004; Oreland, Hallman et al. 2004; Nilsson, Wargelius et al. 2008; Gau, Ni et al. 2010). The hypothesis that low platelet MAO-B activity is associated with high scores of ADHD and DBD phenotype scores was only verified for ODD as well as DBD symptoms in girls. Anti-social behaviours are more believed to be under-diagnosed in girls (Newcorn, Halperin et al. 2001; Biederman, Monuteaux et al. 2006). It is therefore possible that the girls exhibiting symptoms in the present study had more severe symptoms or more deviant personalities than boys reporting the same behaviour. This explanation is in agreement with Cederblad et al (Cederblad, Oreland et al. 1992).

The MAO-A VNTR has been linked to personality traits such as impulse control and antagonistic behaviour (Manuck, Flory et al. 2000; Meyer-Lindenberg, Buckholtz et al. 2006). The hypothesis was that MAO-A genotype in boys would be associated with subtypes of ADHD in which aggressive and impulsive behaviours are especially prominent. The findings are in line with those of Lawson et al. (Lawson, Turic et al. 2003) who found a correlation between the short MAO-A allele and CD in boys with ADHD. There are other studies reporting an association with ADHD related behaviours and the long MAO-A allele (Kim-Cohen, Caspi et al. 2006), as well as studies in which no association were found (Faraone, Perlis et al. 2005).

In both boys and girls, the 5-HTT LPR heterozygosity was associated with high scores for the DBD phenotype. Such molecular heterosis at the 5-HTT gene has been observed

in some previous studies, where heterozygote subjects were found to have greater influences on the binding capacity of the 5-HTT as well as on behavioural traits, e.g. social drinking and depression, than homozygotes (Comings and MacMurray 2000). Furthermore, in a study, by van Dick et al. (van Dyck, Malison et al. 2004) central 5-HTT availability was found to be lowest in heterozygous subjects. Heterosis has also been reported for other monoamine receptor genes (Comings and MacMurray 2000). In a recent article, by Sonuga-Barke et al. (Sonuga-Barke, Kumsta et al. 2011) arrived at the same result as in Paper I. These authors discussed our findings and explained how the following effects could be involved: 1) an inverted U-shaped response curve in which either too little or too much gene expression is harmful; 2) an independent third factor causing a hidden stratification of the sample so that the two homozygote genotypes (s/s and l/l) are independently associated with the highest phenotype score relative to the heterozygote (e.g., s/l); and 3) greater fitness in heterozygotes, because they show a broader range of gene expression than both homozygotes (for a review see (Comings and MacMurray 2000). The findings from Sonuga-Barke et al (Sonuga-Barke, Kumsta et al. 2011) were that the 5-HTT LPR genotype was associated with delay aversion, where s-allele carriers were more delay averse than non carriers in individuals with ADHD. This confirmed the observation of a somewhat of a different pattern of results for the two groups and an unexpected heterosis effect in the ADHD group (the s/l group being the most delay averse). This raises the possibility that the 5-HTT LPR genotype influences impulsive behaviour. With regard to the 5-HTT LPR polymorphism, there are also conflicting results as some studies found associations between an ADHD phenotype and both the long and the short allele while others observed no such association (Faraone and Khan 2006; Thapar, Langley et al. 2006).

The gender ratio for ADHD and DBD is somewhat lower than previously reported. One explanation could be that the sample consisted of adolescents with mean age of 16 years, who are between childhood and adulthood, whereas in other studies the subjects were usually either children or adults. Meta-analyses of childhood ADHD have shown a male:female ratio of 3:1 in non referred populations (Gaub and Carlson 1997). The gender differences in this study were of 1.6:1 for subthreshold diagnoses of ADHD combined type. Studies of adult ADHD have shown ratios of 1.6:1 (Kessler, Adler et al. 2006) and even 1:1 (Faraone and Biederman 2005). One possible explanation of the

gender difference might be an effect of sex hormones on the activity of several genes within the monoamine field (Doyle, Biederman et al. 2003).

Compounds in cigarette smoke exert an inhibitory effect on MAO activity, but are only reported to be significant in quantities exceeding 300 cigarettes per month (Snell, Glanz et al. 2002). In the present study smoking was controlled for and to further investigate its potential effects of smoking on the results, by using the information from the K-SADS-PL to identify the subjects who had smoked more than two cigarettes per day in combination with the group who had smoked during a 24 hours prior the blood sample.

Paper II: A more accurate picture emerged when the genetic markers (the 5-HTT LPR and MAO-A VNTR genotypes with platelet MAO-B activity) were combined, compared with the cases to when these markers were analysed separately. Among boys (not girls) carrying either the short 5-HTT LPR or the short MAO-A VNTR allele, subjects with high platelet MAO-B activity scored higher on the ADHD-related phenotypes.

Most reports indicate that low MAO-B activity is associated with deviant behaviour such as sensation seeking and type II alcoholism (Oreland 2004; Oreland, Hallman et al. 2004), but there are some reports suggesting different dimensions of deviant behaviour are associated with extreme levels of platelet MAO-B activity (high or low) (Klinteberg, Levander et al. 1987; Stoff, Friedman et al. 1989). High platelet MAO-B activity may reflect anxiety-related traits as opposed to impulsive traits which are linked to low platelet MAO-B activity. Comorbidity with anxiety is prevalent in ADHD (Schatz and Rostain 2006; Jarrett and Ollendick 2008), which agrees with the present results of high platelet MAO-B activity in individuals with high ADHD scores, thus it may be that affected individuals have coexisting anxiety. MAO-B knock-out mice have been found to exhibit behavioural disinhibition and a reduction of anxiety-like behaviours (Bortolato, Godar et al. 2009).

Are subthreshold diagnoses of ADHD and DBD related to other symptoms of child and adolescent psychiatric disorders?

Paper III: Subthreshold diagnoses of ADHD and DBD were found to coexist with various psychiatric symptoms based on the K-SADS-PL interview; depression, mania,

panic attacks, phobias, anorexia nervosa, motor tics and PTSD in girls while in boys, these subthreshold diagnoses were found to coexist with symptoms of depression and PTSD.

Psychiatric diagnoses are classified by means of structured interviews based on symptoms from categorical diagnosis. Another way to describe mental health is to use the summary score of psychiatric symptoms that is a dimensional scale of symptoms. ADHD and DBD phenotypes are complex and there is an increased tendency for researchers to refine the phenotype in the secondary analysis (Thapar, Langley et al. 2006). In the study, the phenotypes were determined by subthreshold diagnoses of ADHD_comb and DBD (ODD or CD). Subthreshold diagnoses of ADHD and DBD in adolescents are important, as these conditions are often accompanied by coexisting psychiatric/ psychological problems. In a large study of adolescents and young adults, more than half (52.5%) of the participants had experienced a subthreshold state at some point in their life and 40% of those with at least one subthreshold condition had one or several additional conditions, while over 36.4% of the participants with a subthreshold condition also had a comorbid full syndrome diagnosis. Thus, subthreshold conditions are precursors of full syndrome disorders (Lewinsohn, Shankman et al. 2004; Shankman, Lewinsohn et al. 2009). A series of papers (Faraone, Biederman et al. 2006; Faraone, Wilens et al. 2007; Faraone, Kunwar et al. 2009) found that late-onset and full ADHD subjects had similar psychiatric comorbidity patterns; neuropsychological impairment, substance use disorders and familial transmission. Subthreshold ADHD was milder than late-onset adult ADHD and might be a milder a less severe condition. In adults, subthreshold ADHD cases showed deviations in novelty seeking and self-directiveness (Faraone, Perlis et al. 2005).

Thus the findings were that, mild and non-clinical symptoms of ADHD and DBD are not isolated conditions, but part of other psychiatric/psychological problems. These findings are in agreement with several clinical studies showing that ADHD and DBD coexist with other psychiatric symptoms and impairments and that comorbidity is a clinical feature of both childhood and adult ADHD (Biederman, Petty et al. 2010). ADHD frequently coexists with DBD in the same way as functional impairment coexists with anxiety and/or depression. Children suffering from both CD and ADHD exhibit greater ADHD and CD symptom severity, a stronger association with

neurobiological correlates and poorer outcome than those with either disorder separately (Thapar, Langley et al. 2006; Bussing, Mason et al. 2010; Mordre, Groholt et al. 2011). Comorbidity and coexisting psychiatric symptoms and impairments were found in population-based studies of children with ADHD (Scahill and Schwab-Stone 2000), ODD and CD (Kutcher, Aman et al. 2004). Population-based studies of adults demonstrated that both adult women and men with ADHD are at increased risk of other psychiatric disorders (Friedrichs, Igl et al. 2010) as well as the fact DBD is associated with comorbidity with other psychiatric conditions (Reef, Diamantopoulou et al. 2010).

There were gender differences in that girls exhibited more symptoms of depression, mania, panic anxiety, social phobia, agoraphobia and specific phobia, anorexia nervosa and motor tics in association with subthreshold diagnoses of ADHD and DBD. These findings are similar to the results of Kopp et al. (Kopp, Kelly et al. 2010), who revealed that girls referred to a psychiatric outpatient clinic with social and/or attention deficits often had serious psychiatric comorbidities and low global levels of functioning. Boys with subthreshold diagnoses of ADHD and DBD reported more symptoms of PTSD. A possible explanation may be that novelty seeking has been reported to be associated with DBD and other reports of overlapping of symptoms between PTSD and ADHD (Schmeck and Poustka 2001; Daud and Rydelius 2009).

Subthreshold diagnoses appear to be important from both a clinical and research, perspective, as many with such diagnoses exhibit a great deal of comorbidity with other psychological and psychiatric symptoms. However, studying subthreshold diagnoses may be a difficult way to get a pure phenotype. In the present study comorbidity between these diagnoses was as follows 10 had subthreshold ADHD_comb, 31 had subthreshold DBD while 19 individuals fulfilled the criteria for both subthreshold ADHD_comb and subthreshold DBD. This pattern may be similar to a clinical population as developmental and psychiatric diagnoses often coexist with other problems and are not isolated conditions. One difficulty is that subthreshold diagnoses criteria differ from late-onset or full syndrome criteria and that there is a general lack of consensus. Furthermore individuals with subthreshold diagnoses may need a similar degree of help and treatment as those with the full syndrome.

Are high scores of ADHD and DBD risk behaviour related to smoking, alcohol consumption and use of various drugs?

Paper III: We were in this study able to show that the section of the in-depth K-SADS-PL interview that dealt with smoking and high alcohol consumption was related to subthreshold diagnoses of ADHD and DBD in both boys and girls. This result is similar to Faraone et al. (Faraone, Wilens et al. 2007), who found that full syndrome and late onset ADHD groups had identical cigarette smoking and substance use profiles. Heffner et al. (Heffner, Johnson et al. 2010) demonstrated that childhood symptoms of inattention and hyperactivity/impulsivity are related to cigarette smoking and nicotine dependence among alcohol-dependent individuals at levels below the ADHD diagnostic threshold. A recent study by Baker et al. (Baker, Prevatt et al. 2011) found that students with difficulties in self-regulation and with ADHD are at increased risk for problematic alcohol intake and subsequent risk for alcohol-use disorders when compared their peers. Responses in the section of the K-SADS-PL interview that dealt with use of various drugs revealed that 21/312 of the adolescents had tried cannabis but only one parent had knowledge about this. The results highlight the importance of a broad clinical assessment of adolescents with preliminary diagnoses of ADHD and DBD, including questions about alcohol consumption, smoking and the use of various drugs.

What is the association between individual psychiatric symptoms and behavioural problems based on the K-SADS-PL interview and polymorphism in the 5-HTT LPR genotype and MAO-B activity in platelets?

Paper IV: This study showed that **s**everal psychiatric symptoms in the K-SADS-PL were associated with the 5-HTT genotype and with platelet MAO-B activity. When the two markers were combined another picture emerged with regard to several of the symptoms. In line with previous findings on ADHD, when two markers were combined a more conclusive picture of several symptom emerged (Wargelius, Malmberg et al. 2011).

It should be noted that none of the symptoms tested were associated with the short 5-HTT allele alone but when the short 5-HTT allele was seen in combination with MAO-B activity in platelets another picture of symptoms of depressive disorder emerged.

Low MAO-B activity in platelets was associated with the items "Irritability and anger", "Fears harm befalling attachment figure" and "Disobeys rules a lot". In Malmberg et al. (Malmberg, Wargelius et al. 2008) found that symptoms of ODD and DBD were associated with low MAO-B platelet activity in girls. Nilsson et al. (Nilsson, Wargelius et al. 2008) demonstrated that girls with low platelet MAO-B activity had an increased risk of alcohol-related behaviour in an unfavourable environment. In summary, low MAO-B platelet activity seems to indicate vulnerability to several types of behavioural problem.

Homozygosity of the long allele in 5-HTT was related to the items "Irritability and anger", "Anhedoni, lack of interest", "Fears being alone at home", and "Ever tried cannabis". Heterozygosity of the 5-HTT gene was associated with the item "Impulsiveness". Although we used a non-clinical sample of adolescents, our findings are in line with previous studies of adult patients as well as a large population based study showing that variations in the serotonin transporter are considered to be an important factor in anxiety-depression related personality traits as they are linked to violent behaviour and aggression (Collier, Stober et al. 1996; Merikangas, He et al. 2010).

7.1 METHODOLOGICAL CONSIDERATION

Some limitations should be mentioned. First of all the study population was not a clinical one and individual symptoms that were studied were both clinical and subclinical, making it difficult to know if the findings are representative of specific psychiatric diagnoses. The sample is fairly small and there is a risk that the results could lead to false positives or false negatives, hence, the positive associations reported here need to be validated in larger samples. Another limitation in Paper IV is that we did not separately analyse the results for boys and girls because of the small sample.

The generalizability of this population-based study may be limited because apart from the small sample size, the subjects were twins that were treated as individuals and thus twin methodology was not used. However, studies of twins have shown that twins'

behavioural problems are representative of a general population. Gjone et al. (Gjone and Novik 1995) compared behavioural problems in a general and a twin population and found that the level of attention problems and externalizing behaviour was similar and that the likelihood of developing a disorder is influenced by similar factors in twins and singletons. Thus the generalizability in this study does not seem to differ from that of other studies.

When the interviews were conducted the first Swedish version of K-SADS-PL was used, which does not include a screening question about autism spectrum disorder (ASD). White et al. (White, Ollendick et al. 2011) examined the prevalence of ASD in students at a university and depending on method used, found that between 0.7 and 1.9 per cent fulfilled the criteria for High Functioning ASD. It is difficult to envision that out of 312 individuals so many suffered from ASD. However, it may have been the case that some of them had coexisting problem. One girl had already been diagnosed with Asperger's syndrome at the time of the interview.

However, the strength of the study is that the interviews constituted a clinical assessment where the answers from both adolescents and parents were evaluated by a trained physician, which favourable contrast with studies that rely on self-reported questionnaires.

8 CONCLUSIONS AND CLINICAL IMPLICATIONS

Measurements of MAO-B activity in platelets as well as the 5-HTT LPR and the MAO-A VNTR genotypes revealed that individuals with these genotypes alone and in combination are more likely to have high ADHD and DBD score. Several of psychiatric symptoms based on the K-SADS-PL interview were associated with the 5-HTT LPR genotypes and with platelet MAO-B activity alone and in combination.

These results suggest that the serotonin system is involved in the etiology of ADHD, DBD and other psychiatric symptoms, thus requiring further investigation. The heterosis effect found in ADHD and CD highlights the importance of conducting separate analyses of all genotype groups, rather than pooling alleles together as is frequently the case in association studies (Comings and MacMurray 2000; Malmberg, Wargelius et al. 2008; Sonuga-Barke, Kumsta et al. 2011). More and larger studies are needed to validate these results.

The complexity of the ADHD and DBD phenotypes was shown by the association with several psychiatric and behavioural problems. Subthreshold diagnoses of ADHD and DBD were associated with positive findings with regarding to symptoms from the K-SADS-PL screening interview related to the following psychiatric problem areas: depression, mania, psychosis, anxiety, eating disorders and trauma. However, there were more symptoms of anxiety and trauma related to DBD compared to ADHD. In both boys and girls, smoking and high alcohol consumption where seen as to risk behaviours in association with subthreshold diagnoses. The gender differences in the symptoms found in Paper III are similar to those reported by Laukkanen (Laukkanen, Hintikka et al. 2010), which suggests that the assessment of symptoms based on the K-SADS-PL is valid in the light of Swedish and international studies. This emphasizes that ADHD and DBD constitute a significant risk to mental health. A broad clinical assessment is therefore necessary for adolescents with a preliminary diagnosis of ADHD and DBD to detect other psychiatric/psychological problems and provide target treatment as well as to identify individuals with risk behaviour related to alcohol, smoking and use of various drugs, for which the K-SADS-PL is a good assessment instrument.

Although this is a small sample, the present study indicates that the serotonergic system is involved in the etiology of ADHD and DBD as well as other psychiatric symptoms. Pharmacological and genetic studies have demonstrated the importance of the dopaminergic, serotonergic and noradrenergic systems in the pathogenesis of ADHD and DBD. Thus knowledge of how various symptoms can be explained by different candidate genes and neurotransmitter systems enhance the opportunity to identify the most appropriate pharmacological treatment. However, larger samples are needed as well as more pharmacological studies.

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