# Genetic and neurobiological mechanisms underlying aggression subtypes

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**Genetic and neurobiological mechanisms underlying aggression subtypes**

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# Chapter 1

## Introduction

## OVERVIEW

Aggression poses a substantial burden for society. It is estimated that world-wide, between 1 and 2 million people die on a yearly basis as a direct result of violence. Many millions more suffer other consequences as a result of aggression, such as disabilities, mental health problems, and employment difficulties, placing a considerable emotional and financial burden on society (WHO, 2007). Most interventions designed to reduce aggression - pharmacological or non-pharmacological - typically have small effects, reflecting our limited understanding of its causes (McGuire, 2008; van Schalkwyk et al., 2017). While aggression has multiple determinants, including environmental factors, characterization of the genetic and neural correlates of aggression is needed to better understand the biological basis of aggression, and may ultimately lead to improved prevention and treatment options (Fergusson et al., 2005a; Rosell and Siever, 2015).
This thesis is aimed at gaining improved insight into the genetic and neurobiological architecture of aggression. More specifically, the focus is on exploring behavioral subtypes of aggression and related etiological heterogeneity, as well as on combining knowledge at the levels of genetic and neural architectures underlying the subtypes. In the following sections, different conceptualizations of aggression are discussed, followed by an overview of the current knowledge of the underlying genetics and neurobiology of aggression. An outline of the thesis chapters and the used study samples is given in the final section of this introduction.

## THE AGGRESSION PHENOTYPE

### Heterogeneity in the aggression phenotype

When we think of the word 'aggression', many different types of behavior might come to mind; anything from a child throwing a temper tantrum in anger, to threatening or intimidating acts, to physical violence, or even criminals murdering for money. Aggressive behaviors can be adaptive, and have an important role in survival (for example in the case of self-defense) and competition for resources (Georgiev et al., 2013). However, aggression is often maladaptive and associated with negative consequences in society, causing psychological and somatic burden to victims as well as to aggressive individuals themselves (Fergusson et al., 2005b; Reef et al., 2010). Traditionally, aggression has been defined as any behavior directed toward the goal of causing harm or injury to others (Baron and Richardson, 1994). This definition of aggression relies heavily on the intent to harm. More psychological accounts of aggressive behavior put emphasis on emotions and motivations postulated to mediate aggressive behaviors, such as fear or irritability (Miczek et al., 2002). The *Diagnostic and Statistical Manual of Mental Disorders*, created to provide a common language for describing psychopathology, is widely used to classify psychiatric disorders (APA, 2013). It recognizes disruptive, impulse-control, and conduct disorders, providing categorical definitions for aggression based on frequently co-occurring clinical symptoms. Although this has proven useful in clinical communication and in the treatment of

psychiatric illness, a categorical system implies discrete differences between diagnosed and healthy individuals or even between different disorders. However, there is increasing evidence that categorical behavioral diagnoses fail to align with underlying biological mechanisms. Most mental disorders are now thought to represent the impairing tail on a continuum of normal behavior (Coghill and Sonuga-Barke, 2012). Moreover, pathophysiological mechanisms have been shown to overlap between different disorders, for example at the genetic level (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). Therefore, a rigid classification system may hamper scientific progress, as case-control study designs may be suboptimal models to study the biological causes of psychiatric behaviors. Rather, the incorporation of dimensional conceptualizations in research may provide a way forward into understanding underlying pathophysiology (Coghill and Sonuga-Barke, 2012). Looking at dimensional aspects has the advantages of tackling issues of comorbidity between disorders, as well as tackling the problem of heterogeneity within psychiatric disorders. Different subgroups of patients experience different symptoms and impairments, and might respond differently to treatment. Especially in the field of aggression research, a wide range of behaviors has been investigated in different contexts and populations. There has been increasing evidence that clinical diagnoses of aggression encompass a notably heterogeneous group of behaviors (Moffitt et al., 2008). This heterogeneity in the aggression phenotype has let to inconsistencies between studies investigating the underlying mechanisms of aggression. Heterogeneity as a potential source of inconsistencies between studies will be highlighted throughout this thesis.

The above described heterogeneity issues may be tackled by the use of consistent subtyping approaches (Hyde et al., 2015). Different subtyping approaches have been proposed in aggression literature, for example those based on age of onset (Moffitt, 1993), or occurrence of overt aggressive versus covert rule-breaking behaviors (Burt, 2012; Loeber and Stouthamer-Loeber, 1998). In this thesis, the focus is on a subtyping approach, which is based on biological hypotheses and distinguishes reactive and proactive types of aggression. Additionally, other, data-driven subtyping strategies of aggressive behavior in the context of neurodevelopmental disorders are investigated, as well as cross-disorder comorbidity. In the next sections, the most prominent aggression disorders are discussed in the context of their comorbidity with other neurodevelopmental disorders, followed by an overview of reactive and proactive subtyping in aggression research.

**Aggression as a comorbidity in neurodevelopmental disorders**

The two most investigated categories of psychiatric disruptive behaviors are conduct disorder (CD) and oppositional defiant disorder (ODD). These disorders are highly comorbid, and in many cases CD is preceded by ODD (Buitelaar et al., 2013). Conduct disorder is characterized by a repetitive and persistent pattern of behavior that violates the rights of others or leads to conflict with societal norms or authority figures (APA, 2013). In DSM5, a specifier of CD with callous-unemotional (CU) presentation has been added, distinguishing a subgroup of children capable of premeditated aggressive behaviors and at risk for developing psychopathy in adulthood. CU traits include symptoms related to lack of remorse, lack of empathy, unconcern about performance and shallow affect (Frick and

Viding, 2009; Viding et al., 2012). These traits where also shown to distinguish children with more severe ODD symptoms (Hawes et al., 2013). In ODD, two symptom clusters have been recognized. An affective symptom cluster associated with irritability and temper tantrums, and a defiant headstrong cluster that is associated with a pattern of negativistic, defiant, disobedient and hostile behavior towards authority figures (APA, 2013). These types of aggressive behaviors have in common that they tend to be more prevalent in males than females, and that first onset predominantly occurs in childhood or adolescence. They are also an often occurring comorbidity in other psychiatric disease, such as attention-deficit/hyperactivity disorder (ADHD) or autism spectrum disorders (ASD) (Goldin et al., 2013; King and Waschbusch, 2010; Yamamuro et al., 2017) and have been investigated as such.
ADHD is a common neurodevelopmental disorder characterized by developmentally inappropriate levels of inattentiveness, and/or increased impulsivity and hyperactivity (APA, 2013). It affects about 5% of children and adolescents and 2.5% of adults, with a male-to-female sex ratio of around 4:1 in childhood and adolescence, which levels out during adulthood (Faraone et al., 2015). Co-morbid presence of oppositional defiant disorder (ODD) is a clinically important dimension of ADHD heterogeneity, and occurs in up to 60% of individuals with ADHD (Connor and Doerfler, 2008). Individuals with ADHD, who show comorbid aggression-related problems, have a considerably worse prognosis than individuals without them. A defiant/vindictive behavioral pattern is associated with an increased risk for criminal outcomes later in life (Aebi et al., 2013), and irritable mood is thought to underlie the developmental link between ODD and later affective disorders (Loeber et al., 2000; Stringaris et al., 2009). This emphasizes the need to identify risk factors for aggression co-morbid with ADHD. However, little is known yet about the etiological basis of this comorbidity. Shared and unique genetic influences between these disorders have been postulated to play a role (Dick et al., 2005; Faraone et al., 1991).
Other neurodevelopmental disorders, in which aggressive behavior is highly prevalent, are ASDs and intellectual disabilities (ID). Children with ASD - a neurodevelopmental disorder associated with a specific pattern of behavioral, communication, and social problems - are at higher risk for displaying aggressive and oppositional behavior compared to other populations, with prevalence estimates of up to 68% (Hill et al., 2014). In intellectual disabilities (ID), such as in Fragile X syndrome, a high prevalence of aggressive behaviors is also observed (Newman et al., 2015). Fragile X syndrome, in which the Fragile X Mental Retardation 1 (*FMR1*) gene is mutated, is one of the most common causes of inherited ID, with a 85% incidence of ID in males and a 25% incidence in females (Hagerman and Hagerman, 2002; Loesch et al., 2004). It has been estimated that 38% of males and 14% of females with the full mutation engage in aggressive behavior (Bailey et al., 2008). Like in ADHD, disruptive behaviors in ASD and ID are risk factors for poor outcomes, family stress and greater functional impairment later in life (Lecavalier, 2006), making them an important target for early prevention and treatment opportunities. Identification of underlying risk factors will be indispensible to the development of such interventions.

**Reactive and proactive subtypes of aggression**

Dodge and Coie introduced first introduced the distinction between reactive and proactive aggression in 1987, assimilating different previous accounts of aggression and recognizing that aggressive behavior manifests itself in multiple forms (Dodge and Coie, 1987; Kempes et al., 2005). Proactive aggression, also referred to as instrumental aggression, is defined as goal-oriented, organized behavior often associated with low autonomic arousal and affect. Reactive aggression on the other hand, also known as impulsive or affective aggression, occurs in response to threat, provocation, or a negative emotional state (Raine et al., 2006; Stanford et al., 2003). A distinction of proactive and reactive subtypes of aggression may help to shed light on different etiological pathways to aggression. These subtypes differ significantly in their behavioral correlates. For example, the reactive subtype of aggression has been associated with constructs like impulsivity, anxiety, and hostile interpretation bias (Brugman et al., 2015; Bubier and Drabick, 2009), while proactive aggression has been related to psychopathic traits and delinquent behavior (Cima and Raine, 2009; Cima et al., 2013).

Reactive and proactive subtypes of aggression can be measured by use of the Reactive Proactive Questionnaire (RPQ), a self-report questionnaire consisting of 23 items (Raine et al., 2006). Recently, Smeets and coworkers conducted an exploratory factor analysis of this questionnaire in an adolescent sample, and proposed a further subdivision of the reactive subtype based on the resulting best fit for a three-factor model (Smeets et al., 2016). Besides a proactive factor, the analysis suggested a subdivision of reactive aggression into one subtype associated with external provocation or threat (based on items like for example 'Hit others to defend yourself' and 'Reacted angrily when provoked by others') and another subtype associated with internal frustration (based on items like for example 'Gotten angry when frustrated' and 'Gotten angry or mad when you lost a game'). Although it should be noted that these proactive and reactive subtypes often co-occurred in the same individuals (no individuals with predominantly proactive aggression (without reactive aggression) where found), subtypes did differ in their association with behavioral correlates. Reactive aggression due to internal frustration showed stronger association with anxiety compared to reactive aggression due to external provocation or threat, and internalizing problems were uniquely predicted by frustration-induced aggression (Smeets et al., 2016). The three-factor model may further reduce phenotypic heterogeneity in the assessment of aggression. Importantly, these subtypes are thought to be associated with distinct genetic, neurocognitive, and neural characteristics (see below for a summary of the current knowledge), and they may therefore direct and facilitate the hitherto difficult search for genes and neural circuits involved in aggression etiology.

**Sex differences in aggression**

Important inter-individual differences in the etiology of aggression are thought to arise from sex differences. Both in terms of prevalence and type of aggression displayed, males and females differ markedly. A striking difference in crime rate statistics exists, with males more likely to commit serious offenses than females. For example, over 70 percent of the persons arrested in the United States in 2015 were males (www.fbi.gov). Males are also more

likely to display antisocial behavior than females, and are overrepresented in aggression-related disorders (Stephenson et al., 2014). One example is CD, where the male to female gender ratio is approximately 2.5 (Hill, 2002). Sex differences are also found in the type of aggressive behavior displayed (Collett et al., 2003). Males have an increased risk for physical aggression (Baillargeon et al., 2007; Côté, 2007; Hill et al., 2006), while females may show slightly more indirect aggression (also termed social aggression, usually relating to non-physical forms of aggression) compared to males (Card et al., 2008). Partly, sex differences in the expression of aggression may be confounded by social and cultural aspects. However, the clear gender-specificity of aggression is thought to have evolved by sexual selection, and to reflect differences in optimal strategies in the competition for resources for males and females (Georgiev et al., 2013). Incorporation of sex in aggression studies may facilitate the identification of underlying biological mechanisms of aggressive behaviors. Knowledge on any biological aspects of sexual dimorphism may be important in the management of the social consequences of aggression.

## GENETICS OF AGGRESSION

### Heritability

Twin studies can provide useful insights into the contribution of genetic and environmental factors to behavior, such as aggression. These types of studies make use of the fact that monozygotic twins share 100% of their genetic material as well as their family environments, whereas dizygotic twins share on average 50% of their genetic material and their family environments. Concordance rates for aggression are higher in monozygotic twins compared to dizygotic twins, which points to the fact that genetic factors indeed play a role in this phenotype. Heritability of aggression in general is estimated to be around 50% (Tuvblad and Baker, 2011; Veroude et al., 2016). These estimates differ as a function of the population and the type of aggression that is investigated (Waltes et al., 2016). With regard to reactive and proactive aggression subtypes, there is significant heritability for both, confirmed in study samples including individuals as young 9 years of age. Heritability estimates are slightly higher for proactive aggression (32-50%) than for reactive aggression (20-38%) (Baker et al., 2008). Aggressive behavior in children with high CU score was show to be under stronger genetic influence (81%) than aggressive behavior in children without elevated levels of CU-traits (30%) (Viding et al., 2005). Some twin studies have found gender effects for heritability estimates. Especially when using self-report measures and around or after adolescence, heritability estimates are higher for boys than for girls (Baker et al., 2008; Wang et al., 2013). Highest heritability estimates (up to 68%) have been found for physical aggression (Burt and Klump, 2012; Chen et al., 2015; Lacourse et al., 2014; Yeh et al., 2010).

## Challenges in gene finding

Despite the considerable heritability of aggression, the identification of specific genetic risk factors has been difficult. As discussed in the above sections, heterogeneity in the aggression phenotype presents a huge complication. Different subtypes of aggression might link to quantitatively or qualitatively different underlying genetic risk factors. For example, in the case of aggression comorbid with ADHD, the presence of aggression in ADHD has been shown to index higher genetic loading (Hamshere et al., 2013). An additional factor complicating gene-finding is the largely polygenic nature of aggression. While some monogenic disorders leading to aggression phenotypes do exist (the most well-known example perhaps being Brunner syndrome (Brunner et al., 1993)), multiple genetic variants, each with a small effect size, contribute to the aggression phenotype in most individuals. Moreover, different combinations of these variants can lead to similar phenotypes (Franke et al., 2009). The involvement of epigenetics and gene-environment interactions further complicate the matter. Besides genetic factors, environmental influences early in life (for example family dysfunction, low parental income and hostile parenting styles) are predictive of aggression (Tremblay, 2004). These early life events and other external conditions are able to cause changes in epigenetic modifications, such as DNA methylation (McGowan et al., 2009), which in turn regulate the activity of gene expression throughout life (Bird, 2007). The study of epigenetic marks in aggression is still in its early stages, but may identify genes that are differentially regulated in individuals with high and low levels of aggression (van Dongen et al., 2015).

Because of the hypothesized polygenic model of multiple common variants with small effects underlying aggression, studies have investigated the role of these common variants by conducting association studies. Early studies investigated single candidate genes and variants suspected to play a role in aggression. Next to these candidate genetic approaches, which rely on *a priori* biological hypotheses, genome-wide hypothesis-generating approaches to gene finding exist. These circumvent the need for prior selection. Candidate gene studies and genome-wide association studies (GWAS) for aggression will be discussed in the next sections.

## Candidate gene studies

With regard to candidate gene studies of aggression, the main focus has been on genes related to monoaminergic neurotransmission, like the genes underlying serotonergic and dopaminergic signaling, and on genes related to neuroendocrine signaling. Alterations in serotonergic, dopaminergic, and neuroendocrine systems are thought to play a key role in aggression for several reasons. First, they are important regulators of several cognitive functions related to aggression. For example, the serotonergic system plays an important role in social cognition, emotion regulation, and cognitive control (Lesch et al., 2012), while the dopaminergic system is relevant for understanding aggression, because of its effects on reward signaling, motivated behavior, and decision making (Costa et al., 2012). Second, central serotonin and dopamine neurotransmission levels have been shown to be associated with aggressive behavior: levels of the serotonin metabolite 5-HIAA in cerebrospinal fluid (e.g. (Brown et al., 1979; Coccaro and Lee, 2010), or manipulations of central serotonin

function through tryptophan depletion/loading (e.g. (Bjork, 2000), have revealed a highly significant relationship between serotonin availability and aggression (Rosell and Siever, 2015). Dopamine D2-receptor antagonists have been used effectively to treat aggressive behavior (Nelson and Trainor, 2007). The neuroendocrine system, which includes both stress-related hypothalamic-pituitary-adrenal (HPA) axis signaling and sex-hormone-related hypothalamo-pituitary-gonadal (HPG) axis signaling, is a major candidate system for the development of aggressive behaviors as well, e.g. because early life stress is known to increase risk for the development of mood and aggression-related disorders (Agid et al., 1999; Éthier et al., 2004; Fonagy, 2006; Heim et al., 2001). Levels of the stress hormone cortisol have repeatedly been related to aggression (Alink et al., 2012; Loney et al., 2006; Popma et al., 2007; Shirtcliff et al., 2005; van Bokhoven et al., 2004). The HPG axis involves signaling between hypothalamus, pituitary, and the gonadal glands, which produce estrogen and testosterone. These steroid hormones have been related to human aggression repeatedly as well (Book et al., 2001; Brown et al., 2008; Chichinadze et al., 2010; Yu and Shi, 2009), and it has been hypothesized that the interplay between cortisol and sex steroids in particular is important in determining aggression liability (Pavlov et al., 2012; Terburg et al., 2009). While initial studies supported a model of increased aggression risk for higher testosterone in combination with lower cortisol levels (Popma et al., 2007), more recent studies have revealed that the story may be more complex, as the nature of testosterone-cortisol interactions likely differs as a function of gender as well as specific aggression phenotype of interest (Cima et al., 2008; Denson et al., 2013; Welker et al., 2014).

Extensive reviews of aggression candidate gene studies list the specific candidate genes in the monoaminergic and neuroendocrine systems that have been investigated for association with aggressive behaviors so far (Pavlov et al., 2012; Veroude et al., 2016; Waltes et al., 2015). Although a moderate number of studies has been conducted, a meta-analysis including 12 candidate genes did not reveal any significant associations with aggressive behavior (Vassos et al., 2014). However, this meta-analysis might have suffered from the extensive heterogeneity of assessed phenotypes. A later meta-analysis, which investigated candidate variants in the *SLC6A4* and *MOAO genes specifically, did* confirm association *with antisocial behaviour (Ficks and Waldman, 2014).* Specifically for the *MAOA* gene, more meta-analysic evidence for association of genetic variation with aggression is available. MAOA is an enzyme that breaks down monoamine neurotransmitters like serotonin and dopamine through oxidative deamination (Youdim and Bakhle, 2006). Meta-analysis showed significant interaction between *MAOA* genotype and environmental adversity, affecting antisocial behavior in a sex-dependent manner. Males with an allele of a common variable number tandem repeat (VNTR) polymorphism in the promoter region of this gene, causing low enzyme activity, appear to experience a stronger effect of maltreatment on antisocial behavior compared to males with the high activity variant. In females, an opposite and weaker effect of *MAOA* genotype was suggested by the data, with the high activity variant increasing risk for antisocial behavior after maltreatment (Byrd and Manuck, 2014; Caspi, 2002). The specific association pattern of this gene with aggression again nicely illustrates the heterogeneity issues discussed earlier in this introduction.

**Genome-wide studies**

Hypothesis-free genome-wide association studies (GWAS) of aggression have not yet identified genome-wide significant single nucleotide polymorphisms (SNPs), but the top-findings from these studies have pointed towards novel pathways and functions potentially relevant to aggressive behaviors (Fernandez-Castillo and Cormand, 2016). Only one large-scale GWAS has been conducted to date (within the framework of the EArly Genetics and Lifecourse Epidemiology (EAGLE) consortium), including a large cohort of children and adolescents (N=18,988) from the general population. Common genetic variation was found to contribute to the phenotypic variation in aggressive behavior, with SNP-based heritability estimates ranging between 10 and 54%. In this study, aggressive behavior assessed using parent-report questionnaires in nine population-based studies was combined. The GWAS meta-analysis of the total cohort identified a region on chromosome 2p12 reaching near genome-wide significance. The top-SNP of this analysis is located near a gene involved in the regulation of excitatory synapse development (*LRRTM4*). A candidate gene-based association test (genes were selected as candidates based on previously having been tested for association with aggressive behavior (Craig and Halton, 2009; Vassos et al., 2014)), using the summary statistics of the total sample, showed association of the *AVPR1A* gene with childhood aggression after correcting for 21 candidate genes tested (Pappa et al., 2015). Only a few other GWAS of aggression have been performed (Anney et al., 2008; Merjonen et al., 2011; Mick et al., 2014; Mick et al., 2011; Tielbeek et al., 2012; Viding et al., 2010), and one GWAS studied the interaction between genes and environmental risk factors (GxE) (Sonuga-Barke et al., 2008). These studies investigated a wide range of aggression related phenotypes, including conduct problems in ADHD, hostility, proneness to anger, dysregulated behavior, antisocial behavior and antisocial behavior with callous-unemotional personality traits. Interestingly, two genes showed evidence for association based on more than one GWAS, *NFKB1* and *A2BP1*. *NFKB1* encodes the nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, a transcription regulator involved in axonal regeneration and degeneration (Haenold et al., 2014). *A2BP1* (also called *RBFOX1*) encodes the RNA binding protein, fox-1 homolog (C. elegans) 1, a neuron-specific RNA splicing factor that regulates the expression of large genetic networks during early neuronal development (Fogel et al., 2012). Enrichment analyses, using the top-signals of the combined GWAS, allowed the identification of overrepresented pathways and functions associated with aggressive behavior. These bioinformatics approaches identified signaling pathways involved in axon guidance, neuroactive ligand-receptor interaction, MAPK signaling, and plasma membrane estrogen receptor signaling, and, more generally, functions related to neuronal development (Fernandez-Castillo and Cormand, 2016). Such genome-wide approaches thus highlight the importance of neurodevelopmental and synaptic plasticity genes for aggression risk. Nevertheless, larger sample sizes and in-depth homogeneous phenotyping will be required to further identify and confirm responsible genes, as well as genetic variation contributing to specific types of aggressive behavior. Future genetic association studies might focus on the reactive - proactive distinction of aggression, to reduce wide heterogeneity in investigated phenotypes to date.

## AGGRESSION AND THE BRAIN

### Connecting neuro-cognition with behavioral subtypes

Aggression has been recognized as a neurodevelopmental phenotype, meaning that symptom development is thought to be related to abnormal brain development. Imaging studies have identified structural and functional brain differences in aggressive compared to control subjects. Several neuro-cognitive systems have been recognized to play a role in aggression. Efforts have been made to distinguish different forms of neuro-cognitive dysfunction associated with different subtypes of aggressive behavior. Below is a summary of these efforts in the context of the reactive - proactive distinction, along with an overview of specific imaging findings for implicated neural systems. Emphasis is on a framework that integrates neural, cognitive, and symptom levels (Blair et al., 2016).

The main forms of cognitive dysfunction that have been recognized in the context of aggression are *decreased empathy*, an *increased acute threat response*, and *impaired decision-making*. Different neural systems underlie these cognitive impairments, which associate with different sets of aggressive symptoms linked to different aggression subtypes (**Figure 1**).

The main neural substrates of *empathic processing* are the ventromedial prefrontal cortex (vmPFC) (Corradi-Dell'Acqua et al., 2014; Shamay-Tsoory et al., 2010) and the amygdala. The amygdala, which is important in the processing of emotional expressions, is of specific interest (Fusar-Poli et al., 2009). Reduced responses of this structure to fearful and sad expressions have been found particularly in subjects with psychopathic or callous and unemotional (CU) traits (Jones et al., 2009; Lozier et al., 2014; Marsh et al., 2008; Passamonti et al., 2010; Viding et al., 2012; White et al., 2012). Increased CU traits are associated with increased proactive aggression. Interestingly, this relation was found to be mediated by amygdala response to distress cues of others (Lozier et al., 2014). Reduced activity of the amygdala was also found in individuals with psychopathy, while observing others in pain (Marsh et al., 2013). Thus, functional studies support an important role for amygdala dysfunction in proactive forms of aggression.

The *acute threat response* is mediated by the amygdala-hypothalamus-periaqueductal gray neural system (Coker-Appiah et al., 2013; Mobbs et al., 2010). Dysfunction in this system is associated with increased reactive aggression (Blair, 2004; Yu et al., 2014). While both reduced and increased amygdala responses to visual threat stimuli have been found for aggressive compared to comparison subjects (Herpertz et al., 2008; Stadler et al., 2007; Sterzer et al., 2005), these inconsistencies have been attributed to the presence or absence of CU traits, in such a way that individuals with low CU traits have increased amygdala responses to social threats or provocations (Sebastian et al., 2014; Viding et al., 2012; White et al., 2016b). All in all, increased amygdala responsiveness to threat or provocation is associated with increased reactive forms of aggression (Choe et al., 2015).

Impairments in reward sensitivity, processing of punishment, and regulation of avoidance behaviors are thought to be the basis of poor *decision-making* in individuals with aggressive behavior (Fairchild et al., 2009). The neural loci thought to underlie dysfunction in these systems are the striatum and vmPFC. Functional imaging studies indicate that aggressive individuals show a reduced response to reward both within the striatum and the vmPFC

(Cohn et al., 2015; Crowley et al., 2010; Finger et al., 2011; Rubia et al., 2009; White et al., 2013), but an increased response to punishment (Crowley et al., 2010; Finger et al., 2011; Finger et al., 2008; White et al., 2013). Poor decision-making might lead to increased reactive aggression due to frustration, but also to proactive forms of aggression, for example when an individual does not learn to avoid actions that harm others (Blair et al., 2016; White et al., 2016a).

The functional imaging studies described above, indicate an important role for subcortical structures in aggression etiology, most notably amygdala and striatum. Studies investigating functional connectivity of these subcortical structures with the vmPFC (Marsh et al., 2011; Marsh et al., 2008), support the idea that vmPFC has a regulatory function in response selection through its role in representing value information (Hare et al., 2009). Anatomical imaging studies support the importance these structures for aggressive behavior risk as well. Differences in amygdala volume between aggressive and healthy individuals have been reported in many studies. Volume reductions of the amygdala were found predominantly (Caldwell et al., 2015; Fairchild et al., 2013; Noordermeer et al., 2016; Pardini et al., 2014; Sterzer et al., 2007; Thijssen et al., 2015; Wallace et al., 2014; Zhang et al., 2013). For striatum, both volume reductions and volume increases have been related to aggressive phenotypes, especially with regard to the caudate nucleus and nucleus accumbens substructures (Cha et al., 2015; Ducharme et al., 2011; Fairchild et al., 2013; McAlonan et al., 2007; Nosarti et al., 2005; Schiffer et al., 2011). For orbitofrontal/vmPFC, volume reductions have been observed in people with aggressive behavior, and vmPFC lesions are associated with increased aggressiveness compared to lesions in other regions of the brain (Ducharme et al., 2011; Grafman et al., 1996; Young et al., 2010).

**Hypothesized pathways from gene to brain to behavior**

The above-mentioned neuro-cognitive systems can be hypothesized to be regulated by different genetic influences. However, strict categorical lines cannot likely be drawn, as genetic overlap as well as differences may exist between aggression subtypes, and different genetic systems are likely to interact with each other. For example, the serotonin and dopamine neurotransmitter systems have been shown to interact intricately (Oades, 2008). Nevertheless, specific genetic systems are likely to play a more important role in one cognitive function than another. **Figure 1** represents a hypothetical representation of gene-brain-behavior relationships adapted from a schematic of Blair et al., which illustrates the proposed neuro-cognitive relationships described above (Blair et al., 2016). It was adapted to incorporate the dimensional relationship of these neuro-cognitive mechanisms to reactive and proactive symptoms as well as the main candidate genetic systems currently implicated in aggression. It has been speculated that serotonergic and dopaminergic neurotransmission regulate both reactive and proactive aggression, whereas endocrine signaling seems to be more involved in the regulation of reactive aggression (Waltes et al., 2016). As dopamine is part of the neural reward system, dopaminergic genes are thought to be involved in aggression etiology primarily through effects on the reward neurotransmitter system (Chen et al., 2005), thus affecting decision-making. However, because it is also involved in hormonal regulation through the tuberoinfundibular pathway of dopamine transmission, as

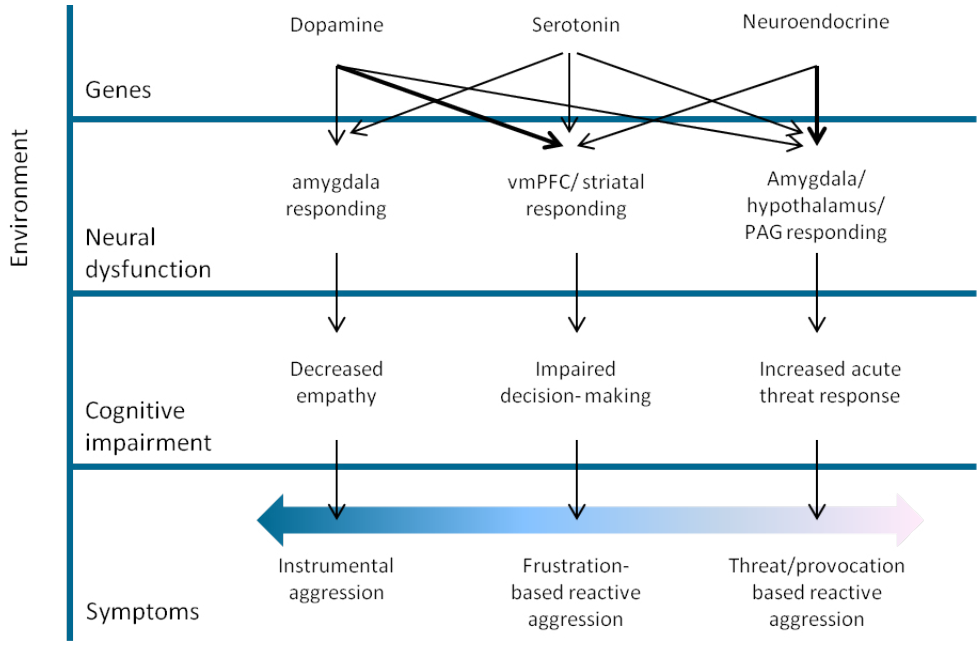


**Figure 1:** Tentative representation of genetic, neuro-cognitive, and behavioral relationships in aggression (adapted from Blair et al. (2016)). See text for a detailed description of the relationships between genes, neuronal functioning, cognition, and behavior.

well as in mood regulation through the mesolimbic pathway, dopamine likely plays a role in connecting reward signaling and the stress response (Pivonello et al., 2007). Dopaminergic genes may thus be involved in both proactive and reactive aggression. The same is true for serotonergic genes, candidate variants in which have been associated with very diverse aggressive phenotypes (Veroude et al., 2016). The endocrine systems, specifically the HPA axis, are involved in regulation of impulsivity and the stress response, which are closely related to reactive aggression (Waltes et al., 2016). However, system-specificity of effects needs to be further investigated before we can make conclusive statements on gene-subtype associations. While neuroimaging genetics studies, investigating genetic influences on neuroimaging measures, are still scarce for aggression, they can be of particular interest in elucidating specific pathways from gene to behavior via the brain.

## THESIS OUTLINE

### Aim and structure of this thesis

The overall aim of this thesis was to improve our understanding of the genetic and neurobiological architecture of aggression. In this, my work contributes to the reduction of heterogeneity issues in aggression research by looking into subtype- and sex- specific gene identification and by investigating specific pathways to disease. A combination of

well-phenotyped local study cohorts and large scale consortium-based samples was used to overcome power issues related to the polygenic nature of aggression. This work also adds to the scarce imaging genetics literature on aggression and formulates recommendations for future imaging genetics studies. The thesis in divided into two parts.

In **PART 1**, I focused on the potential of **reducing phenotypic heterogeneity** to elucidate **genetic mechanisms** involved in aggression. Different subtyping approaches were employed, followed by genetic association studies aiming at the identification of the genetic mechanisms underlying these subtypes. In **chapter 2**, I report the results of a latent class analysis that was conducted to identify conceptually meaningful subtypes of oppositionality in childhood ADHD. Additionally, I present a novel genetic landscape for oppositional behavior based on the results of a multivariate GWAS on identified subtypes. In **chapter 3**, I describe our findings from a GWAS meta-analysis conducted in adult patients with ADHD. The phenotype of interest was childhood aggression, a predictor of worse outcomes in adulthood. In **chapter 4**, I aimed to confirm the existence of reactive and proactive subtypes of aggressive behavior in the general population as well as to identify subtype-specific association of candidate gene-sets with aggressive behavior. I report the results of a confirmatory factor analysis followed by gene-set association analysis for serotonergic, dopaminergic, and neuroendocrine genes.

In **PART 2**, the focus is on neuroimaging genetics approaches to study associations between genes implicated in externalizing behaviors and brain phenotypes. **Chapter 5** is a review of the existing imaging genetics studies performed for aggression as well as for several neurodevelopmental phenotypes with high comorbidity with aggression (ADHD, ASD, and selected IDs). I provide a wide overview of the imaging genetics field and formulate specific recommendations for future research. In **chapter 6**, I used large-scale GWAS meta-analysis data on aggression and subcortical brain volumes from several large consortia, to conduct cross-trait meta-analysis. I used gene-wide association statistics with the aim of identifying genes with pleiotropic effects on both subcortical brain volume and aggression risk. Additionally, I report subtype-specific association of identified genes with aggression.

## STUDY COHORTS AND CONSORTIA

**Aggressotype:** All work in this thesis was conducted in the context of the Aggressotype consortium, which focuses on aggression subtyping for improved insight and treatment innovation in paediatric psychiatric disorders (www.aggressotype.eu). Its main aims are 1) to gain new insights into the mechanisms underlying pathological aggression by improving the subtyping of aggression and building a knowledge chain for aggression aetiology from the molecular level via cellular, brain-network, and cognitive levels to behavior, and 2) to translate preclinical findings into predictive, preventive, and therapeutic strategies for the benefit of vulnerable patients with paediatric conduct disorders. Aggressotype is funded by the European Community's Seventh Framework Programme (FP7/2007 – 2013) and has twenty-eight participating research groups from eleven countries.

**IMAGE:** The International Multicenter ADHD Genetics study was conducted by a consortium of seven European countries and Israel, with the aim to identify genetic risk factors for ADHD (Brookes et al., 2006; Muller et al., 2011a; Muller et al., 2011b). Families with at least one child with ADHD as well as at least one biological sibling (irrespective of ADHD diagnostic status) were recruited. IMAGE collected extensive phenotyping, neuropsychological and genotypic information of these families. The IMAGE project participated in the Genetic Association Information Network (GAIN) to enable genome-wide genetic studies. In the Netherlands, it was followed-up by the NeuroIMAGE study, enriching the data-set with structural and functional MRI data, current clinical status, and additional collection of neuropsychological measures and DNA (von Rhein et al., 2015).

**IMpACT:** The International Multicentre persistent ADHD CollaboraTion is a consortium of clinical and basic researchers from several European countries (The Netherlands, Germany, Spain, Norway, The United Kingdom, Sweden), from the United States of America, and from Brazil (www.impactadhdgenomics.com). The aim of IMpACT is to perform and promote high quality research in ADHD across the lifespan. It includes the identification of novel genetic variants for adult ADHD and improving the understanding the mechanisms underlying the effect of these genetic variants on disease risk. IMpACT currently coordinates biosamples and phenotypic information of over 4000 cases with persistent ADHD and over 8000 controls. In this thesis, data from Germany, Norway and Spain was used.

**BIG:** The Brain Imaging Genetics study was set up in 2007 by the Human Genetics department of the Radboud university medical center and the Donders Centre for Cognitive Neuroimaging of the Radboud University (www.cognomics.nl/big). In 2010, the Max Planck Institute for Psycholinguistics in Nijmegen also joined. BIG aims to study relations between genes, brain structure and function, and cognition and behavior in healthy individuals. For this, a continuously growing database has been created with data of structural and functional magnetic resonance imaging (MRI)-based brain scans and DNA as well as cognitive and behavioral data derived from internet-based testing.

**ENIGMA:** The Enhancing NeuroImaging Genetics through Meta Analysis network brings together researchers in imaging genomics to understand brain structure, function, and disease, based on brain imaging and genetic data (http://enigma.ini.usc.edu/). Currently, more than 30 working groups have been formed within the ENIGMA consortium. In this thesis, summary statistic data of the ENIGMA GWAS meta-analysis on subcortical volumes and intracranial volume is used (Hibar et al., 2015).

**EAGLE:** The EArly Genetics and Lifecourse Epidemiology Consortium is a consortium of pregnancy and birth cohorts that aims to investigate the genetic basis of phenotypes in antenatal and early life and childhood. All participating cohorts have GWAS data available. In this thesis, summary statistic data of the EAGLE GWAS meta-analysis on childhood aggressiveness is used (Pappa et al., 2015).

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# Part 1

## Subtyping and genetic association studies of aggression

# Chapter 2

## Gene-set and multivariate genome-wide association analysis of oppositional defiant behavior subtypes in attention-deficit/ hyperactivity disorder

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## ABSTRACT

Oppositional Defiant Disorder (ODD) is a frequent psychiatric disorder seen in children and adolescents with Attention-Deficit-Hyperactivity Disorder. ODD is also a common antecedent to both affective disorders and aggressive behaviors. Although the heritability of ODD has been estimated to be around 0.60, there has been little research into the molecular genetics of ODD. The present study examined the association of irritable and defiant/vindictive dimensions and categorical subtypes of ODD (based on latent class analyses) with previously described candidate gene polymorphisms (*DRD4* exon3 VNTR, *5-HTTLPR,* and seven *OXTR* SNPs) as well as with dopamine, serotonin and oxytocin genes and pathways in a clinical sample of children and adolescents with ADHD. In addition, we performed a multivariate genome-wide association study (GWAS) and integrated the top-ranked findings into a landscape of functionally interacting proteins and molecules that regulate biological signaling cascades. Apart from adjusting the analyses for age and sex, we controlled for "parental ability to cope with disruptive behavior". None of the hypothesis-driven analyses revealed a significant association with ODD dimensions and subtypes. Parenting behavior was significantly associated with all ODD dimensions and subtypes, most strongly with defiant/vindictive behaviors. The GWAS did not result in genome-wide significant findings. Bioinformatics and literature analyses revealed that the proteins encoded by 28 of the 53 top-ranked genes interact in a molecular landscape centered around β-catenin signaling and involved in the regulation of neurite outgrowth. Our findings provide new insights into the molecular basis of ODD and inform future genetic studies of oppositional behavior.

## INTRODUCTION

Oppositional Defiant Disorder (ODD) shows strong comorbidity with Attention-Deficit-Hyperactivity Disorder (ADHD), Conduct Disorder (CD), and mood disorders (Angold et al., 1999), in both epidemiological and clinical samples. To date, the etiological basis of this comorbidity is unclear, although shared genetic influences between these disorders have been postulated to play a role (Dick et al., 2005; Faraone et al., 1991). Research into ODD has gained momentum due to its relation to later psychopathology such as affective disorders (Copeland et al., 2009) and antisocial personality disorder (Langbehn et al., 1998). Youths with ADHD frequently show severe impulse control problems and are at high risk for developing ODD. A better understanding of the developmental pathways from ADHD to ODD is crucial to prevent further antisociality and psychopathology. However, there has been little research on the genetics of ODD, perhaps because this disorder has been viewed primarily as the result of ineffective parenting (Frick et al., 1992). Nevertheless, the heritability of ODD has been estimated to be around 0.60 (Coolidge et al., 2000; Nadder et al., 1998) and ODD is familial among families of ADHD youth (Petty et al., 2009).

ADHD has been the focus of considerable genetic research. Meta-analyses of candidate gene studies of ADHD have yielded evidence for a number of genes involved in the dopaminergic, serotonergic, noradrenergic, and nicotinergic neurotransmission and receptor function (Gizer et al., 2009). Genome-wide Association Studies (GWAS) of ADHD did not yet reveal any significant association (Neale et al., 2010). There is comparatively little work into the molecular genetics of oppositional and disruptive behaviors in children and adolescents . A recent meta-analysis showed a significant association of the short allele of the polymorphic region (5-HTTLPR) in the promoter region of the serotonin transporter gene *5-HTT/SLC6A4* with antisocial behaviors (including aggression) (Ficks and Waldman, 2014), although evidence for this association is conflicting (Vassos et al., 2014). The short allele has been found to affect negatively the transcription rate of the gene compared to the long allele (Heils et al., 1996), putatively affecting the availability of serotonin in the synaptic cleft and thus increasing the risk for aggressive behavior. Further studies also support the role of dopamine genes in the development of ODD and/or CD. The variable number tandem repeat polymorphism (VNTR) within exon 3 of the dopamine receptor D4 gene (*DRD4)* has been frequently investigated in psychiatric genetic studies and the 7-repeat allele was found to lead to less efficient dopamine binding and reduced receptor sensitivity. Several studies found individuals with the 7-repeat allele to have an increased risk for ODD and CD symptoms (DiLalla et al., 2009; Holmes et al., 2002). In accordance with the findings for *DRD4* and *5-HTTLPR*, high levels of dopamine and low levels of serotonin were associated with aggression and irritability in humans (Duke et al., 2013; Ryding et al., 2008). Deregulation of oxytocin (OXT) signaling – for example as a consequence of genetic variability - also predisposes an individual to antisocial and aggressive behaviors and disrupts prosocial behaviors (Malik et al., 2012). Two studies found that low levels of OXT are linked to aggressive behaviors in adult males (Fetissov et al., 2006; Lee et al., 2009). In genetic studies, single nucleotide polymorphisms (SNPs) within the oxytocin

receptor gene (*OXTR*) were associated with callous-unemotional and aggressive behaviors in males and females (Malik et al., 2012; Zai et al., 2012). To date, seven *OXTR* SNPs (rs1042778, rs6770632, rs237885, rs4564970, rs1488467, rs53576, rs13316193) have been found to be related to aggression, CU behaviors, and/or behavior problems (Beitchman et al., 2012; Campbell et al., 2011; Johansson et al., 2012a; Johansson et al., 2012b; Malik et al., 2012; Park et al., 2010). Most of the molecular genetic studies of *OXTR* have been limited by small sample sizes, though, and therefore warrant replication.

The phenotypic heterogeneity of ODD complicates the identification of genetic involvement with the occurrence of the disorder. An increasing number of studies supports the need for discrimination of ODD irritable and defiant/vindictive dimensions in community samples of preschoolers, school-aged children, and adolescents (Ezpeleta et al., 2012; Krieger et al., 2013; Stringaris and Goodman, 2009a, b) as well as in children and adolescents referred for ADHD or autism (Aebi et al., 2010; Mandy et al., 2014), which may inform genetic studies. Irritable mood has been suggested to underlie the developmental link between ODD and later affective disorders (Stringaris et al., 2009), and a defiant/vindictive behavioral pattern of ODD is associated with CD and the presence of callous unemotional (CU) traits (Kolko and Pardini, 2010) as well as later criminal outcomes in adulthood (Aebi et al., 2013). A genetic link between ODD irritable behavior and depression, on the one hand, and between ODD defiant/vindictive aspects and delinquent behavior, on the other, was found in a UK twin sample (Stringaris et al., 2012).

In this study, we aim to investigate the genetic underpinnings of ODD using data from the International Multicentre ADHD Genetics (IMAGE) study (Müller et al., 2011a, b) including 750 subjects. We first defined conceptually meaningful dimensions/subtypes of oppositionality, in order to improve the power of our analyses by reducing the known heterogeneity of the ODD phenotype (Burke et al., 2005). We subsequently tested genetic variants in dopamine, serotonin and oxytocin signaling pathways for their association with the two dimensions and the two categorical subtypes. We first tested individual polymorphisms earlier found related to such traits, i.e. the *DRD4* VNTR 7-repeat allele, the *5-HTTLPR* short allele and variants in the *OXTR* gene. In a second step, gene-wide analysis for *DRD4*, *5-HTT*, *OXTR* and gene-set analysis of the dopamine, serotonin and oxytocin pathways was performed to test their association with the two dimensions and the two categorical subtypes. Besides adjusting the analyses for age and sex, we also controlled for "parental ability to cope with disruptive behavior", because parenting behavior has been identified as a major source of ODD (e.g. Burke et al., 2008). We also tested the interaction between genetic polymorphisms and "parental ability to cope with disruptive behavior" and ODD subtypes/dimensions. In addition to the hypothesis-driven analyses, we aimed to generate new hypotheses about genetic involvement in ODD. Because genetic overlap as well as differences can be expected to exist between the two dimensions and the two categorical ODD subtypes (Dowell et al., 2010) and to maximize power of our analyses (Galesloot et al., 2014), we used a multivariate genome-wide association testing framework.

Employing bioinformatics and literature mining we integrated top-ranked findings from the GWAS into a landscape of proteins and molecules that regulate biological signaling cascades, providing important new insights into the genetic etiology of ODD.



## MATERIALS AND METHODS

### Sample

The present study is based on 750 probands and their parents from the International Multicentre ADHD Genetics (IMAGE) study. Participants of the IMAGE study were European Caucasians aged 5-17 years, who had been recruited in 12 child and adolescent psychiatry clinics representing eight countries: Belgium, Germany, Switzerland, Holland, Ireland, Israel, Spain, and the United Kingdom. Approval was obtained by the Institutional Review Board of SUNY Upstate Medical University and from ethical review boards within each country. A detailed description of the study design and assessment procedures has been provided in previous publications (Müller et al., 2011a, b). In short, entry criteria for probands were a clinical diagnosis of ADHD based on DSM-IV criteria and access to one or both biological parents and one or more full siblings for DNA collection and clinical assessment. Exclusion criteria applying to both probands and siblings included autism, epilepsy, IQ < 70, brain disorders, and any genetic or medical disorder associated with externalizing behaviors that might mimic ADHD. The full sample of the IMAGE project amounts to 1067 subjects. Out of this sample with ADHD combined type, 774 subjects with full information on ODD phenotypes and covariates (see below) were included in the analyses. Genome-wide imputed genotypes (HAPMAP2) and Variable Number of Tandem Repeats (VNTR) were available for 750 subjects. Attrition analyses showed that the 317 subjects who were not included in the analyses, did not differ from the participating 750 subjects in terms of sex (male sex 86.8% vs. 87.7%; $\chi^2$=0.20, *df*=1, *p*=*n.s.*), age (10.94 vs. 10.67 years; *t*=1.43, *df*=1065, p=*n.s.*), and ODD diagnosis (69.0% vs. 64.1%; $\chi^2$=2.32, *df*=1, p=*n.s.*).

### Measures

The long form of the revised Conners Parent Rating Scale (CPRS-R:L) was used in the present study (Conners, 1997; Conners et al., 1998). Subtypes and dimensions of oppositionality were assessed by use of the 10 items (0 = not true, 1 = little true, 2 = much true, 3 = very much true) of the CPRS-R:L oppositional scale. In total, four different phenotype (two dimensional and two categorical) measures were included in the present study and tested for differences in the candidate-based and hypothesis-free analyses (see below). The use of dimensional as well as categorical measures of ODD is in line with previous research confirming (a) separate but correlated dimensions of ODD (Aebi et al., 2010; Aebi et al., 2013; Ezpeleta et al., 2012; Krieger et al., 2013; Stringaris and Goodman, 2009a, b) and (b) distinct subtypes of irritable and severe forms of ODD (Althoff et al., 2014; Burke, 2012; Kuny et al., 2013).

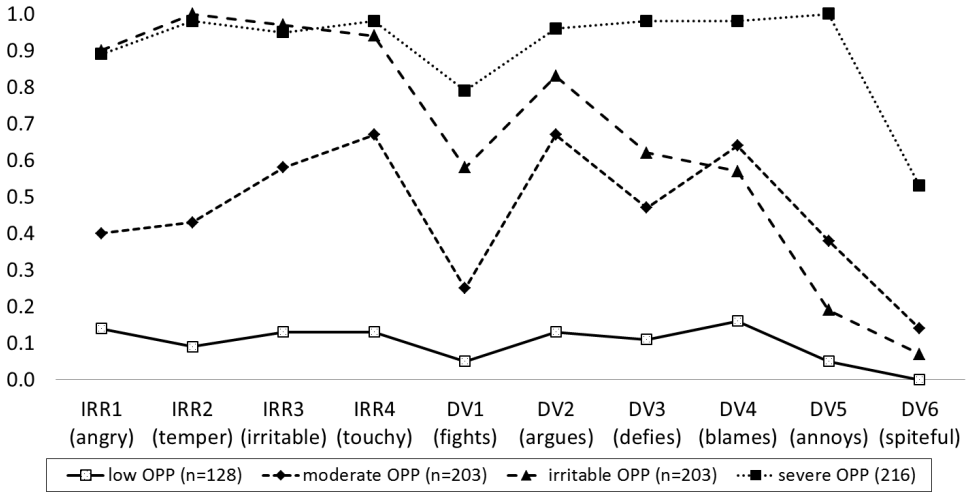



**Figure 1:** Mean scores of dichotomized items of the Conners Parent Scale (CPRS-R:L) oppositional scale assessing irritable (IRR1- IRR4) and defiant/vindictive (DV1 – DV6) behaviors as a function of latent classes for children and adolescents with ADHD combined type (N = 750). OPP = oppositionality.

a) Two dimensions were defined on theoretical grounds, which reflected the two previously described dimensions of ODD (Aebi et al., 2013; Stringaris et al., 2012), namely defiant/vindictive (P1) and irritable (P2). The items related to P1 with scores ranging from 0 to 18 and P2 with scores from 0 to 12 for P2 are shown in Figure 1. Internal consistencies (Cronbach alpha) amounted to 0.79 and 0.82 for the defiant/vindictive and the irritable dimension, respectively. Because of a right skewed distribution, a Blom transformation (Blom, 1958) of P2 was performed.
b) Two further dichotomous subtypes were based on findings from a latent class analysis (LCA). LCA was performed using poLCA package (Linzer and Jeffrey, 2011) in R statistic software (R Development Core Team, 2011). All of the 10 dichotomized CPRS-oppositionality items (0 and 1 were scored as absent; 2 and 3 were scored as present) were included in analysis. One to five class models were compared, and the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC) were used to determine the number of classes. The four class solution, which fitted the data best (BIC=11169; AIC= 10955), contained classes labeled *low oppositionality (OPP), moderate OPP, irritable OPP,* and *severe OPP* (see Figure 1). Because of our interest in severe forms of ODD, we defined the following dichotomous phenotypes: a dichotomous subtype P3, with 0 representing 'low OPP/moderate OPP' (n=331) and 1 representing 'irritable OPP/severe OPP' (n=419), and a dichotomous subtype P4, with 0 representing 'low OPP/moderate OPP/irritable OPP' (n=534) and 1 representing 'severe OPP' (n=216).

The DSM-IV diagnoses of ODD / CD and parental ability to cope with disruptive behaviors was coded from the diagnostic interview (Parental Account of Childhood Symptoms, [PACS]; Chen and Taylor, 2006; Taylor et al., 1986) A parent (usually the mother) responded to a 7-point Likert-scale ranging from 0 (efficient coping) to 7 (abusive parental behavior) measuring maternal and paternal coping with disruptive behaviors. A mean score was used when information for both parents was available. Furthermore, the oppositional scale of the Conners' Teacher Rating Scale (CTRS- R:L;Conners, 1997) and the conduct problem scale of the Strengths and Difficulties Questionnaire (Goodman, 1997) were used for phenotype description.

**DNA collection and genotype assays**

Sample collection and DNA isolation has been described previously (Brookes et al., 2006). Genome-wide genotyping and data cleaning was performed as part of the GAIN study using the Perlegen 600K genotyping platform, as described in (Neale et al., 2008). To increase genomic coverage, imputation was performed using MACH and the Hapmap 2 (Release 22 Build 36) reference data set (Li et al., 2010). Quality control was performed on the imputed data, and SNPs with imputation quality scores lower than 0.30, a minor allele frequency lower than 0.01, and those failing the Hardy-Weinberg equilibrium test at a threshold of $p \leq 10^{-5}$ were excluded. In addition, SNPs and subjects with missingness rates higher than 0.05 were removed from the data. Distributed over 22 autosomes, 1,871,025 SNPs were left for analysis.

Genotyping of candidate polymorphisms (DRD4 exon 3 VNTR; 5-HTTTLPR) was performed at the SGDP laboratories in London or at the Human Genetics department of the Radboudumc in Nijmegen, the Netherlands. Standard PCR protocols were used, as previously described (Brookes et al., 2006; Thissen et al., 2015).

**Statistical analyses**

*Analysis of candidate polymorphisms*

Linear and logistic regression analyses were used to test the effects of the *DRD4* exon 3 variant (presence/absence of the 7-repeat allele: 7R/7R and 7R/other versus other/other) and the *5-HTT* variant (presence/absence of the *5-HTTLPR* short allele: S/S and S/L versus L/L) on the ODD dimensions/subtypes. Variables included in the model were age, sex, and parental ability to cope with disruptive behaviors, as well as the interaction of *DRD4* and *5-HTTLPR* genotype with parental ability to cope with disruptive behaviors. For the oxytocin receptor gene *OXTR*, only one of the seven SNPs previously linked with aggression was present in the data (rs1488467). Therefore, outcome of the association analysis of all SNPs located in that region was plotted to find out if an association signal was presented by closely related linked SNPs.

*Gene-wide and gene-set analyses*

Gene-wide analysis was applied for *5-HTT* as well as for *DRD4* and *OXTR* using a mass-univariate approach, to take potential allelic heterogeneity into account and test if a combination of SNPs located in these genes showed association with the ODD dimensions/subtypes. Similarly, gene-set analysis was performed for all genes involved in serotonin, dopamine and oxytocin neurotransmission. A list of genes included in each pathway-wide analysis can be found in Supplementary Table S1. All available variants of each gene were extracted, including variants within a 100 kilobase (kb) flanking region of each gene to capture regulatory sequences. The effect of common variants of each gene or gene-set of interest on the two dimensions and the two categorical subtypes was investigated using the statistical approach described by Bralten et al. (2013) consisting of SNP-by-SNP regression and estimation of the effect of the whole gene or gene-set. For both gene-wide and gene-set based analyses, linkage disequilibrium-pruned genotyping data were prepared, using the 'indep' command in Plink (Purcell et al., 2007) with a $r^2$ threshold of 0.8.

*Correction for multiple testing*

Results were considered to be significant if they reached the Bonferroni corrected p-value threshold for multiple testing (0.05 divided by the number of phenotypes, polymorphisms and gene(-sets) tested; p-value threshold=1.4E-3).

*Multivariate genome-wide association study*

We performed a multivariate GWAS to capture covariance among the different correlated ODD dimensions/subtypes and to increase the power for finding genetic associations. Using only a single test for association instead of four, has the additional advantage of a reduced multiple testing burden. Following analysis of correlation between traits we assessed association between genetic markers and the two dimensions and the two categorical subtypes using the MQFAM multivariate extension of PLINK (Ferreira and Purcell, 2009). Residuals obtained for each subtype after adjustment for age, sex, parental ability to cope with disruptive behavior, and four population components derived from multidimensional scaling analysis were used as input. The MQFAM method uses canonical correlation analysis to identify the linear combination of traits that maximizes the covariance between a marker and the traits. It can be used for analysis of a combination of quantitative and binary traits (Ferreira and Purcell, 2009; Galesloot et al., 2014). For each SNP included in the analysis, a loading is calculated in the output which reflects the contribution of each phenotype to the association results. Top-SNPs (p<1.00E-5) from the multivariate GWAS were investigated for their location in or around genes and for their performance in univariate analysis, which provided information on the direction of effect.

*Molecular landscape building: bioinformatics and literature analyses*

To increase the understanding of the molecular basis of ODD, we aimed at integrating the top findings from the GWAS into a landscape of functionally interacting proteins and molecules that regulate biological signaling cascades. First, a list of independent association

regions was obtained by clumping the results using PLINK (Purcell et al., 2007). SNPs in LD ($r^2 \geq 0.2$) within 10000 kb of a more significant index SNP were discarded. Second, a threshold of p<1.00E-04 was applied for index SNPs, resulting in 75 LD-independent regions. The chosen statistical cut-off for association of p<1.00E-04 is often used to designate 'suggestive' association and has been previously used in studies of neurodevelopmental disorders (ADHD and autism) (Poelmans et al., 2013; Poelmans et al., 2011b). Third, a list of top genes was compiled. Gene annotation was performed when an index SNP was located within an exon, an intron or untranslated region of the gene, or when an index SNP was located within a region 100 kb downstream or upstream of the gene to capture regulatory sequences (Gherman et al., 2009; Nicolae et al., 2010; Pickrell et al., 2010; Veyrieras et al., 2008).

We then conducted a canonical pathway analysis of the list of top-ranked genes from the multivariate GWAS, using the Ingenuity software package (http://www.ingenuity.com). For this pathway enrichment analysis, Ingenuity draws on the Ingenuity Knowledge Base which is based on information from published literature as well as on various other sources including gene expression and gene annotation databases. An enrichment P-value is calculated for each pathway with the right-tailed Fisher's exact test and correction for multiple testing is performed using the Benjamini-Hochberg correction. Subsequently, we searched the literature for the function of the proteins encoded by all the top-ranked genes from the multivariate GWAS, using UniProtKb (http://www.uniprot.org/uniprot) and Pubmed (http://www.ncbi.nlm.nih.gov/). The landscape building approach described here has been used in earlier studies of neurodevelopmental disorders (Poelmans et al., 2011a; Poelmans et al., 2013; Poelmans et al., 2011b) Lastly, the genes from the list with top findings were investigated for previous implication in the etiology of neurodevelopmental or neuropsychiatric disorders using Ensembl release 75 (Flicek et al., 2014) and the NCBI databases (http://www.ncbi.nlm.nih.gov/).

## RESULTS

### Descriptives

The final sample (N=750) consisted of 680 boys (87.9%) and 94 girls (12.1%) aged 5 to 18 years (mean 10.67 years, SD=2.77). According to the PACS interview, 481 (64.1%) children and adolescents fulfilled DSM-IV criteria for ODD and 170 (22.7%) for CD. Bivariate correlations of the two dimensions and the two categorical subtypes are shown in Supplementary Table S2. All correlations were significant and moderate. Furthermore, all dimensions/subtypes were slightly correlated to teacher ratings of oppositionality (CTRS), and moderately correlated to SDQ conduct problems and DSM-IV diagnosis of ODD / CD (also shown in Supplementary Table S2).

### Candidate polymorphisms

No associations of *DRD4* and *5-HTTLPR* were observed for any of the four measures, nor were any interactions of parental ability to cope with disruptive behaviors with these genotypes observed (Table 1). Parental ability to cope with the child's disruptive behaviors was significantly associated with all four ODD measures (except for *5-HTTLPR* analysis of severe oppositionality (P4). Age was positively associated with irritability (P2) and irritable/severe oppositionality (P3), both in the *DRD4* model as well as in the *5-HTTLPR* model. There appeared to be no SNPs closely located to, and in high linkage disequilibrium with, *OXTR* SNP rs1488467 that show association with the ODD dimensions/subtypes (Supplementary Figure S1).

### Gene-wide and gene-set analyses

Findings for the *5-HTT, DRD4* and *OXTR* genes and the neurotransmission pathways are shown in Table 2. None of the analyses revealed a significant association with any of the four ODD phenotypes.

**Table 1:** Linear and logistic regressions of the DRD4 genotype (presence/absence of the seven repeat allele: 7R7R and 7R/other versus other/other) and of the HTTLPR genotype (presence/absence of the short allele: S/S and S/L versus L/L) predicting the four phenotypes of ODD.

| **Phenotypes** | **P1** | **P2 (transformed)** | **P3** | **P4** |
|---|---|---|---|---|
| **Variables** | B | B | B | B |
| DRD4 genotype | | | | |
| DRD4 (7R7R and 7R/other vs other/other) | -0.39 n.s. | -0.08 n.s. | 0.00 n.s. | -0.10 n.s. |
| Parent coping (centered) | 0.65*** | 0.16*** | 0.28*** | 0.27*** |
| DRD4 (7R7R and 7R/other vs other/other) x parent coping (centered) | 0.02 n.s. | -0.08 n.s. | -0.10 n.s. | -0.12 n.s. |
| Sex (0=female, 1=male) | 0.69 n.s. | 0.19 n.s. | 0.33 n.s. | 0.35 n.s. |
| Age | 0.09 n.s. | 0.04** | 0.07*. | 0.05 n.s. |
| HTTLPR genotype | | | | |
| 5-HTTLPR (S/S and S/L vs. L/L) | 0.51 n.s. | -0.02 n.s. | -0.03 n.s. | 0.20 n.s. |
| Parent coping (centered) | 0.73** | 0.16** | 0.25* | 0.23 n.s. |
| 5-HTTLPR (S/S and S/L vs. L/L) x parent coping (centered) | -0.13 n.s. | -0.04 n.s. | 0.00 n.s. | -0.02 n.s. |
| Sex (0=female, 1=male) | 0.64 n.s. | 0.19 n.s. | 0.34 n.s. | 0.28 n.s. |
| Age | 0.08 n.s. | 0.04** | 0.07* | 0.04 n.s. |

Note: P1= defiant vindictive dimension, P2= irritable dimension, P3= irritable/severe oppositionality, P4= severe oppositionality, +=significance (two sided), p<.010, *=significance (two sided), p<.05, **=significance (two sided), p<.01, ***=significance (two sided), p<.001.

**Table 2:** P-values of gene-wide and gene-set based analysis of 5-HTT, DRD4 and OXTR genes and the neurotransmission pathways for serotonin, dopamine and oxytocin.

| Phenotype | Gene-wide analysis | | | Gene-set analysis | | |
|---|---|---|---|---|---|---|
| | **5-HTT** (20 SNPs) | **DRD4** *(14 SNPs)* | **OXTR** (71 SNPs) | **Serotonin** (942 SNPs) | **Dopamine** (2568 SNPs) | **Oxytocin** (360 SNPs) |
| P1 | 0.2508 | 0.2756 | 0.3101 | 0.3458 | 0.5612 | 0.6798 |
| P2 | 0.6463 | 0.9455 | 0.5737 | 0.5493 | 0.4726 | 0.9272 |
| P3 | 0.9445 | 0.3128 | 0.9649 | 0.515 | 0.276 | 0.9991 |
| P4 | 0.1632 | 0.7257 | 0.5579 | 0.5012 | 0.274 | 0.9377 |

Note: P1= defiant vindictive dimension, P2= irritable dimension, P3= irritable/severe oppositionality, P4= severe oppositionality.



**Multivariate genome wide association study**

As expected given the modest sample size (n=750), multivariate GWAS did not result in genome-wide significant findings (p< 5.0E-08; Dudbridge and Gusnanto, 2008) (see Figure 2, and Supplementary Figure S2 for the Quantile- Quantile plot). Supplementary Table S3 presents the 65 SNPs showing association with the ODD dimensions and subtypes at p<1.00E-5, together with their respective loadings reflecting the contribution of each phenotype to the association results and their performance in univariate analysis. The top three findings were for rs7204436 (p= 1.98E-07) located in an intergenic region on chromosome 16, rs1278352 (p=1.24E-06) located in an intronic region of the *ADAM12* gene on chromosome 10, and rs12370275 (p=2.41E-06) located in an intergenic region on chromosome 12 (Figure 2). Also of interest is a region on chromosome 20 with a large number of SNPs in high LD showing a strong association signal. This region is located on chromosome 20q11.21 and is spanning several genes (*COX4I2, BCL2L1, TPX2, MYLK2, FOXS1, TTLL9*) (also depicted in Figure 2).

**Molecular landscape building**

From 76 independent SNPs with a p<1.00E-04, a list of fifty-three top-ranked genes was derived using the criteria as described in the Methods section (Supplementary Table S4). The bioinformatics analysis with Ingenuity revealed significant enrichment of the canonical pathways 'Inhibition of matrix metalloproteases' ($P_{corrected}$=1.19E-2), 'Axonal guidance signaling' ($P_{corrected}$=2.60E-02), and 'Wnt/ B-catenin signaling' ($P_{corrected}$=2.60E-02), with the proteins encoded by nine of the top-ranked genes belonging to one more of these pathways (Table 3). Importantly, all proteins encoded by these nine genes play a role in neurite outgrowth. In addition, the subsequent literature analysis revealed that in total, 28 of the 53 top-ranked ODD genes (53%) interact in a molecular landscape centered around β-catenin signaling and involved in regulating neurite outgrowth (depicted in Figure 3). This landscape encompasses signaling cascades that are important for the

neural modulations necessary for the growth of axons in a specific direction. The evidence linking the molecules in the landscape to neurite outgrowth is described in detail in the Supplementary Information.

Fifteen of the top-ranked genes have also been implicated previously in the etiology of neurodevelopmental and/or neuropsychiatric disorders. A summary of these genes and previous findings from literature can be found in Supplementary Table S5.

**Table 3:** Three canonical pathways that were significantly enriched in the top 53 ODD GWAS genes, using Ingenuity pathway analysis (www.ingenuity.com). The genes encoding proteins that could be directly placed in the ODD landscape are indicated in bold.

| Canonical pathway | Genes | Significance* | Adjusted significance** |
|---|---|---|---|
| Inhibition of Matrix Metalloproteases | *ADAM10, ADAM12, MMP7* | 1.20E-04 | 1.19E-02 |
| Axonal Guidance Signaling | *ABLIM2, ADAM10, ADAM12, MMP7, PAK7, SLIT1* | 6.46E-04 | 2.60E-02 |
| Wnt/$\beta$-catenin Signaling | *MMP7, RARB, SFRP4, SOX5* | 7.86E-04 | 2.60E-02 |

* Single test P value calculated with the right-tailed Fisher's exact test and taking into consideration both the total number of molecules from the analysed dataset and the total number of molecules that is linked to the same gene category according to the Ingenuity Knowledge Base.

** Multiple test-corrected P values using the Benjamini-Hochberg correction ($p<0.05$).

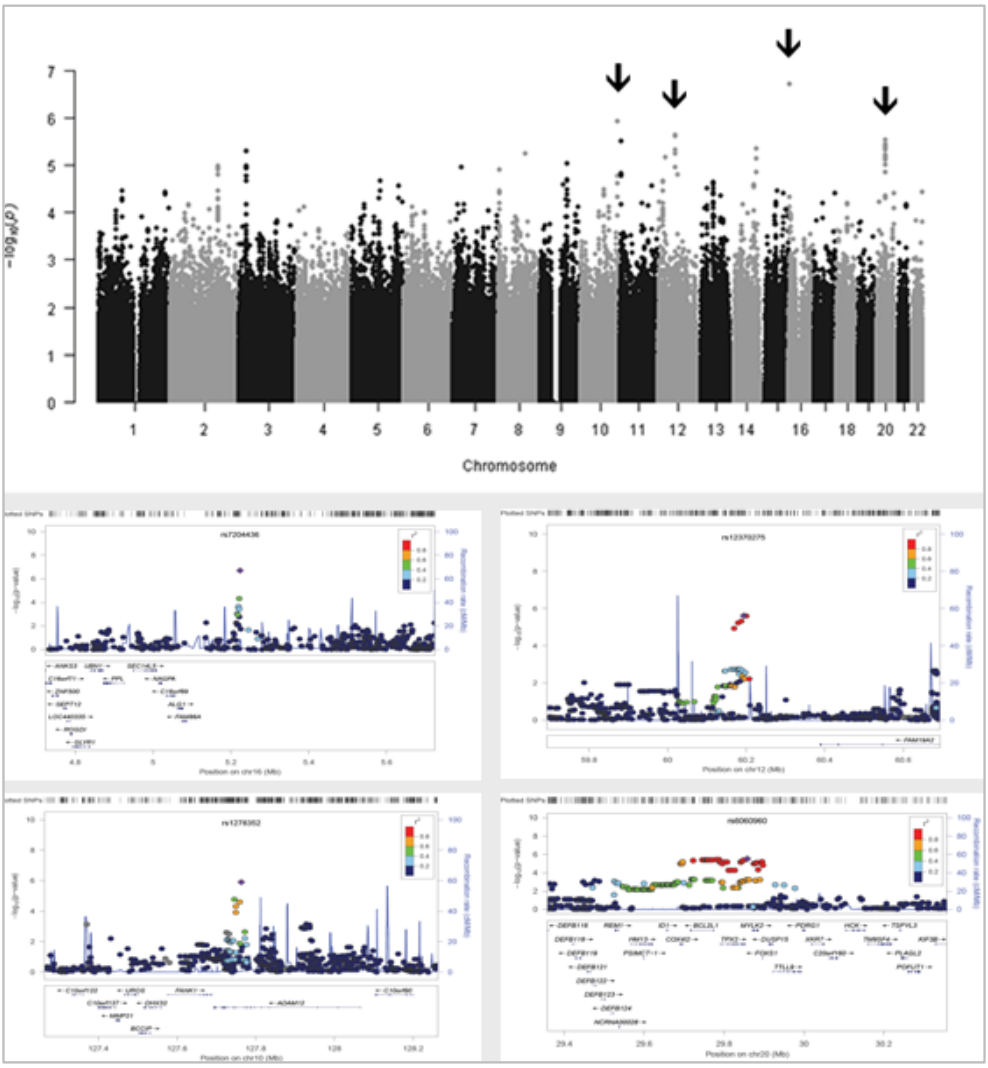
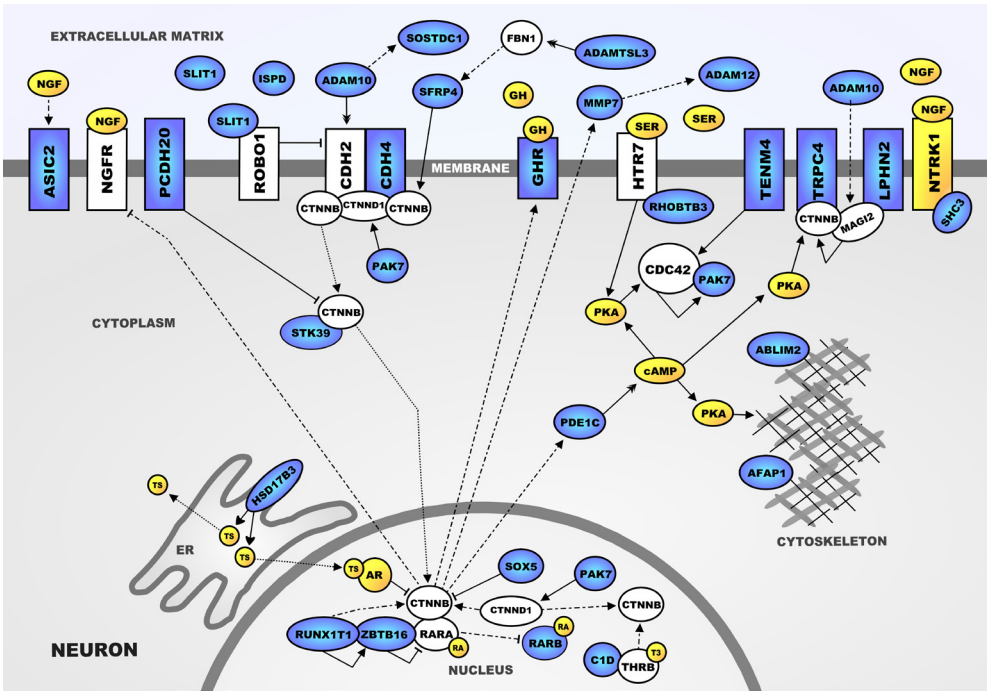



**Figure 2:** Top: Manhattan plot of multivariate GWAS including ODD subtypes P1 (defiant vindictive), P2 (irritable), P3 (0 representing 'low OPP/moderate OPP' and 1 representing 'irritability/severe OPP') and P4 (0 representing 'low OPP/moderate OPP/irritability' and 1 representing 'severe OPP'). Bottom: Top four regions (indicated by arrows in the manhattan plot) containing SNPs showing association at p<1.00E-5 in the multivariate GWAS. Top SNPs for each region are depicted in purple; rs7204436 on chromosome 16 (p=1.98E-07) , rs1278352 on chromosome 10 (p=1.24E-06), rs60193286 on chromosome 12 (p=2.41E-06) and rs6060960 on chromosome 20 (p=3.00E-06). OPP = oppositionality.



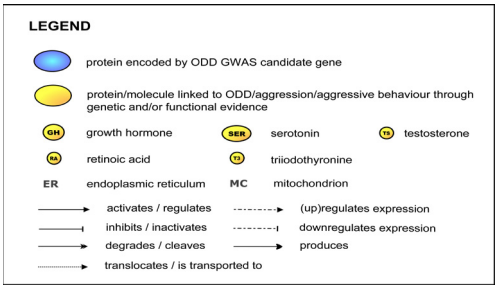




**Figure 3:** Neurite outgrowth- regulating molecular landscape implicated in ODD.The evidence linking the molecules in the landscape to neurite outgrowth can be found in the Supplementary Information.

## DISCUSSION

The aim of this study was to reduce the known heterogeneity in the ODD phenotype in order to improve the power to detect the genetic underpinnings. We first identified four conceptually meaningful subtypes and dimensions of oppositionality in the IMAGE sample. We then tested the VNTRs and genes/gene-sets that have been previously implicated in aggression/disruptive behavior for their effect on the two dimensions and the two categorical subtypes. In addition to these hypothesis-driven analyses, we aimed to generate new hypotheses about genetic involvement in ODD by performing multivariate GWAS. By using bioinformatics analysis and literature mining, we found that top findings obtained from the GWAS fit into a neurite outgrowth- regulating molecular landscape.

Previous research has focused on various dimensions within oppositional defiant behaviors (Aebi et al., 2010; Stringaris and Goodman, 2009b). Further studies have attempted to identify discrete classes of children and adolescents according to their oppositional behavior profiles. Consistent with previous research (Althoff et al., 2014; Kuny et al., 2013), LCA in the present study revealed a low symptom endorsement type, an irritable type, and a severe type with elevated scores on all symptoms. In contrast to these previous findings, we additionally found a moderate oppositional type with intermediate scores on all symptoms, but not a specific defiant/vindictive type. Considering the large sample size and the multi-site data collection for the sample of the present study (Müller et al., 2011a, b) one may conclude that, most probably, children with ADHD more often show the full range of ODD symptoms rather than defiant/vindictive symptoms only. In contrast, irritability symptoms are frequently co-occurring in ADHD children and may represent a specific subtype of ADHD (Fernandez de la Cruz et al., 2015).

Although we tried to reduce the heterogeneity of ODD by identifying conceptually meaningful subtypes and dimensions of oppositionality, we did not observe any significant associations or interactions with previously postulated candidates (SNPs, genes, and pathways). This is not surprising in light of inconsistent reports of *DRD4*, *5-HTT*, and *OXTR* effects on externalizing behaviors (e.g. Beitchman et al., 2012; Kirley et al., 2004; Lavigne et al., 2013; Malik et al., 2012), and the small effect sizes of most genetic risk factors for behavioral measures. A recent meta-analysis did not confirm a relation of *DRD4* exon3 and *5-HTTLPR* to aggression and violence (Vassos et al., 2014). Furthermore, our findings mirror those of a previous study that did not find a *DRD4*/*5-HTTLPR*- interaction with parental support for ODD in 4 year old children (Lavigne et al., 2013). Parenting behavior was moderately to strongly associated with the defined ODD dimensions and subtypes. In line with behavioral theories on negative parent-child interactions (e.g. coercive behaviors; Patterson, 1982), parenting behavior was most strongly associated with defiant/vindictive behaviors. Since parental ability to cope with the child's disruptive behavior was rated by PACS interviewers, and symptoms of oppositionality were rated by parents, confounding of these variables by rater-effects is unlikely.

In order to obtain new insights into genetic risk factors for ODD that can inform future investigations of the neurobiology related to oppositional behavior, we also conducted a multivariate GWAS using the four ODD subtypes. We found 65 markers that showed association with at least one of the four phenotypes at p<1.00E-5. The strongest association with oppositional behavior was found for rs7204436 (p=1.98E-07) located in an intergenic region on chromosome 16. Although no genes are located nearby, a novel microRNA was found 30 kb from the marker which might regulate genes involved in the etiology of oppositional behavior.

Out of 65 markers with p<1.00E-05, 46 were located in a region on chromosome 20q11.21 spanning the genes *COX4I2, BCL2L1, TPX2, MYLK2, FOXS1* and *TTLL9*. It can be hypothesized that of these genes, *BCL2L1* is the most likely candidate causing suggestive

association of the region with oppositional behavior. The long isoform Bcl-S(L) is an anti-apoptotic regulator expressed at high levels in both the developing and the adult brain (Krajewska et al., 2002). Interestingly, it regulates neurotransmitter release and retrieval of vesicles in neurons, thereby influencing presynaptic plasticity (Li et al., 2013). Recently, it has also been shown that *BCL2L1* is associated with volume of the putamen in a GWAS of subcortical volumes in 30,717 individuals from 50 cohorts (Hibar et al., 2015). *BCL2L1* is not present in our top gene list because of filtering during the clumping procedure.

Genomewide studies of aggression phenotypes are starting to emerge. A GWAS of CD had been performed before in the current ADHD sample (Anney et al., 2008), where one of the three phenotypes used was defined as the sum score for 12 CPRS-R:L items, giving perhaps a better representation of ODD than CD. In contrast, we assumed in the present study that combining biologically valid and less heterogeneous subtypes of ODD through a multivariate approach would improve power to define new hypotheses about the genetics of ODD. The top SNPs reported by Anney et al. (2008), who performed family-based Transmission Disequilibrium Tests (TDT), did not reach suggestive significance ($p<1.00E-04$) in our study (Supplementary Table S6). A few other GWAS of aggression- related phenotypes have been reported to date. We compared our association results for the oppositional phenotypes to the top results of four published aggression related genome-wide association studies (Alliey-Rodriguez et al., 2011; Dick et al., 2011; Mick et al., 2014; Tielbeek et al., 2012). None of the SNPs in a 100 kb region surrounding these reported top results reached the threshold for suggestive association in our study ($p<1.00E-4$) (supplementary figure S3). Interestingly though, among our list of top genes is *EPDR1* (ependymin related 1). Ependymin is involved in control of aggressive behavior in fish, where it is a neurotrophic factor that plays a role in neuronal regeneration and adhesion (Sneddon et al., 2011). The mammalian ependymin related protein 1 shows significant sequence similarity to piscine ependymins and has been proposed to be the human homologue of the piscine ependymin (Nimmrich et al., 2001). These findings make *EPDR1* an interesting candidate gene for future investigations of genetic contributions to aggression phenotypes.

As could be expected based on sample size, our multivariate approach did not retrieve any region that yielded genome-wide significant association with ODD. Nevertheless, using the described landscape building approach, we have integrated the top-ranked findings of the GWAS into a molecular landscape involved in regulating neurite outgrowth. More than half of our top-ranked ODD genes were found to interact functionally within this landscape, identifying neurite outgrowth as a biological process that is important for the etiology of ODD. This is in line with neuroimaging studies indicating that aggressive behavior is associated with dysfunctional brain circuitry involved in emotion regulation and decision making (Blair, 2013). Moreover, current models of aggression postulate an impaired structural and functional connectivity between prefrontal areas and subcortical structures such as the amygdala (Rusch et al., 2007; Saxena et al., 2012; Siever, 2008). Indeed, alterations in the efficiency or direction of neurite outgrowth may underlie these dysfunctions.

The identified molecular landscape centers around β-catenin (CTNNB) signaling. CTNNB has a pivotal function in an important signaling cascade leading to neurite outgrowth. The process of neurite outgrowth can be initiated at the neuronal cell membrane, where the binding of ligands from the extracellular matrix to their receptors leads to the modulation of downstream molecular cascades in the cytoplasm, cytoskeleton and nucleus that are involved in regulating neurite outgrowth. Importantly, several proteins and signaling molecules in the landscape (highlighted in yellow in figure 3) –including serotonin, testosterone, triiodothyronine, growth hormone and retinoic acid - have been associated with ODD or aggressive behavior through genetic or functional evidence. Starting with the discovery of a nonsense mutation in the *MAOA* gene leading to a syndrome characterized by violent behaviour (Brunner et al., 1993), the key role of monoamines and especially serotonin in aggression has been demonstrated in a wide variety of human and animal studies (Anholt and Mackay, 2012). Several studies also show a correlation of levels of the male hormone testosterone and aggression (Pavlov et al., 2012) and it has been proposed that an altered testosterone-to-cortisol ratio may be associated with aggression in humans (Haller, 2012; Montoya et al., 2012). Further, thyroid hormones are associated with stress, and elevated levels of the active thyroid hormone triiodothyronine (T3) are associated with conduct disorder and criminal behavior (Ramklint et al., 2001; Stalenheim, 2004). In addition, several animal studies suggest that growth hormone (GH) influences aggressive behavior. For example, *GHRH* knock- out mice with GH deficiency show reduced aggressive behavior which can be normalized by GH replacement (Sagazio et al., 2011). Lastly, chronic administration of synthetic retinoic acid to rats reduced aggression- and increased flight-related behaviors in the resident-intruder paradigm (Trent et al., 2009). The fact that these and other molecules active within our landscape have been associated previously with aggressive behavior provides corroborating evidence for the involvement of neurite outgrowth in aggression etiology.

Of note, alterations in neurite outgrowth are not specific to the etiology of ODD, as neurite outgrowth has also been shown to play a role in the pathogenesis of other neurodevelopmental disorders such as ADHD, Autism spectrum disorders (ASD), dyslexia and schizophrenia (Penzes et al., 2011; Poelmans et al., 2011a; Poelmans et al., 2013; Poelmans et al., 2011b). It has been hypothesized in these studies that each of these disorders may in part be explained by different functional consequences and different primarily affected brain regions of disturbed neurite outgrowth. Psychiatric disorders, including ODD, are currently classified based on clinical presentation rather than underlying etiology. Hence, shared genetic etiology can be expected to exist not only between definable subtypes of psychiatric disorders, but also between different psychiatric disorders as currently classified in clinical practice. This notion is also supported by a recent study (Cross-Disorder Group of the Psychiatric Genomics et al., 2013) that detected substantial genetic correlations between five major psychiatric disorders and by the fact that 15 out of the 53 top ranked genes of our study have previously been associated with neuropsychiatric and neurodevelopmental disorders.

This study is based on a representative clinical sample from eight European countries. Psychometrically reliable and valid measures and methods (e.g. LCA) were used for phenotype definitions and advanced methods were performed in gene-set and genome wide analyses. However, the present study is limited to data obtained from children and adolescents with ADHD combined type (which is often comorbid with ODD) and although our findings may not be generalized to other clinical and community samples, the overlap of our top findings with results in other genetic studies of psychiatric disorders suggests a broader validity. Our results were based on Caucasian subjects only and the sample consisted mostly of male subjects. Due to missing information in the PACS and other instruments, our sample was reduced to 750 probands. However, attrition analyses did not show significant differences between probands included in the sample and drop-outs.

A potential source of bias in our bioinformatics analysis arises from the fact that brain-expressed genes are relatively large. Therefore, brain-expressed genes may be over-represented in our GWAS results. If large genes are more likely found to be associated by chance (because they contain more SNPs), this should be the case in GWASs of both psychiatric disorders and non-psychiatric disorders that do not originate in the brain. However, previous studies have compared enrichment results for psychiatric disorders with results from Crohn's disease and diabetes mellitus (Poelmans et al., 2013; Poelmans et al., 2011b) and showed that the 'neurological disease' category enriched in the psychiatric GWASs showed very little or no enrichment in Crohn's disease or diabetes. Combined with the fact that 53% of our ODD top genes also fitted in the molecular landscape for neurite outgrowth based on extensive literature mining, we argue that although some genes in the landscape may have been chance findings, most candidate genes from the GWAS represent true findings contributing to our phenotype. Future studies conducting pathway analyses using algorithms that address potential confounders such as the large size of brain genes will be of additional information (Holmans et al., 2009; Lee et al., 2012).

In summary, the present findings confirmed the existence of various subgroups of youths with different oppositional symptom profiles. However, against our expectations the examined ODD dimension and subtypes were not associated with previously described candidate genes and pathways. By employing a multivariate genome-wide association approach, we identified several genetic susceptibility loci that may inform future theories on the etiology of oppositional behavior. We also identified a biological landscape of molecular signaling cascades involved in neurite outgrowth providing new insights into the etiology of ODD. In part, our findings may reflect shared genetic risk factors for psychiatric disorders. We hope to encourage further investigations towards a biologically informed classification of psychopathology.

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## SUPPLEMENTARY MATERIAL

**Supplemental description of the molecular ODD landscape and legend (Figure 3)**
The ODD candidate gene/proteins that were implicated by our GWAS (**Supplementary Table S4**) and the other ODD/aggressive behavior candidate genes/proteins/molecules (**Supplementary Table S7**) are indicated in bold.

Signaling through the landscape can be initiated at the neuronal cell membrane, where the binding of ligands from the extracellular matrix to their receptors leads to the modulation of downstream molecular cascades in the cytoplasm, cytoskeleton and nucleus that are involved in regulating neurite outgrowth. Furthermore, modulation of the neuronal extracellular matrix is necessary for the neurite to grow in a certain direction. The main signaling cascade in the landscape centers around β-catenin (CTNNB), a protein that has a dual function as a peripheral membrane/cytoplasmic protein and nuclear transcription factor and regulates neurite outgrowth (Bhardwaj et al., 2013; Votin et al., 2005; Yanagisawa et al., 2010).

CDH2 and **CDH4**, two proteins that mediate cell adhesion (UniProt, 2014) and regulate neurite outgrowth (Hansen et al., 2008) (Burden-Gulley et al., 2010; Oblander & Brady-Kalnay, 2010), are anchored in the neuronal cell membrane through forming a functional complex with each other and the peripheral membrane proteins CTNNB and CTNND1 (Agiostratidou et al., 2009; Hazan & Norton, 1998; Ishiyama et al., 2010; Rubio et al., 2005; Shan et al., 2000; UniProt, 2014). The activity of CTNND1 is regulated by the neurite outgrowth-implicated kinase **PAK7** (Dan et al., 2002; Wong et al., 2010) and both CTNNB and CTNND1 can function as a transcription factor (see below). Moreover, **SLIT1** binding to the ROBO1 axon guidance receptor inactivates CDH2, which leads to CTNNB being released from CDH2 and targeted to the cytoplasm and subsequently the nucleus (Rhee et al., 2007). Through degrading CDH2, the extracellular metalloprotease **ADAM10** also promotes the translocation of CTNNB from the peripheral membrane to the nucleus (Kohutek et al., 2009; Maretzky et al., 2005). In addition, **ADAM10** is involved in regulating the expression of MAGI2 (Prinzen et al., 2009), a scaffolding protein that binds and regulates the activity of CTNNB (J. Xu et al., 2001) and that has been implicated in neurite outgrowth (UniProt, 2014; X. Wu et al., 2000). MAGI2 also binds and functionally modulates the neuronal membrane receptor **LPHN2** (Tobaben et al., 2000) and, through binding and regulating CTNNB, it is functionally linked to **TRPC4**, a calcium channel that directly binds and interacts with CTNNB (Graziani et al., 2010) and stimulates neurite outgrowth (Weick et al., 2009; D. Wu et al., 2008), and the CDH2-**CDH4**-CTNND1 complex (see above; not shown). Furthermore, extracellular **SFRP4** regulates the activity and localization of CTNNB (Berndt et al., 2003) while **PCDH20**, a membrane protein that like CDH2 and **CDH4** mediates (neuronal) cell adhesion (UniProt, 2014), inhibits the cytoplasmic/nuclear translocation and function of CTNNB (Lv et al., 2015) and the **STK39** kinase binds and functionally interacts with CTNNB (Miyamoto-Sato et al., 2010).

After having translocated to the nucleus, CTNNB functions as a transcription factor. The transcriptional activity of CTNNB is inhibited by two other transcription factors, i.e., **SOX5** (Martinez-Morales et al., 2010) and the **androgen receptor (AR)** bound to and activated by **testosterone** (Pawlowski et al., 2002)**,** the male sex hormone that positively regulates neurite outgrowth (Estrada et al., 2006; Marron et al., 2005), and is produced by a number of enzymes in the endoplasmic reticulum, including **HSD17B3** (UniProt, 2014). Further, the expression of CTNNB is upregulated by nuclear and **PAK7**-activated (see above) CTNND1 (Gavard et al., 2004) and **RUNX1T1** (Muller-Tidow et al., 2004), a transcription factor that binds and activates **ZBTB16**, another transcription factor (A. Melnick et al., 2000a; A. M. Melnick et al., 2000b). In turn, **ZBTB16** binds and inhibits the transcriptional activity of retinoic acid receptor alpha (RARA) (Martin et al., 2003), a nuclear receptor that, when bound and activated by **retinoic acid,** forms a functional complex with CTNNB (Easwaran et al., 1999) and downregulates the expression of the **retinoic acid receptor beta (RARB)** (Q. Wu et al., 1997), a transcription factor with an established role in stimulating neurite outgrowth (Agudo et al., 2010; Hoecker et al., 2013; Puttagunta et al., 2011; So et al., 2006). In addition, **C1D** binds and modulates the transcriptional activity of THRB (UniProt, 2014; Zamir et al., 1997), a nuclear receptor that when bound and activated by **triiodothyronine (T3)** - the active thyroid hormone that promotes neurite outgrowth (Walter, 1996) - upregulates the expression of CTNNB (O'Shea et al., 2012; UniProt, 2014).

When activated, CTNNB (up)regulates the expression of the **growth hormone (GH) receptor** (**GHR)** (Renou et al., 2003), **PDE1C** (Morkel et al., 2003) and **MMP7** (Dey et al., 2013) while it downregulates the expression of the **nerve growth factor (NGF)** receptor (NGFR) (Grigoryan et al., 2013). The **GH-GHR** complex is involved in inducing neurite outgrowth (Baudet et al., 2008; Grimbly et al., 2009) while **NGF** regulates this process through binding specifically to NGFR or **NTRK1** (E. J. Huang & Reichardt, 2001; UniProt, 2014), with the **NGF-NTRK1** complex also being bound and functionally modulated by the adaptor protein **SHC3** (Nakamura et al., 1998; UniProt, 2014). In addition, **NGF** promotes neurite outgrowth through upregulating the expression of the sodium channel **ASIC2** (Drummond et al., 2006; Mamet et al., 2002). Furthermore, **PDE1C** is a cytoplasmic enzyme that degrades **cyclic AMP (cAMP)** (UniProt, 2014), a second messenger molecule that regulates many physiological processes through activating **protein kinase A (PKA)** (Jones & Kuhar, 2006), which itself is an important regulator of neurite outgrowth (Aglah et al., 2008; Kao et al., 2002; Shea et al., 1992). **PKA** is activated through **serotonin** binding to the HTR7 receptor (Gervasi et al., 2007; UniProt, 2014), which is directly bound and modulated by **RHOBTB3** (Matthys et al., 2012), and activates/stabilizes CTNNB (Hino et al., 2005) and CDC42 (Chen et al., 2003), an important mediator of directed neurite outgrowth (Brown et al., 2000; Nikolic, 2002) that binds and activates **PAK7** (Dan et al., 2002). CDC42 is also activated downstream of **TENM4**, a membrane protein that promotes neurite outgrowth (Suzuki et al., 2014).

Furthermore, **PKA** is directly involved in remodelling the neuronal cytoskeleton - which is essential for neurite outgrowth to take place - through regulating the stability of actin filaments that together with microtubules form the cytoskeleton (Juliano, 2002), and both **ABLIM2** (Barrientos et al., 2007; Klimov et al., 2005) and **AFAP1** (UniProt, 2014; X. Xu et al., 2009) have a similar effect through directly binding and affecting the stability of actin filaments in the (neuronal) cytoskeleton.

As already indicated above and in addition to cytoskeletal remodelling, the neuronal extracellular matrix has to be modulated for the neurite to grow in a certain direction (Ma et al., 2008). In this respect, the brain-expressed metalloproteinases **ADAM10, ADAM12**, **ADAMTSL3** and **MMP7** - which are also upregulated by CTNNB (see above) - regulate neurite outgrowth through degrading the neuronal extracellular matrix (Malinin et al., 2005; Seetharaman et al., 2011; Szklarczyk et al., 2007; UniProt, 2014; J. Y. Wang et al., 2014). Moreover, **MMP7** is involved in upregulating the expression of **ADAM12** (X. Wang et al., 2009) and **ADAMTSL3** degrades fibrillin-1 (FBN1) (Sengle et al., 2012) which in turn regulates the expression of **SFRP4** (Bayle et al., 2008). Furthermore, in addition to the interactions already described above, **ADAM10** regulates the expression of **SOSTDC1** (Prinzen et al., 2009), a brain-expressed extracellular matrix protein (Park et al., 2009). Lastly, **ISPD** is an extracellular enzyme that, similar to the **SLIT1**-ROBO1 complex (see above), is involved in regulating axon guidance and hence neurite outgrowth (Wright et al., 2012).

**Supplementary Table S1:** Lists of genes included in each of the gene-set analyses.

| Dopamine gene-set | | | | | Serotonin gene-set | | Oxytocin gene-set |
|---|---|---|---|---|---|---|---|
| *ADCY1* | *DRD3* | *PPP1R14A* | *PPP2R2C* | *PRKAR2A* | *DDC* | *HTR6* | *EZH2* |
| *ADCY10* | *DRD4* | *PPP1R14B* | *PPP2R3A* | *PRKAR2B* | *GCH1* | *HTR7* | *GNAQ* |
| *ADCY2* | *DRD5* | *PPP1R14C* | *PPP2R4* | *PTS* | *HTR1A* | *IL4I1* | *GRK5* |
| *ADCY3* | *GCH1* | *PPP1R14D* | *PPP2R5A* | *QDPR* | *HTR1B* | *PCBD1* | *HTT* |
| *ADCY4* | *IL4I1* | *PPP1R1B* | *PPP2R5B* | *SLC18A1* | *HTR1D* | *PTS* | *IGF1* |
| *ADCY5* | *NCS1* | *PPP1R3A* | *PPP2R5C* | *SLC18A2* | *HTR1E* | *QDPR* | *OXT* |
| *ADCY6* | *PCBD1* | *PPP1R3C* | *PPP2R5D* | *SLC18A3* | *HTR2A* | *SLC18A1* | *OXTR* |
| *ADCY7* | *PPM1J* | *PPP1R3D* | *PPP2R5E* | *SLC6A3* | *HTR2B* | *SLC18A2* | *PPARA* |
| *ADCY8* | *PPM1L* | *PPP1R7* | *PRKACA* | *SMOX* | *HTR3A* | *SLC18A3* | |
| *ADCY9* | *PPP1CA* | *PPP2CA* | *PRKACB* | *SPR* | *HTR3B* | *SLC6A4* | |
| *CALY/ DRD1IP* | *PPP1CB* | *PPP2CB* | *PRKACG* | *TH* | *HTR3C* | *SMOX* | |
| *COMT* | *PPP1CC* | *PPP2R1A* | *PRKAG1* | | *HTR3D* | *SPR* | |
| *DDC* | *PPP1R10* | *PPP2R1B* | *PRKAG2* | | *HTR3E* | *TPH1* | |
| *DRD1* | *PPP1R11* | *PPP2R2A* | *PRKAR1A* | | *HTR4* | *TPH2* | |
| *DRD2* | *PPP1R12A* | *PPP2R2B* | *PRKAR1B* | | *HTR5A* | | |

**Supplementary Table S2:** Bivariate correlations of ODD subtypes.

| | P1 | P2 | P3 | P4 | CTRS OPP | SDQ CP | PACS ODD | PACS CD |
|---|---|---|---|---|---|---|---|---|
| P1 defiant vindictive dimension | 1 | 0.68 | 0.62 | 0.73 | 0.21 | 0.63 | 0.42 | 0.31 |
| P2 irritable dimension | | 1 | 0.75 | 0.53 | 0.10 | 0.56 | 0.42 | 0.25 |
| P3 irritable/severe oppositionality | | - | 1 | 0.57 | 0.14 | 0.52 | 0.38 | 0.23 |
| P4 severe oppositionality | | | | 1 | 0.16 | 0.45 | 0.32 | 0.26 |

Note: CTRS OPP = Conners' Teacher Rating Scale Oppositionality, SDQ CP = Strengths and Difficulties Questionnaire Conduct Problems, PACS = Parental Account of Childhood Symptoms, ODD = Oppositional Defiant Disorder, CD = Conduct Disorder, all correlations were significant at a threshold of $p<0.05$.

**Supplementary Table S3:** Top SNPs with p < 1.00E-05 for association in the multivariate GWAS and their performance in univariate analysis. Associated genes are presented for the SNPs that are located within exonic, intronic or untranslated regions of genes and for the SNPs located within 100 kilobases (kb) of downstream and upstream regions flanking a gene. The SNPs that do not fulfill these criteria are designated as 'intergenic'.

| | | | | Multivariate analysis | | | | | | | Univariate analyses | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | P1 | P2 | P3 | P4 | | | P1 | | P2 | | P3 | | P4 | |
| CHR | SNP | BP * | Band | P value | loading | loading | loading | loading | Gene | Position ~ gene | Beta | P | Beta | P | OR | P | OR | P |
| 3 | rs9822411 | 25338947 | p24.2 | 5.29E-06 | -0.04124 | -0.5858 | -0.7042 | 0.1419 | *RARB* | intronic | 0.07 | 8.25E-01 | 0.24 | 1.43E-03 | 1.95 | 1.79E-04 | 0.86 | 4.36E-01 |
| 3 | rs9833653 | 25344352 | p24.2 | 5.29E-06 | -0.04124 | -0.5858 | -0.7042 | 0.1419 | *RARB* | intronic | 0.07 | 8.25E-01 | 0.24 | 1.43E-03 | 1.95 | 1.79E-04 | 0.86 | 4.36E-01 |
| 8 | rs4500123 | 93039329 | q21.3 | 5.93E-06 | 0.5288 | 0.2728 | 0.3982 | 0.9613 | *RUNX1T1* | 3'UTR | 1.12 | 4.23E-03 | 0.13 | 1.41E-01 | 1.56 | 3.16E-02 | 2.82 | 4.39E-07 |
| 8 | rs4734962 | 93039798 | q21.3 | 5.93E-06 | 0.5288 | 0.2728 | 0.3982 | 0.9613 | *RUNX1T1* | 3'UTR | 1.12 | 4.23E-03 | 0.13 | 1.41E-01 | 1.56 | 3.16E-02 | 2.82 | 4.39E-07 |
| 9 | rs10821428 | 92227628 | q22.2 | 9.70E-06 | 0.3505 | 0.7513 | 0.2141 | 0.4764 | *(-)* | intergenic | 0.41 | 6.19E-02 | 0.20 | 6.48E-05 | 1.14 | 2.52E-01 | 1.36 | 1.08E-02 |
| 10 | rs1278352 | 127763366 | q26.2 | 1.24E-06 | -0.3312 | -0.8576 | -0.8092 | -0.1843 | *ADAM12* | intronic | 0.40 | 6.08E-02 | 0.24 | 9.40E-07 | 1.67 | 4.97E-06 | 1.13 | 2.88E-01 |
| 11 | rs4758132 | 6739977 | p15.4 | 3.15E-06 | 0.002295 | 0.3843 | 0.7613 | 0.3524 | *OR2AG2* | 5 kb downstream | 0.00 | 9.90E-01 | -0.18 | 3.38E-02 | 0.44 | 3.33E-05 | 0.65 | 5.24E-02 |
| 11 | rs4758132 | 6739977 | p15.4 | 3.15E-06 | 0.002295 | 0.3843 | 0.7613 | 0.3524 | *OR2AG1* | 23 kb upstream | 0.00 | 9.90E-01 | -0.18 | 3.38E-02 | 0.44 | 3.33E-05 | 0.65 | 5.24E-02 |
| 12 | rs17399946 | 23968776 | p12.1 | 6.95E-06 | 0.2993 | 0.6779 | 0.5185 | 0.7186 | *SOX5* | intronic | -0.49 | 1.05E-01 | -0.25 | 2.58E-04 | 0.65 | 5.16E-03 | 0.49 | 1.48E-04 |
| 12 | rs10784227 | 60178960 | q14.1 | 5.77E-06 | 0.07303 | 0.06676 | 0.6157 | -0.1548 | *(-)* | intergenic | 0.09 | 6.96E-01 | 0.02 | 7.38E-01 | 1.52 | 8.68E-04 | 0.89 | 3.99E-01 |
| 12 | rs1453714 | 60187685 | q14.1 | 4.74E-06 | 0.05256 | 0.06777 | 0.6182 | -0.162 | *(-)* | intergenic | 0.06 | 8.11E-01 | 0.02 | 7.64E-01 | 1.53 | 7.69E-04 | 0.88 | 3.46E-01 |
| 12 | rs12370275 | 60193289 | q14.1 | 2.41E-06 | 0.0263 | 0.03506 | 0.6045 | -0.1592 | *(-)* | intergenic | 0.01 | 9.51E-01 | 0.01 | 9.07E-01 | 1.53 | 7.45E-04 | 0.88 | 3.35E-01 |
| 12 | rs11174055 | 60195641 | q14.1 | 2.41E-06 | 0.0263 | 0.03506 | 0.6045 | -0.1592 | *(-)* | intergenic | 0.01 | 9.51E-01 | 0.01 | 9.07E-01 | 1.53 | 7.45E-04 | 0.88 | 3.35E-01 |
| 12 | rs1453708 | 60200018 | q14.1 | 2.49E-06 | -0.0328 | -0.03922 | -0.6107 | 0.1501 | *(-)* | intergenic | 0.02 | 9.20E-01 | 0.01 | 8.88E-01 | 1.54 | 6.62E-04 | 0.88 | 3.64E-01 |
| 14 | rs11844114 | 93530282 | q32.13 | 4.52E-06 | -0.1947 | -0.3355 | -0.5466 | 0.3344 | *DDX24* | 54 kb downstream | 0.23 | 2.92E-01 | 0.09 | 6.84E-02 | 1.39 | 3.00E-03 | 0.80 | 6.69E-02 |
| 14 | rs11844114 | 93530282 | q32.13 | 4.52E-06 | -0.1947 | -0.3355 | -0.5466 | 0.3344 | *ASB2* | 17 kb upstream | 0.23 | 2.92E-01 | 0.09 | 6.84E-02 | 1.39 | 3.00E-03 | 0.80 | 6.69E-02 |
| 14 | rs4905137 | 93535834 | q32.13 | 7.49E-06 | -0.01004 | -0.1563 | -0.5016 | 0.4062 | *DDX24* | 49 kb downstream | 0.01 | 9.58E-01 | 0.04 | 4.05E-01 | 1.34 | 7.49E-03 | 0.77 | 2.92E-02 |
| 14 | rs4905137 | 93535834 | q32.13 | 7.49E-06 | -0.01004 | -0.1563 | -0.5016 | 0.4062 | *ASB2* | 23 kb upstream | 0.01 | 9.58E-01 | 0.04 | 4.05E-01 | 1.34 | 7.49E-03 | 0.77 | 2.92E-02 |
| 16 | rs7204436 | 5223492 | p13.3 | 1.98E-07 | -0.1103 | 0.2759 | 0.2562 | 0.5517 | *(-)* | intergenic | -0.15 | 5.23E-01 | 0.09 | 8.95E-02 | 1.20 | 1.14E-01 | 1.53 | 8.25E-04 |
| 20 | rs6088857 | 29690425 | q11.21 | 9.88E-06 | 0.7255 | 0.5855 | 0.5625 | 0.9935 | *COX4I2* | intronic | 0.98 | 1.13E-04 | 0.18 | 1.87E-03 | 1.49 | 2.92E-03 | 2.04 | 2.20E-07 |
| 20 | rs6088864 | 29691818 | q11.21 | 9.88E-06 | 0.7255 | 0.5855 | 0.5625 | 0.9935 | *COX4I2* | intronic | 0.98 | 1.13E-04 | 0.18 | 1.87E-03 | 1.49 | 2.92E-03 | 2.04 | 2.20E-07 |
| 20 | rs6060446 | 29695298 | q11.21 | 9.88E-06 | 0.7255 | 0.5855 | 0.5625 | 0.9935 | *COX4I2* | intronic | 0.98 | 1.13E-04 | 0.18 | 1.87E-03 | 1.49 | 2.92E-03 | 2.04 | 2.20E-07 |
| 20 | rs6120970 | 29696113 | q11.21 | 6.42E-06 | 0.7426 | 0.5865 | 0.5787 | 0.995 | *COX4I2* | intronic | 1.02 | 6.03E-05 | 0.18 | 1.56E-03 | 1.52 | 1.91E-03 | 2.07 | 1.42E-07 |
| 20 | rs7272062 | 29722806 | q11.21 | 4.44E-06 | 0.6186 | 0.4683 | 0.4755 | 0.989 | *BCL2L1* | intronic | 0.85 | 6.63E-04 | 0.14 | 1.08E-02 | 1.40 | 9.07E-03 | 2.08 | 8.70E-08 |
| 20 | rs6119651 | 29722907 | q11.21 | 4.44E-06 | 0.6186 | 0.4683 | 0.4755 | 0.989 | *BCL2L1* | intronic | 0.85 | 6.63E-04 | 0.14 | 1.08E-02 | 1.40 | 9.07E-03 | 2.08 | 8.70E-08 |
| 20 | rs6060763 | 29745884 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs1994251 | 29750989 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs1994250 | 29751155 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs6060793 | 29752491 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs6060812 | 29756464 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs6060821 | 29757695 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs6058421 | 29758674 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |

| | | | | Multivariate analysis | | | | | | | Univariate analyses | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | P1 | P2 | P3 | P4 | | | P1 | | P2 | | P3 | | P4 | |
| CHR | SNP | BP * | Band | P value | loading | loading | loading | loading | Gene | Position ~ gene | Beta | P | Beta | P | OR | P | OR | P |
| 20 | rs7354225 | 29760542 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs6060870 | 29766960 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs3181073 | 29770860 | q11.21 | 3.82E-06 | 0.608 | 0.4439 | 0.4689 | 0.9882 | *BCL2L1* | intronic | 0.84 | 7.58E-04 | 0.14 | 1.41E-02 | 1.40 | 9.55E-03 | 2.08 | 7.77E-08 |
| 20 | rs2376996 | 29780328 | q11.21 | 3.63E-06 | 0.612 | 0.4547 | 0.4644 | 0.9876 | *COX4I2* | 84 kb downstream | 0.85 | 7.05E-04 | 0.14 | 1.22E-02 | 1.40 | 1.04E-02 | 2.08 | 8.33E-08 |
| 20 | rs2376996 | 29780328 | q11.21 | 3.63E-06 | 0.612 | 0.4547 | 0.4644 | 0.9876 | *BCL2L1* | 6 kb upstream | 0.85 | 7.05E-04 | 0.14 | 1.22E-02 | 1.40 | 1.04E-02 | 2.08 | 8.33E-08 |
| 20 | rs6089055 | 29787706 | q11.21 | 3.63E-06 | 0.612 | 0.4547 | 0.4644 | 0.9876 | *COX4I2* | 91 kb downstream | 0.85 | 7.05E-04 | 0.14 | 1.22E-02 | 1.40 | 1.04E-02 | 2.08 | 8.33E-08 |
| 20 | rs6089055 | 29787706 | q11.21 | 3.63E-06 | 0.612 | 0.4547 | 0.4644 | 0.9876 | *TPX2* | 3 kb upstream | 0.85 | 7.05E-04 | 0.14 | 1.22E-02 | 1.40 | 1.04E-02 | 2.08 | 8.33E-08 |
| 20 | rs6060912 | 29789416 | q11.21 | 6.61E-06 | 0.6199 | 0.4657 | 0.4838 | 0.9901 | *COX4I2* | 93 kb downstream | 0.84 | 7.95E-04 | 0.14 | 1.22E-02 | 1.40 | 9.02E-03 | 2.05 | 1.39E-07 |
| 20 | rs6060912 | 29789416 | q11.21 | 6.61E-06 | 0.6199 | 0.4657 | 0.4838 | 0.9901 | *TPX2* | 1 kb upstream | 0.84 | 7.95E-04 | 0.14 | 1.22E-02 | 1.40 | 9.02E-03 | 2.05 | 1.39E-07 |
| 20 | rs6089058 | 29789999 | q11.21 | 6.87E-06 | 0.6134 | 0.4555 | 0.4796 | 0.9893 | *COX4I2* | 94 kb downstream | 0.83 | 8.85E-04 | 0.14 | 1.38E-02 | 1.40 | 9.53E-03 | 2.05 | 1.44E-07 |
| 20 | rs6089058 | 29789999 | q11.21 | 6.87E-06 | 0.6134 | 0.4555 | 0.4796 | 0.9893 | *TPX2* | 566 bp upstream | 0.83 | 8.85E-04 | 0.14 | 1.38E-02 | 1.40 | 9.53E-03 | 2.05 | 1.44E-07 |
| 20 | rs6058448 | 29796057 | q11.21 | 9.87E-06 | 0.6208 | 0.4391 | 0.4712 | 0.991 | *TPX2* | intronic | 0.84 | 8.50E-04 | 0.14 | 1.85E-02 | 1.39 | 1.17E-02 | 2.05 | 1.93E-07 |
| 20 | rs6060923 | 29800316 | q11.21 | 6.87E-06 | 0.6134 | 0.4555 | 0.4796 | 0.9893 | *TPX2* | intronic | 0.83 | 8.85E-04 | 0.14 | 1.38E-02 | 1.40 | 9.53E-03 | 2.05 | 1.44E-07 |
| 20 | rs6058450 | 29807572 | q11.21 | 6.87E-06 | 0.6134 | 0.4555 | 0.4796 | 0.9893 | *TPX2* | intronic | 0.83 | 8.85E-04 | 0.14 | 1.38E-02 | 1.40 | 9.53E-03 | 2.05 | 1.44E-07 |
| 20 | rs6089070 | 29841434 | q11.21 | 6.87E-06 | 0.6134 | 0.4555 | 0.4796 | 0.9893 | *TPX2* | intronic | 0.83 | 8.85E-04 | 0.14 | 1.38E-02 | 1.40 | 9.53E-03 | 2.05 | 1.44E-07 |
| 20 | rs6089071 | 29842045 | q11.21 | 6.87E-06 | 0.6134 | 0.4555 | 0.4796 | 0.9893 | *TPX2* | intronic | 0.83 | 8.85E-04 | 0.14 | 1.38E-02 | 1.40 | 9.53E-03 | 2.05 | 1.44E-07 |
| 20 | rs3203770 | 29845976 | q11.21 | 4.86E-06 | 0.6053 | 0.4463 | 0.4686 | 0.9871 | *TPX2* | synonymous coding | 0.83 | 8.82E-04 | 0.14 | 1.49E-02 | 1.39 | 1.06E-02 | 2.06 | 1.21E-07 |
| 20 | rs6058463 | 29848853 | q11.21 | 4.08E-06 | 0.6039 | 0.4477 | 0.474 | 0.9871 | *TPX2* | intronic | 0.83 | 8.47E-04 | 0.14 | 1.42E-02 | 1.40 | 9.73E-03 | 2.07 | 1.03E-07 |
| 20 | rs6119725 | 29849218 | q11.21 | 4.08E-06 | 0.6039 | 0.4477 | 0.474 | 0.9871 | *TPX2* | intronic | 0.83 | 8.47E-04 | 0.14 | 1.42E-02 | 1.40 | 9.73E-03 | 2.07 | 1.03E-07 |
| 20 | rs6060960 | 29858178 | q11.21 | 3.00E-06 | 0.6045 | 0.4489 | 0.4816 | 0.9877 | *TPX2* | 5 kb downstream | 0.84 | 7.68E-04 | 0.14 | 1.29E-02 | 1.41 | 8.07E-03 | 2.08 | 8.08E-08 |
| 20 | rs6060960 | 29858178 | q11.21 | 3.00E-06 | 0.6045 | 0.4489 | 0.4816 | 0.9877 | *MYLK2* | 13 kb upstream | 0.84 | 7.68E-04 | 0.14 | 1.29E-02 | 1.41 | 8.07E-03 | 2.08 | 8.08E-08 |
| 20 | rs6121242 | 29874538 | q11.21 | 9.79E-06 | 0.626 | 0.4589 | 0.4747 | 0.9905 | *MYLK2* | intronic | 0.84 | 8.41E-04 | 0.14 | 1.51E-02 | 1.38 | 1.25E-02 | 2.03 | 2.40E-07 |
| 20 | rs6119729 | 29876824 | q11.21 | 9.79E-06 | 0.626 | 0.4589 | 0.4747 | 0.9905 | *MYLK2* | intronic | 0.84 | 8.41E-04 | 0.14 | 1.51E-02 | 1.38 | 1.25E-02 | 2.03 | 2.40E-07 |
| 20 | rs6060979 | 29881183 | q11.21 | 8.07E-06 | 0.624 | 0.4533 | 0.4737 | 0.9904 | *MYLK2* | intronic | 0.85 | 7.98E-04 | 0.14 | 1.58E-02 | 1.39 | 1.20E-02 | 2.05 | 1.90E-07 |
| 20 | rs4518038 | 29882712 | q11.21 | 8.07E-06 | 0.624 | 0.4533 | 0.4737 | 0.9904 | *MYLK2* | intronic | 0.85 | 7.98E-04 | 0.14 | 1.58E-02 | 1.39 | 1.20E-02 | 2.05 | 1.90E-07 |
| 20 | rs17093657 | 29892555 | q11.21 | 8.07E-06 | 0.624 | 0.4533 | 0.4737 | 0.9904 | *FOXS1* | 3 kb downstream | 0.85 | 7.98E-04 | 0.14 | 1.58E-02 | 1.39 | 1.20E-02 | 2.05 | 1.90E-07 |
| 20 | rs17093657 | 29892555 | q11.21 | 8.07E-06 | 0.624 | 0.4533 | 0.4737 | 0.9904 | *TTLL9* | 30 kb upstream | 0.85 | 7.98E-04 | 0.14 | 1.58E-02 | 1.39 | 1.20E-02 | 2.05 | 1.90E-07 |
| 20 | rs6060989 | 29893424 | q11.21 | 8.07E-06 | 0.624 | 0.4533 | 0.4737 | 0.9904 | *FOXS1* | 2 kb downstream | 0.85 | 7.98E-04 | 0.14 | 1.58E-02 | 1.39 | 1.20E-02 | 2.05 | 1.90E-07 |
| 20 | rs6060989 | 29893424 | q11.21 | 8.07E-06 | 0.624 | 0.4533 | 0.4737 | 0.9904 | *TTLL9* | 29 kb upstream | 0.85 | 7.98E-04 | 0.14 | 1.58E-02 | 1.39 | 1.20E-02 | 2.05 | 1.90E-07 |
| 20 | rs6060992 | 29894916 | q11.21 | 6.26E-06 | 0.6228 | 0.4548 | 0.4758 | 0.9902 | *FOXS1* | 848 bp downstream | 0.85 | 7.26E-04 | 0.14 | 1.45E-02 | 1.39 | 1.08E-02 | 2.06 | 1.44E-07 |
| 20 | rs6060992 | 29894916 | q11.21 | 6.26E-06 | 0.6228 | 0.4548 | 0.4758 | 0.9902 | *TTLL9* | 27 kb upstream | 0.85 | 7.26E-04 | 0.14 | 1.45E-02 | 1.39 | 1.08E-02 | 2.06 | 1.44E-07 |
| 20 | rs6089094 | 29895432 | q11.21 | 6.26E-06 | 0.6228 | 0.4548 | 0.4758 | 0.9902 | *FOXS1* | 332 bp downstream | 0.85 | 7.26E-04 | 0.14 | 1.45E-02 | 1.39 | 1.08E-02 | 2.06 | 1.44E-07 |
| 20 | rs6089094 | 29895432 | q11.21 | 6.26E-06 | 0.6228 | 0.4548 | 0.4758 | 0.9902 | *TTLL9* | 27 kb upstream | 0.85 | 7.26E-04 | 0.14 | 1.45E-02 | 1.39 | 1.08E-02 | 2.06 | 1.44E-07 |

Note: P1= defiant vindictive dimension, P2= irritable dimension, P3= irritable/severe oppositionality, P4= severe oppositionality. * Positions according to HAPMAP 2 Release 22.

**Supplementary Table S4:** Index SNPs showing association with ODD at p < 1.00E-04 after clumping of multivariate association results. These SNPs include those that are located within exonic, intronic or untranslated regions of genes or that are located within 100 kilobases (kb) of downstream and upstream regions flanking a gene. The genes in bold have already been implicated in the etiology of neurodevelopmental or neuropsychiatric disorders.

| CHR | SNP | BP * | Band | P value | N clumped** | Gene | Position ~ gene | Gene name |
|---|---|---|---|---|---|---|---|---|
| 1 | rs11806028 | 81768458 | p31.1 | 3.57E-05 | 17 | LPHN2 | intronic | latrophilin 2 |
| 1 | rs10910623 | 231005322 | q42.2 | 3.88E-05 | 38 | MAP10 | 2 kb upstream | microtubule-associated protein 10 |
| 1 | rs7542425 | 245616954 | q44 | 8.49E-05 | 5 | **NLRP3** | 29 kb upstream | NLR family, pyrin domain containing 3 |
| 1 | rs7542425 | 245616954 | q44 | 8.49E-05 | 5 | OR2B11 | 64 kb downstream | olfactory receptor, family 2, subfamily B, member 11 |
| 2 | rs17034837 | 68089376 | p14 | 7.00E-05 | 1 | C1D | 32 kb downstream | C1D nuclear receptor corepressor |
| 2 | rs6712239 | 168557693 | q24.3 | 1.07E-05 | 65 | **STK39** | intronic | serine threonine kinase 39 |
| 2 | rs6710235 | 168622325 | q24.3 | 9.66E-05 | 2 | **STK39** | intronic | serine threonine kinase 39 |
| 3 | rs9822411 | 25338947 | p24.2 | 5.29E-06 | 41 | **RARB** | intronic | retinoic acid receptor, beta |
| 4 | rs11732351 | 8006388 | p16.1 | 9.08E-05 | 41 | ABLIM2 | 12 kb downstream | actin binding LIM protein family, member 2 |
| 4 | rs11732351 | 8006388 | p16.1 | 9.08E-05 | 41 | AFAP1 | 14 kb upstream | actin filament associated protein 1 |
| 4 | rs11724348 | 25593163 | p15.2 | 7.72E-05 | 1 | SMIM20 | 53 kb downstream | small integral membrane protein 20 |
| 5 | rs13188386 | 42509312 | p13.1 | 7.06E-05 | 200 | GHR | intronic | growth hormone receptor |
| 5 | rs3797207 | 95195665 | q15 | 5.12E-05 | 18 | **GLRX** | 11 kb upstream | glutaredoxin (thioltransferase) |
| 5 | rs3797207 | 95195665 | q15 | 5.12E-05 | 18 | **RHOBTB3** | 10 kb downstream | Rho-related BTB domain containing 3 |
| 5 | rs2973725 | 177736638 | q35.3 | 6.14E-05 | 23 | COL23A1 | intronic | collagen, type XXIII, alpha 1 |
| 6 | rs11967472 | 31379264 | p21.33 | 7.55E-05 | 110 | **HLA-C** | intronic | major histocompatibility complex, class I, C |
| 6 | rs7739455 | 74477349 | q13 | 9.83E-05 | 82 | CD109 | intronic | CD109 molecule |
| 7 | rs7812175 | 16447568 | p21.2 | 6.81E-05 | 52 | ISPD | 20 kb upstream | isoprenoid synthase domain containing |
| 7 | rs7812175 | 16447568 | p21.1 | 6.81E-05 | 52 | SOSTDC1 | 20 kb downstream | sclerostin domain containing 1 |
| 7 | rs7811079 | 31909609 | p14.3 | 1.13E-05 | 37 | PDE1C | intronic | phosphodiesterase 1C, calmodulin-dependent 70kDa |
| 7 | rs1721393 | 38043347 | p14.1 | 6.95E-05 | 68 | EPDR1 | 85 kb downstream | ependymin related 1 |
| 7 | rs1721393 | 38043347 | p14.1 | 6.95E-05 | 68 | SFRP4 | 12 kb upstream | secreted frizzled-related protein 4 |
| 8 | rs2013938 | 6463312 | p23.1 | 1.31E-05 | 29 | **MCPH1** | intronic | microcephalin 1 |
| 8 | rs4500123 | 93039329 | q21.3 | 5.93E-06 | 3 | RUNX1T1 | 3'UTR | runt-related transcription factor 1; translocated to, 1 (cyclin D-related) |
| 9 | rs4075163 | 90788923 | q22.1 | 5.48E-05 | 14 | C9orf47 | 7 kb upstream | chromosome 9 open reading frame 47 |
| 9 | rs4075163 | 90788923 | q22.1 | 5.48E-05 | 14 | **SHC3** | 29 kb downstream | SHC (Src homology 2 domain containing) transforming protein 3 |
| 9 | rs2479828 | 98090615 | q22.32 | 5.59E-05 | 0 | HSD17B3 | intronic | hydroxysteroid (17-beta) dehydrogenase 3 |
| 9 | rs13291423 | 132860593 | q34.12 | 7.55E-05 | 2 | **LAMC3** | 14 kb upstream | laminin, gamma 3 |
| 10 | rs4919079 | 98927898 | q24.1 | 7.93E-05 | 15 | SLIT1 | intronic | slit homolog 1 (Drosophila) |

| CHR | SNP | BP * | Band | P value | N clumped** | Gene | Position ~ gene | Gene name |
|---|---|---|---|---|---|---|---|---|
| 10 | rs1278352 | 127763366 | q26.2 | 1.24E-06 | 42 | **ADAM12** | intronic | ADAM metallopeptidase domain 12 |
| 11 | rs4758132 | 6739977 | p15.4 | 3.15E-06 | 23 | OR2AG1 | 23 kb upstream | olfactory receptor, family 2, subfamily AG, member 1 |
| 11 | rs4758132 | 6739977 | p15.4 | 3.15E-06 | 23 | OR2AG2 | 6 kb downstream | olfactory receptor, family 2, subfamily AG, member 2 |
| 11 | rs4945343 | 78732638 | q14.1 | 6.67E-05 | 6 | TENM4 | intronic | teneurin transmembrane protein 4 |
| 11 | rs10895301 | 101869559 | q22.2 | 7.29E-05 | 30 | MMP7 | 27 kb downstream | matrix metallopeptidase 7 (matrilysin, uterine) |
| 11 | rs10895301 | 101869559 | q22.2 | 7.29E-05 | 30 | TMEM123 | 41 kb upstream | transmembrane protein 123 |
| 11 | rs17116334 | 113466972 | q23.2 | 2.77E-05 | 8 | ZBTB16 | intronic | zinc finger and BTB domain containing 16 |
| 12 | rs2159381 | 3222193 | p13.33 | 6.06E-05 | 5 | TSPAN9 | intronic | tetraspanin 9 |
| 12 | rs17399946 | 23968776 | p12.1 | 6.95E-06 | 14 | **SOX5** | intronic | SRY (sex determining region Y)-box 5 |
| 13 | rs1005720 | 28981187 | q12.3 | 8.30E-05 | 46 | SLC7A1 | 360 bp downstream | solute carrier family 7 (cationic amino acid transporter, y+ system), member 1 |
| 13 | rs10507466 | 37361657 | q13.3 | 8.68E-05 | 82 | TRPC4 | 20 kb upstream | transient receptor potential cation channel, subfamily C, member 4 |
| 13 | rs1330598 | 60831132 | q21.2 | 2.39E-05 | 21 | PCDH20 | 51 kb downstream | protocadherin 20 |
| 14 | rs11844114 | 93530282 | q32.12 | 4.52E-06 | 11 | ASB2 | 17 kb upstream | ankyrin repeat and SOCS box containing 2 |
| 14 | rs11844114 | 93530282 | q32.12 | 4.52E-06 | 11 | DDX24 | 54 kb downstream | DEAD (Asp-Glu-Ala-Asp) box helicase 24 |
| 15 | rs4275799 | 56693437 | q21.3 | 6.18E-05 | 132 | **ADAM10** | intronic | ADAM metallopeptidase domain 10 |
| 15 | rs7164017 | 60675326 | q22.2 | 3.57E-05 | 2 | TLN2 | intronic | talin 2 |
| 15 | rs950168 | 82497201 | q25.2 | 4.07E-05 | 10 | **ADAMTSL3** | intronic | ADAMTS-like 3 |
| 16 | rs1862861 | 4195458 | p13.3 | 7.88E-05 | 6 | SRL | intronic | sarcalumenin |
| 17 | rs17837003 | 29264945 | q12 | 6.51E-05 | 13 | **ASIC2** | intronic | acid-sensing (proton-gated) ion channel 2 |
| 17 | rs590040 | 74030083 | q25.3 | 4.02E-05 | 10 | DNAH17 | intronic | dynein, axonemal, heavy chain 17 |
| 20 | rs6108320 | 9581004 | p12.2 | 6.24E-05 | 34 | **PAK7** | intronic | p21 protein (Cdc42/Rac)-activated kinase 7 |
| 20 | rs6060960 | 29858178 | q11.21 | 3.00E-06 | 150 | MYLK2 | 13 kb upstream | myosin light chain kinase 2 |
| 20 | rs6060960 | 29858178 | q11.21 | 3.00E-06 | 150 | TPX2 | 5 kb downstream | TPX2, microtubule-associated |
| 20 | rs6061345 | 59762911 | q13.33 | 4.04E-05 | 16 | CDH4 | intronic | cadherin 4, type 1, R-cadherin |
| 21 | rs2839417 | 42192806 | q22.3 | 7.00E-05 | 12 | C2CD2 | intronic | C2 calcium-dependent domain containing 2 |

Note: * Positions according to HAPMAP 2 Release 22. ** Total number of SNPs in clump passing LD ($r^2$=.2) and physical distance (10,000 kb) thresholds for clumping.

**Supplementary Table S5:** Top-ranked genes previously implicated in the etiology of neurodevelopmental or neuropsychiatric disorders.

| Gene | Locus | Associated with neurodevelopmental or neuropsychiatric disorders |
|---|---|---|
| *ADAM10* | 15q21.3 | *ADAM10* mutations were found in 11 out of 16 affected individuals from families with late-onset Alzheimer's disease (AD) (Kim et al., 2009). |
| *ADAM12* | 10q26.2 | *ADAM12* resides in a genetic locus that has been linked to schizophrenia (SZ) and reduced numerical density of ADAM12-expressing oligodendrocytes was found in the white matter of SZ patients (Farkas et al., 2010). Significant interaction was found between rs3740473 (in *SH3PXD2A*) and rs11244787 (in *ADAM12*) with respect to the risk of developing AD (Harold et al., 2007), but this interaction was not replicated in a subsequent study (Laumet et al., 2010). ADAM12 levels in urine of bipolar disorder (BPD) patients are elevated compared to healthy controls (Nadri et al., 2007). |
| *ADAMTSL3* | 15q25.2 | The *ADAMTSL3*-located SNPs rs2135551, rs950169 and rs1911155 yielded P-values just below genome-wide significance in a GWAS of SZ (Need et al., 2009), with rs950169 as the most likely causative variant for the GWAS association (Dow et al., 2011). |
| *ASIC2* | 17q12 | SNPs in *ASIC2* showed association with panic disorder in a Faroese case-control sample, but not in an independent Danish sample (Gregersen et al., 2014). |
| *GLRX* | 5q15 | The Glrx protein expression pattern in hippocampus tissue was shown to be altered in AD patients compared to controls (Arodin et al., 2014). |
| *HLA-C* | 6p21.33 | Various strong association signals for SNPs spanning the Major Histocompatibility Complex (MHC) region have been found in SZ GWASs and HLA-C*01:02 has been specifically associated with SZ (Bergen et al., 2012; Genomics, 2013; Irish Schizophrenia Genomics & the Wellcome Trust Case Control, 2012; Stefansson et al., 2009). |
| *LAMC3* | 9q34.12 | Exome sequencing studies of parent-child trios exhibiting sporadic autism spectrum disorders (ASDs) identified de novo mutations in *LAMC3*, implying involvement of this gene in ASD etiology (O'Roak et al., 2011; O'Roak et al., 2012). |
| *MCPH1* | 8p23.1 | *MCPH1* has been implicated in Autosomal recessive primary microcephaly (MCPH) with mental retardation (Garshasbi et al., 2006; Hosseini et al., 2012; Perche et al., 2013; Woods et al., 2005). |
| *NLRP3* | 1q44 | Activation of the NLRP3-dependent inflammasome was demonstrated in blood mononuclear cells from depressive patients (Alcocer-Gomez et al., 2014). NLRP3 polymorphisms have been associated with late-onset AD in a candidate gene association study (Tan et al., 2013) |
| *PAK7* | 20p12.2 | A duplication overlapping *PAK7* showed association with psychosis (Morris et al., 2014). |
| *RARB* | 3p24.2 | The levels of RARB were found to be lower in patients with major depression than in healthy controls (T.-L. Huang et al., 2014). |
| *RHOBTB3* | 5q15 | A study using gene expression profiling to identify blood biomarkers for psychotic symptoms found decreased expression of *RHOBTB3* in patients with SZ or related disorders with high hallucinations states (Kurian et al., 2011). |
| *SHC3* | 9q22.1 | SNP-based and haplotype analyses show associations of *SHC3* variants with nicotine dependence (Li et al., 2007). |
| *SOX5* | 12p12.1 | Copy number variations in *SOX5* have been found in patients with ASDs (Rosenfeld et al., 2010). *SOX5* haploinsufficiency was also found in several patients presenting with with speech delay, intellectual disability and behavior abnormalities (Lamb et al., 2012; Schanze et al., 2013). |
| *STK39* | 2q24.3 | Analysis of candidate autism loci on chromosome 2q24-q33 showed that different haplotypes of SNPs encompassing *STK39* are significantly associated with autism (Ramoz et al., 2008). The STK39 locus shows genome-wide significant association with Parkinson's disease (PD) risk in large-scale meta-analyses of PD GWAS and risk gene studies (International Parkinson Disease Genomics et al., 2011; Lill et al., 2012). |

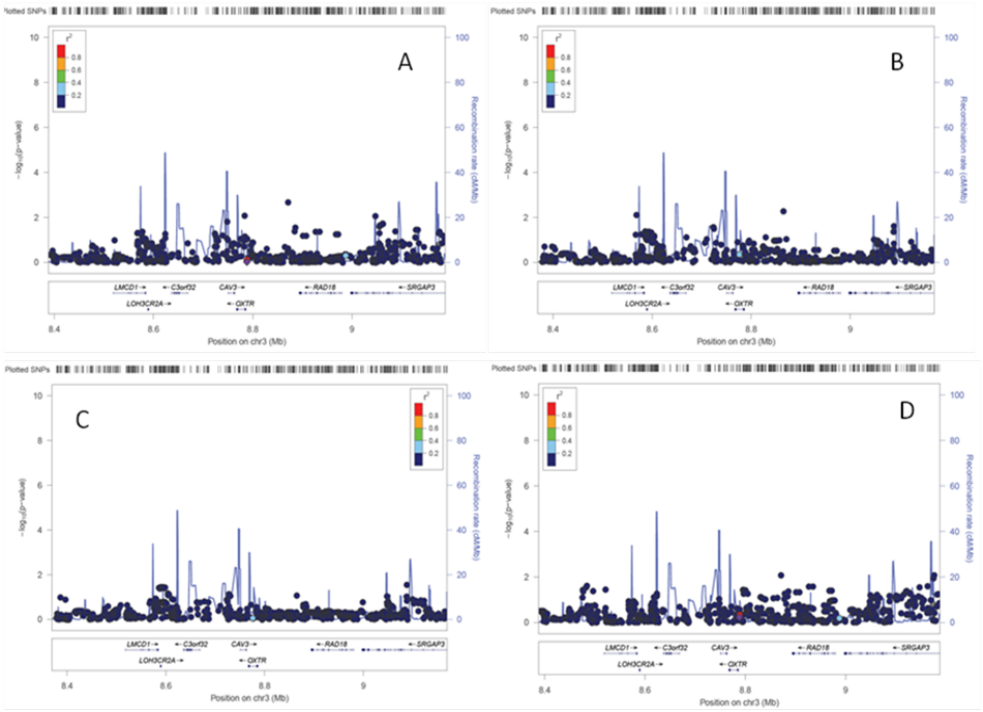
**Supplementary Table S6:** SNPs showing association signal P<1.00E-5 in the GWAS by Anney et al. (Anney et al., 2008) and their performance in our multivariate GWAS.

| CHR | SNP | BP | P value |
|---|---|---|---|
| 9 | rs10815798 | 8225633 | 1.51E-02 |
| 3 | rs13061352 | 22203581 | 1.69E-02 |
| 1 | rs2064648 | 30410603 | 2.43E-02 |
| 1 | rs2180233 | 30400299 | 2.89E-02 |
| 1 | rs4949546 | 30406270 | 2.89E-02 |
| 1 | rs1543424 | 30392051 | 3.31E-02 |
| 7 | rs10229603 | 112415609 | 5.07E-02 |
| 1 | rs6661210 | 155399012 | 5.73E-02 |
| 1 | rs10796972 | 155399761 | 5.73E-02 |
| 1 | rs6700498 | 155399774 | 5.73E-02 |
| 1 | rs1176542 | 155400100 | 5.73E-02 |
| 1 | rs1176543 | 155400219 | 5.73E-02 |
| 1 | rs1176551 | 155402280 | 5.73E-02 |
| 1 | rs1176555 | 155403165 | 5.73E-02 |
| 11 | rs1557488 | 126124400 | 5.87E-02 |
| 11 | rs1557487 | 126124791 | 5.87E-02 |
| 11 | rs10831284 | 94307612 | 6.04E-02 |
| 1 | rs11264625 | 155395329 | 6.14E-02 |
| 1 | rs6427356 | 155397190 | 6.14E-02 |
| 16 | rs4889240 | 79714023 | 8.80E-02 |
| 11 | rs10736554 | 126120771 | 9.29E-02 |
| 8 | rs4734494 | 101986897 | 9.56E-02 |
| 8 | rs4734495 | 101986993 | 9.62E-02 |
| 8 | rs931812 | 101988497 | 9.62E-02 |
| 1 | rs701157 | 228741449 | 1.48E-01 |
| 12 | rs789560 | 68618094 | 1.64E-01 |
| 14 | rs1951082 | 26329883 | 1.92E-01 |
| 14 | rs8021717 | 26333357 | 1.98E-01 |
| 10 | rs2764978 | 3273385 | 2.11E-01 |
| 10 | rs2764980 | 3274007 | 2.11E-01 |
| 10 | rs2814925 | 3274061 | 2.11E-01 |
| 2 | rs1521883 | 202657917 | 2.40E-01 |
| 1 | rs10797919 | 182119537 | 2.44E-01 |
| 1 | rs4079923 | 182113907 | 2.53E-01 |
| 15 | rs4533251 | 95063431 | 2.81E-01 |
| 16 | rs16973500 | 70522697 | 2.85E-01 |
| 12 | rs7297018 | 78586361 | 3.30E-01 |
| 2 | rs939745 | 202649555 | 3.66E-01 |
| 2 | rs1521882 | 202658096 | 3.67E-01 |
| 2 | rs1521879 | 202661472 | 3.72E-01 |
| 4 | rs6536350 | 159660267 | 3.79E-01 |
| 13 | rs10492664 | 107614226 | 4.32E-01 |
| 13 | rs8002852 | 107616886 | 4.70E-01 |

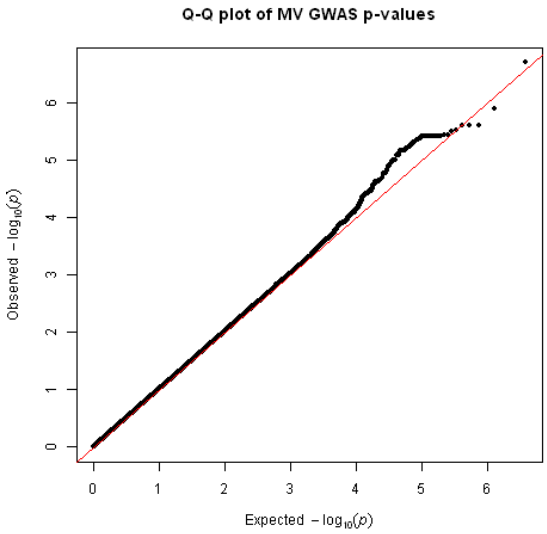
| CHR | SNP | BP | P value |
|---|---|---|---|
| 18 | rs7236632 | 53585200 | 5.07E-01 |
| 2 | rs6733379 | 34333579 | 5.51E-01 |
| 2 | rs7595103 | 77928752 | 6.93E-01 |
| 13 | rs9512900 | 27327738 | 7.16E-01 |
| 5 | rs1644305 | 133231495 | 7.40E-01 |
| 5 | rs1644308 | 133227448 | 7.55E-01 |
| 21 | rs2826340 | 20807173 | 8.38E-01 |
| 16 | rs1381102 | 62512948 | 8.54E-01 |
| 2 | rs1487044 | 77918912 | 9.54E-01 |
| 2 | rs1487045 | 77918966 | 9.54E-01 |
| 16 | rs12921846 | - | - |

**Supplementary Table S7:** Genes/proteins/molecules from the molecular landscape linked to aggressive behavior through genetic and/or functional evidence.

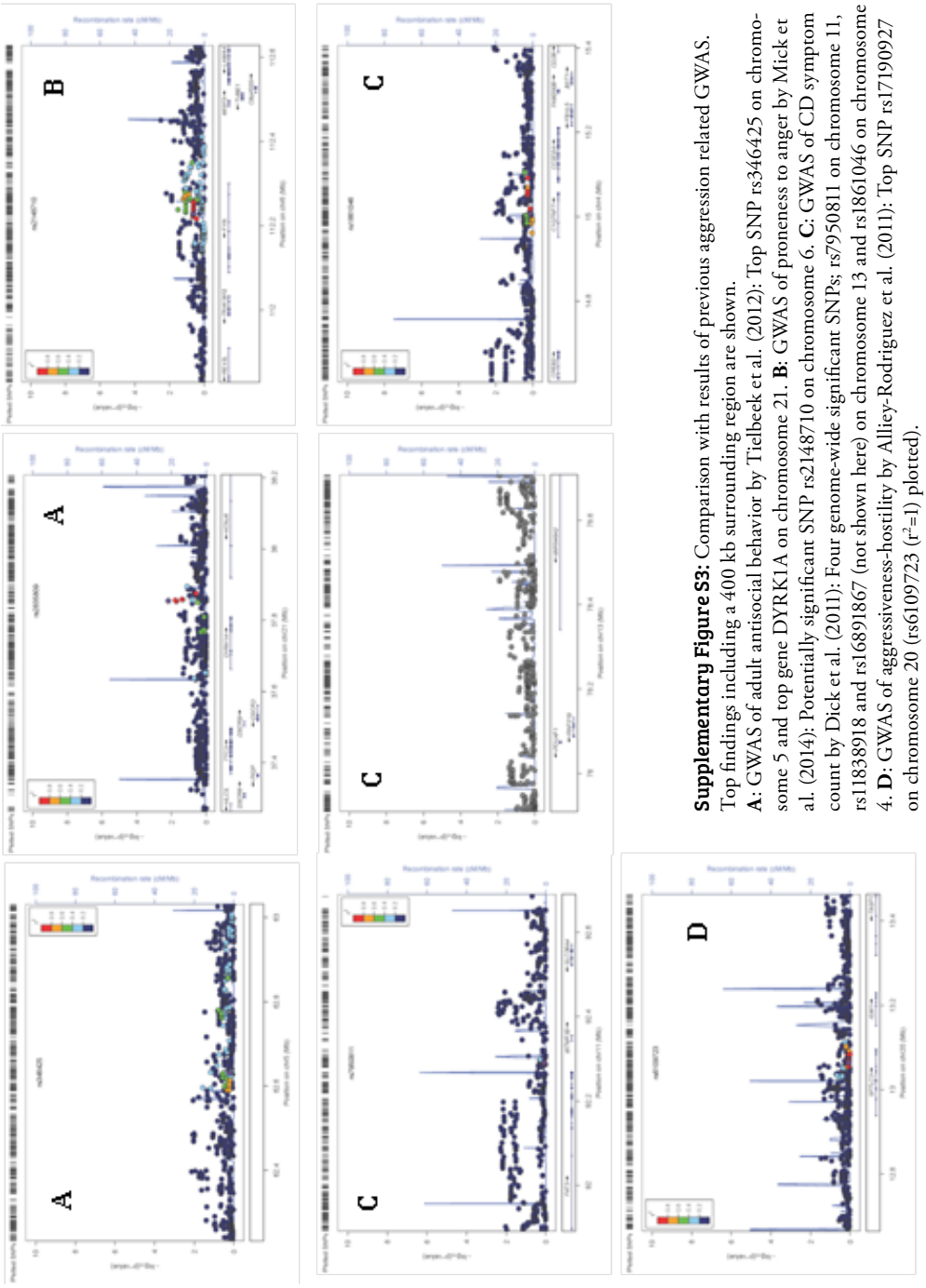
| Genes/proteins/molecules | Link to aggressive behavior |
|---|---|
| Serotonin | The role of serotonin in aggression has been demonstrated in a wide variety of human and animal studies (Anholt & Mackay, 2012). |
| Testosterone | Levels of the male hormone testosterone were shown to be correlated with aggression (Pavlov et al., 2012). Altered testosterone-to-cortisol ratio may be associated with aggression in humans (Haller, 2012; Montoya et al., 2012). |
| Triiodothyronine | Elevated levels of the active thyroid hormone triiodothyronine have been associated with conduct disorder and criminal behavior (Ramklint et al., 2001; Stalenheim, 2004). |
| Growth hormone | *GHRH* knock- out mice with growth hormone (GH) deficiency show reduced aggressive behavior which can be normalized by GH replacement (Sagazio et al., 2011). |
| Retinoic acid | Chronic administration of synthetic retinoid acid to rats reduced aggression in the resident-intruder paradigm (Trent et al., 2009). |
| Nerve growth factor | NGF is thought to be involved in aggression and alcohol dependence and changes in levels of nerve growth factor have been observed in rodents following aggressive intermale interactions (Pardon, 2010). |
| *NTRK1* | Fighting in male mice potentiates mRNA for the high affinitiy nerve growth factor receptor TrkA (encoded by the *NTRK1* gene in humans) in the subventricular zone and hippocampus (Fiore et al., 2005). |
| Protein Kinase A | Coronin 1 deficiency in mouse and human causes severe neurobehavioral defects, including increased aggression and social deficits, through modulation of cAMP/Protein Kinase A Signaling (Jayachandran et al., 2014). |
| cAMP | Coronin 1 deficiency in mouse and human causes severe neurobehavioral defects, including increased aggression and social deficits, through modulation of cAMP/Protein Kinase A Signaling (Jayachandran et al., 2014). |
| *AR* | The androgen receptor (*AR*) CAG repeat motif has been associated with aggressive and violent behavior in men (Craig & Halton, 2009; Hurd et al., 2011). |




**Supplementary Figure S1:** Outcome of the association analysis of the four ODD subtypes for all SNPs located in the OXTR region.
**A.** P1 (defiant vindictive), **B.** P2 (irritability), **C.** P3 (0 representing 'low OPP/moderate OPP' and 1 representing 'irritable OPP/severe OPP') and **D.** P4 (0 representing 'low OPP/moderate OPP/irritable OPP' and 1 representing 'severe OPP'). SNP rs1488467 and linked SNPs are depicted in color.




**Supplementary Figure S2:** Quantile quantile plot for the multivariate GWAS.




**Supplementary Figure S3:** Comparison with results of previous aggression related GWAS. Top findings including a 400 kb surrounding region are shown. **A**: GWAS of adult antisocial behavior by Tielbeek et al. (2012): Top SNP rs346425 on chromosome 5 and top gene DYRK1A on chromosome 21. **B**: GWAS of proneness to anger by Mick et al. (2014): Potentially significant SNP rs2148710 on chromosome 6. **C**: GWAS of CD symptom count by Dick et al. (2011): Four genome-wide significant SNPs; rs7950811 on chromosome 11, rs11838918 and rs16891867 (not shown here) on chromosome 13 and rs1861046 on chromosome 4. **D**: GWAS of aggressiveness-hostility by Alliey-Rodriguez et al. (2011): Top SNP rs17190927 on chromosome 20 (rs6109723 ($r^2$=1) plotted).

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# Chapter 3

## Genome-wide analyses of aggressiveness in attention-deficit hyperactivity disorder

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## ABSTRACT

Aggressiveness is a behavioural trait that has the potential to be harmful to individuals and society. With an estimated heritability of about 40%, genetics is important in its development. We performed an exploratory genome-wide association (GWA) analysis of childhood aggressiveness in attention deficit hyperactivity disorder (ADHD) to gain insight into the underlying biological processes associated with this trait. Our primary sample consisted of 1060 adult ADHD patients (aADHD). To further explore the genetic architecture of childhood aggressiveness, we performed enrichment analyses of suggestive genome-wide associations observed in aADHD among GWA signals of dimensions of oppositionality (defiant/vindictive and irritable dimensions) in childhood ADHD (cADHD). No single polymorphism reached genome-wide significance (p<5.00E-08). The strongest signal in aADHD was observed at rs10826548, within a long noncoding RNA gene (beta = -1.66, standard error (SE) = 0.34, $p$ = 1.07E-06), closely followed by rs35974940 in the neurotrimin gene (beta = 3.23, SE = 0.67, $p$ = 1.26E-06). The top GWA SNPs observed in aADHD showed significant enrichment of signals from both the defiant/vindictive dimension (Fisher's p-value = 2.28E-06) and the irritable dimension in cADHD (Fisher's p-value = 0.0061). In sum, our results identify a number of biologically interesting markers possibly underlying childhood aggressiveness and provide targets for further genetic exploration of aggressiveness across psychiatric disorders.

## INTRODUCTION

Aggressiveness can be defined as any behavior directed towards an individual with the immediate intent to cause harm (Anderson and Bushman, 2002). Violence, which is strongly related to aggressiveness, is the sixth leading cause of burden of disease for people aged 15–44 years worldwide (WHO, 2008). To date, most interventions designed to reduce violence risk typically have small effects, reflecting our limited understanding of its causes and stressing the need for further studies (McGuire, 2008; Moffitt, 2005a).

As a complex phenomenon, aggressiveness spans across numerous facets of human behavior, ranging from emotional lability and temperamental traits (e.g. hot-tempered, short fuse, irritable) to physical violence (Lesch et al., 2012). These traits are frequently found among youth with attention deficit hyperactivity disorder (ADHD), a common child and adolescent psychiatric disorder with a prevalence of about 5% and a rate of persistence into adulthood of about 50% (Faraone et al., 2015). ADHD is defined by symptoms of inattention and hyperactivity/impulsivity, and youth with ADHD often have co-existing disorders, some of which are closely related to aggressiveness and violence, such as conduct disorder (CD) and/or oppositional defiant disorder (ODD) and disorders characterized by symptoms defined within the broader term of antisocial behavior (Dalsgaard et al., 2002). These disorders put youth with ADHD at high risk of problems associated with aggressiveness in adulthood (Klassen et al., 2010), especially when the aggressive behavior has an early onset (Hofvander et al., 2009). This can be illustrated by the fact that around 30% of youth and 25% of adult prison inmates are found to qualify for an ADHD diagnosis (Young et al., 2014). Studies of childhood aggressiveness in adults can, therefore, be of great importance to improve our understanding of adult ADHD.

The etiology of ADHD as well as traits of aggressiveness is complex, with genetics playing an important role. The heritability of ADHD has been estimated to be up to 88% across the lifespan (Larsson et al., 2013), while the estimates of genetic influence on aggression vary across studies, collectively reaching about 40-50% (Brendgen et al., 2006; Tuvblad and Baker, 2011). Such diversity in the estimation of aggression heritability may result from inconsistency in measures across studies. Several different aggression measures have been utilised to assess the genetic and environmental influences on its development (Veroude et al., 2015), reflecting that there is no consensus regarding its definition (Ramirez and Andreu, 2006). Furthermore, the estimates of aggressiveness are influenced by the age of the study participants. The literature reports stability of aggressiveness between childhood and adulthood, with adolescence as a transient period with little stability in this trait (Moffitt, 2005b). Genes seem to explain little variation in adolescent aggression, but are likely to account for individual differences in childhood and adult aggression (Lyons et al., 1995). Also, given higher levels of aggression in males and higher genetic load in males with antisocial behaviour compared to females, it is an open question whether genetic propensity is of greater importance in one sex over the other (Miles and Carey, 1997; Tuvblad and Baker, 2011). Interestingly, similar considerations of age and sex effects are also present in studies of ADHD as well as when ADHD is co-morbid with aggressive behavior (Faraone et al., 2015; Faraone et al., 1991).

Given that ADHD and aggression often co-occur and that both traits are heritable, twin studies have noted the possibility of shared genetic etiology between ADHD and aggression. A common genetic factor has been reported among ADHD and symptoms of aggression in 9-10 year old children (Tuvblad et al., 2009). Likewise, it has been suggested that impulsivity and aggression are genetically mediated to a similar extent (Seroczynski et al., 1999).

Influenced by major theories on neuronal circuits, genetic association studies of ADHD and/or aggression have been dominated by candidate gene studies, focusing on the regulation of monoaminergic transmission (Faraone et al., 2015; Veroude et al., 2015). In line with twin studies, these candidate gene analyses have provided further support towards a shared genetic component between ADHD and aggression. Many genes associated with ADHD point towards the same biological mechanisms as those associated with aggressive behavior, including genes that are involved in the synthesis, binding, transport and degradation of neurotransmitters, especially dopamine and serotonin (Faraone et al., 2015; Veroude et al., 2015). It has been reported, for example, that the genes *MAOA*, *DRD2*, *DRD4, COMT, SLC6A4, TPH1* and *TPH2* may contribute to the development of ADHD as well as aggressive behaviors (Gizer et al., 2009; Vassos et al., 2014). However, these candidate gene studies suffer from the lack of replication in independent samples (where available) and small effect sizes suggest that some of these genes play a more limited role in the susceptibility to ADHD and/or aggressive behavior, or that their involvement may be limited to rare familial cases (Halmoy et al., 2010; McKinney et al., 2008; Tiihonen et al., 2014). Thus, the overall genetic architecture of ADHD and/or aggression remains largely unknown and warrants studies using a hypothesis-free approach (Vassos et al., 2014).

Genome-wide association (GWA) studies allow interrogation of the entire genome to generate new hypotheses. To date, few GWA studies have been performed for ADHD and/or aggressiveness, with no finding passing the stringent Bonferroni-corrected genome-wide significance level ($p<5.00E-08$) for either phenotype (Dick et al., 2011; Mick et al., 2014; Salvatore et al., 2015; Tielbeek et al., 2012). Nonetheless, as these studies were generally underpowered, some understanding of biological processes behind ADHD and/or aggressiveness may emerge from the convergence of identified nominally significant loci. Previous GWA studies on aggressive behaviors in ADHD have noted a number of suggestive association signals, generating biological hypotheses regarding the etiology of ADHD and/or aggression (Aebi et al., 2015; Anney et al., 2008). In addition, a recent GWA study revealed a positive linear correlation between ADHD polygenic scores and comorbid aggression scores, indicating that the presence of aggressive symptoms in ADHD is likely to index a greater genetic load (Hamshere et al., 2013). Similarly hypothesis-free, genome-wide linkage analyses have also reported evidence of significant co-segregation between ADHD and disruptive behavior (Jain et al., 2007).

The lack of robust genetic association signals may be explained by the modest sample sizes and the complex nature of both ADHD and aggressiveness, where genetic factors are intertwined with environmental influences (Brendgen et al., 2006). In addition,

heterogeneity in genetic susceptibility, phenotypic manifestation and operationalization of aggressiveness may depress association signals (Cross-Disorder Group of the Psychiatric Genomics et al., 2013). The phenotypic heterogeneity in ADHD may potentially be exacerbated by its high rates of comorbidity with not only aggressive behaviors, but also mood and anxiety disorders (Biederman et al., 1992). Another possible reason behind the lack of replicable genetic findings is the limited annotation of the human genome. The annotation has mostly been focused on protein-coding genes that represent only ~1% of our genome, making it difficult to evaluate possible biological pathways involved in ADHD and/or aggressiveness, as the majority of GWA findings tend to reside outside the traditional protein-coding regions (Dick et al., 2011; Schizophrenia Working Group of the Psychiatric Genomics, 2014).



In the present study, we aimed to perform exploratory genome-wide association tests to shed light on the genetic susceptibility loci and biological processes possibly involved in the etiology of childhood aggressiveness in ADHD. We utilized the GWA method to analyze childhood aggressiveness in adults with ADHD gathered in studies across Europe. To minimize phenotypic heterogeneity between samples, we derived our measure of childhood aggressiveness in adult ADHD (aADHD) from the Wender Utah Rating Scale (WURS). This questionnaire was used as part of the assessment procedure at all sites. As the WURS reflects childhood recollections, we also explored a possible genetic overlap of association signals observed in aADHD with those of irritable and defiant/vindictive dimensions of ODD in youth with ADHD (cADHD) (Aebi et al., 2015). Finally, we performed an examination of non-protein coding genes in order to obtain a better understanding of the biological processes underlying childhood aggressiveness in aADHD.

## METHODS AND MATERIALS

### Subjects

*aADHD samples*

Recruitment of adult ADHD patients was conducted at three sites within an international multi-center persistent ADHD collaboration (IMpACT, http://www.impactadhdgenomics.com): Germany, Norway and Spain. All individuals were of Caucasian ancestry. Only participants who gave written informed consent were enrolled in the studies, which complied with the Declaration of Helsinki.

*German sample:* Patients with a diagnosis of aADHD were recruited by experienced psychiatrists at the University of Würzburg (Würzburg, Germany). Unrelated in– and outpatients of self-reported central-European descent completed a semi-structured clinical interview according to DSM-IV. Inclusion criteria were onset before the age of 7 years, life-long persistence, current diagnosis and age of recruitment between 18 and 65 years. Exclusion criteria were the appearance of symptoms restricted to the duration of any other Axis I disorder; current diagnosis of active alcohol or other drug abuse or dependence;

lifetime diagnosis of bipolar I disorder, schizophrenia, or any other psychotic disorder; and an IQ score below 80. For a more detailed sample description, please confer previous publications (Franke et al., 2010; Reif et al., 2009). The study was approved by the Ethic Committee of the University of Würzburg (Würzburg, Germany).

*Norwegian sample:* Participants were recruited at the University of Bergen (UiB, Bergen, Norway) as described elsewhere (Halmoy et al., 2009). In short, adult patients with ADHD were recruited through a Norwegian national medical registry as well as by psychologists and psychiatrists working at outpatient clinics. All patients had been previously diagnosed with ADHD using either DSM-IV or ICD-10. The ICD-10 criteria were adapted to the DSM-IV criteria by allowing the inattentive subtype as sufficient for the ADHD diagnosis, and by accepting the coexistence of other neuropsychiatric disorders as long as they appeared after the criteria of ADHD were fulfilled. Individuals with IQ below 70 were excluded from the study. All participants provided either blood or saliva samples for DNA extraction. The study was approved by the regional committee for medical and health research ethics, western Norway.

*Spanish sample:* Participants were recruited at the Department of Psychiatry from the Hospital Universitari Vall d'Hebron (HUVH, Barcelona, Spain) as described elsewhere (Sanchez-Mora et al., 2015). Patients were adults of Caucasian origin and met Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV) criteria for ADHD. The diagnosis of ADHD was evaluated with the Structured Clinical Interview for DSM-IV Axis I and II Disorders (SCID-I and SCID-II) and the Conner's Adult ADHD Diagnostic Interview for DSM-IV (CAADID Parts I and II). Consensus eligibility criteria for the current study were a diagnosis of ADHD according to the diagnostic criteria of DSM-IV, onset before the age of 7 years via retrospective diagnosis (which was confirmed by a family member, wherever possible), lifelong persistence and current diagnosis. DNA was extracted from either peripheral blood or saliva samples. The study was approved by the ethics committee of the institution.

*cADHD sample*

Youth with ADHD were participants in the International Multicentre ADHD Genetics (IMAGE) study, recruited in 12 child and adolescent psychiatry clinics representing eight countries across Europe. Approval was obtained by the Institutional Review Board of SUNY Upstate Medical University and from ethical review boards within each country. A detailed description of the study design and assessment procedures has been provided in previous publications (Muller et al., 2011a, b). In short, entry criteria for probands were a clinical diagnosis of ADHD according to DSM-IV-based structured interviews and access to one or both biological parents and one or more full siblings for DNA collection and clinical assessment. Exclusion criteria included autism, epilepsy, IQ < 70, brain disorders, and any genetic or medical disorder associated with externalizing behaviors that might mimic ADHD.

**Measures of aggressiveness**

*aADHD samples*

The adult measure of childhood aggressiveness in the aADHD samples was derived from the Wender Utah Rating Scale (WURS) (Ward et al., 1993). The WURS is a questionnaire used for retrospective assessment of childhood symptoms of ADHD in adults. An exploratory factor analysis (EFA) was run to determine the latent structure of the WURS. The EFA consisted of a principal component analysis with Varimax rotation and yielded three factors with Eigen values above one. From the main factor explaining the greatest amount of variance in responses to the WURS (30.7%), the top six items with the highest loadings (0.74-0.82) all represented prototypical elements of aggressiveness: "temper outburst/tantrums", "angry", "hot- or short-tempered/low boiling point", "disobedient with parents/rebellious/sassy", "losing control of myself" and "irritable". For each item, the participant was asked to evaluate if she/he as a child was (or had) a specific symptom and to rate it according to the following four response categories: "not at all/very slightly" (0), "mildly" (1), "moderately" (2), quite a bit" (3) or "very much" (4). The arithmetic sum of the responses of the aforementioned items was adopted as a continuous measure of aggressiveness, ranging from 0 to 24. Supplementary Figure 1 shows the distribution of this measure across genders in the three aADHD datasets.

*cADHD sample*

The dimensions of oppositionality were assessed using the long form of the revised Conners Parent Rating Scale (CPRS-R:L) (Conners et al., 1998). The defiant/vindictive and irritable dimensions of ODD were defined on theoretical grounds as described elsewhere (Aebi et al., 2015), and reflect two previously described dimensions of ODD (Aebi et al., 2013; Stringaris et al., 2012).

**Genotype data**

Genotyping of each sample was performed by each of the four participating groups, individually. To maximize available genetic information among examined datasets, genetic imputation was carried out independently at each site.

*aADHD samples*

*German sample:* Genotyping of participants was performed on Illumina's PsychChip array (Illumina, San Diego, CA, USA) at the Broad Institute (Cambridge, MA, USA) using the PsychChip 15048346 B manifest. Genotypes were assigned in Illumina's GenomeStudio v2010.3, using the calling algorithm/genotyping module version 1.8.4. Quality control procedures were performed as described previously, with lightly modified exclusion criteria (SNPs exhibiting missingness above 98%; minor allele frequency below 5%; failing Hardy-Weinberg equilibrium test ($p<10^{-4}$)) (Zayats et al., 2015). Genotype imputation was performed with SHAPEIT/IMPUTE2 pipeline as described elsewhere, using 1000 Genomes Phase 3 data as a reference (Cross-Disorder Group of the Psychiatric Genomics, 2013; Howie et al., 2009; Marchini et al., 2007).

*Norwegian sample:* Participants were genotyped on Human OmniExpress-12v1-1_B (Illumina, San Diego, CA, USA) platform at the deCODE Genetics facility (Reykjavik, Iceland). Genotyping and quality control procedures are described elsewhere (Zayats et al., 2015). Imputation was performed utilizing IMPUTE software as previously detailed (Cross-Disorder Group of the Psychiatric Genomics, 2013; Howie et al., 2009; Marchini et al., 2007).

*Spanish sample:* Genome-wide genotyping was performed with the Illumina HumanOmni1-Quad BeadChip platform. Quality control was implemented at the individual and SNP level using PLINK and included filtering subjects with low call rate (<98%) or gender discrepancy followed by filtering SNPs with minor allele frequency (MAF) < 0.01, Hardy–Weinberg equilibrium test P-values <1e-06 or call rate < 0.99 in either cases or controls. Imputation was performed using BEAGLE software (Browning and Browning, 2007).

*cADHD sample*
Sample collection and DNA isolation has been described previously (Brookes et al., 2006). Genome-wide genotyping and quality control was performed as part of the GAIN study using the Perlegen 600K genotyping platform, as previously described (Neale et al., 2008). The imputation was performed using MACH and the Hapmap 2 (Release 22 Build 36) reference data set (Li et al., 2010). Quality control was performed on the imputed data, and SNPs with imputation quality scores lower than 0.30, a minor allele frequency lower than 0.01, and those failing the Hardy-Weinberg equilibrium test at a threshold of $p \leq 10^{-5}$ were excluded. SNPs and subjects with missingness rates higher than 0.05 were removed from the data.

**Statistical analyses**
The age and gender distributions between the aADHD and cADHD samples were assessed using chi-square for gender and ANOVA for age.

*Genome-wide association (GWA) of aggressiveness*
In the aADHD sample, single nucleotide polymorphisms (SNPs) were tested for association with the WURS-derived measure of aggressiveness in the form of linear regression carried out in PLINK using post-imputation dosage data (Purcell et al., 2007). Regression models were adjusted for age and sex. Genotype data of each site were first processed individually. The results were then combined with the use of fixed-effects inverse variance meta-analysis in METAL (Willer et al., 2010). Only SNPs with minor allele frequency (MAF) equal to or above 1% and imputation INFO measure equal to or above 0.6 were included in the analyses. Genomic control, QQ plotting and regional plotting of top loci was applied to check the integrity of test statistics (Cuellar-Partida et al., 2015; Devlin and Roeder, 1999). The genomic inflation factor was calculated using METAL (Willer et al., 2010). A genome-wide significance threshold of 5.00E-08 was adopted to correct for multiple testing.
GWA analyses of irritable and defiant/vindictive dimensions of ODD in cADHD sample was performed in PLINK software in the form of linear regression adjusted for sex and age (Purcell et al., 2007). Details of the analyses are described elsewhere (Aebi et al., 2015).

*Gene-based and Gene-Set association of aggressiveness in the aADHD meta-analysed sample*
Gene-based and gene-set pathway analysis were performed in the aADHD sample carried out in MAGMA software (de Leeuw et al., 2015). First, a degree of association was calculated for each gene based on METAL-derived individual SNPs' p-values, using 1000 Genomes CEU dataset as a reference panel to correct for linkage disequilibrium (LD) (Genomes Project et al., 2012). To evaluate each gene's contribution to examined gene-sets (gene-set pathway analysis), the p-value of each gene was converted to a Z-value and used as an outcome variable in a regression model with gene-set membership as a predictor. Gene size and gene-sets' gene density were added as covariates to adjust for possible confounding effects and prevent spurious association.



For gene-based tests, we assessed the association with both protein and non-protein-coding genes. The protein-coding gene list was curated from the catalog of known genes downloaded from the Genome Browser of the University of California Santa Cruz (UCSC, California, USA). The non-protein-coding genes were examined in the form of long non-coding RNA (lncRNA) genes detailed in the aforementioned catalog. For gene-set pathway analysis, we examined structural categories of gene ontology (GO, http://geneontology.org), with respect to cellular function, biological process and cellular compartments. To achieve meaningful statistics and interpretation of the results, we restricted our pathway analysis to those GO terms that contained SNPs in at least 10 genes per term in our aADHD data.

*Genome-wide enrichment analyses between GWA results in aADHD and cADHD samples*
Prior to performing enrichment analyses, the genetic data in both aADHD and cADHD samples were pruned to remove correlated loci in linkage disequilibrium (LD) with each other. A pairwise correlation coefficient ($r^2$) threshold of 0.2 and the 1000 Genomes CEU reference dataset were used to identify independent SNPs, as previously described (Genomes Project et al., 2012; Lindgren et al., 2009).

Enrichment was examined by means of Fisher's test performed in R software, assessing the difference in proportion of SNPs revealing association p-values below 0.05 in cADHD sample according to suggestive association in aADHD sample (p-value below or equal to 1.00E-03 versus p-value above 1.00E-03) (Rahmioglu et al., 2015). Consistency in directionality of SNP effects with indication of enrichment between aADHD and cADHD samples was tested as linear regression on the effect (beta) of each SNP for aADHD as an outcome and for cADHD (either irritable or defiant/vindictive dimensions of ODD, respectively) as predictor variables (Do et al., 2013).

*Examination of previously reported aggressiveness-related candidate GWA loci*
We assembled a list of previously reported candidate GWA loci associated with aggressive behavior by systematic literature search the catalog of published genome-wide association studies provided by National Human Genome Research Institute (NHGRI) (https://www.genome.gov/26525384), using key words of "aggression", "anger", "violence" as well as "conduct disorder" and "antisocial personality disorder". Each identified candidate GWA locus was then looked up in meta-analysed aADHD sample.

## RESULTS

### Subjects, measure of aggressiveness and GWA analyses

In total, 1060 adult patients as well as 750 children and adolescents with ADHD were available for the analyses. The age ranges in the aADHD samples were 17 – 75 in the German sample, 18 – 57 in the Norwegian sample and 17 – 60 in the Spanish sample. In the cADHD sample, the age range was 5 – 17. Details of the final samples are summarized in Table 1. Supplementary Figure 1 presents the distribution of the selected measures of aggressiveness in each aADHD dataset.

After quality control of imputed SNPs in the adult samples, 9.301.568 SNPs were available for the analyses in the German sample, 8.910.491 SNPs in the Norwegian sample and 6.683.176 SNPs in the Spanish sample. Among these three datasets, 7.576.458 autosomal SNPs were present in at least two and, thus, were meta-analyzed to assess genetic architecture of childhood aggressiveness in aADHD. In cADHD sample, 1.871.025 autosomal SNPs were available for the analyses.

Individual GWA analyses revealed no genome-wide significant hits ($p \leq 5.00E-08$) in either aADHD sample (not shown) nor in the cADHD sample (Supplementary Table 1; Supplementary Figure 2). None of the variants in the meta-analysis reached the Bonferroni-corrected genome-wide significance level ($p \leq 5.00E-08$) either. The strongest signal was observed at rs10826548 on chromosome 10 located within the transcript of a long noncoding RNA (lncRNA) (beta = -1.66, standard error (SE) = 0.34, p-value = 1.07E-06) (Figure 1), closely followed by rs35974940 in the neurotrimin (*NTM*) gene (beta = 3.23, SE = 0.67, p-value = 1.26E-06) (Figure 2). Top associated markers ($p \leq 1.00E-05$) are summarized in Supplementary Table 2. The genomic inflation factor was close to one for all individual and meta-GWA analyses in aADHD. QQ plots of GWA analyses in aADHD are presented in Supplementary Figure 3.

**Table 1:** *Details of the ADHD patient samples.*

| aADHD samples | | | | | |
|---|---|---|---|---|---|
| **IMpACT site** | **Number of participants** | | **Females (%)** | **Age (mean ± SD)** | **Aggressiveness score (mean ± SD)** |
| Germany | 368 | | 53 | 35.18 ± 10.53 | 11.33 ± 5.17 |
| Norway | 293 | | 52.6 | 32.61 ± 11.00 | 12.10 ± 6.39 |
| Spain | 399 | | 32.3 | 31.31 ± 12.39 | 10.19 ± 6.15 |
| Total | 1,060 | | 45.1 | 33.01 ± 11.51 | 11.11 ± 5.94 |
| **cADHD sample** | | | | | |
| | | | | **ODD scores (mean ± SD)** | |
| | **Number of participants** | **Females a (%)** | **Age b (mean ± SD)** | **Irritable** | **Defiant/vindictive** |
| IMAGE | 750 | 12.3 | 10.67 ± 2.77 | 7.75 ± 3.06 | 8.95 ± 4.18 |

Aggressiveness score was derived from WURS in the aADHD sample. In the cADHD sample, dimensions of oppositionality (irritable and defiant/vindictive dimensions) were examined (Aebi et al., 2015). SD, standard deviation.

[a]Difference in the proportion of females between the aADHD and cADHD samples: $P < 2.2E-16$ ($\chi 2$ test).

[b]Difference in age between the aADHD and cADHD samples: $P < 2.2E-16$ (ANOVA).

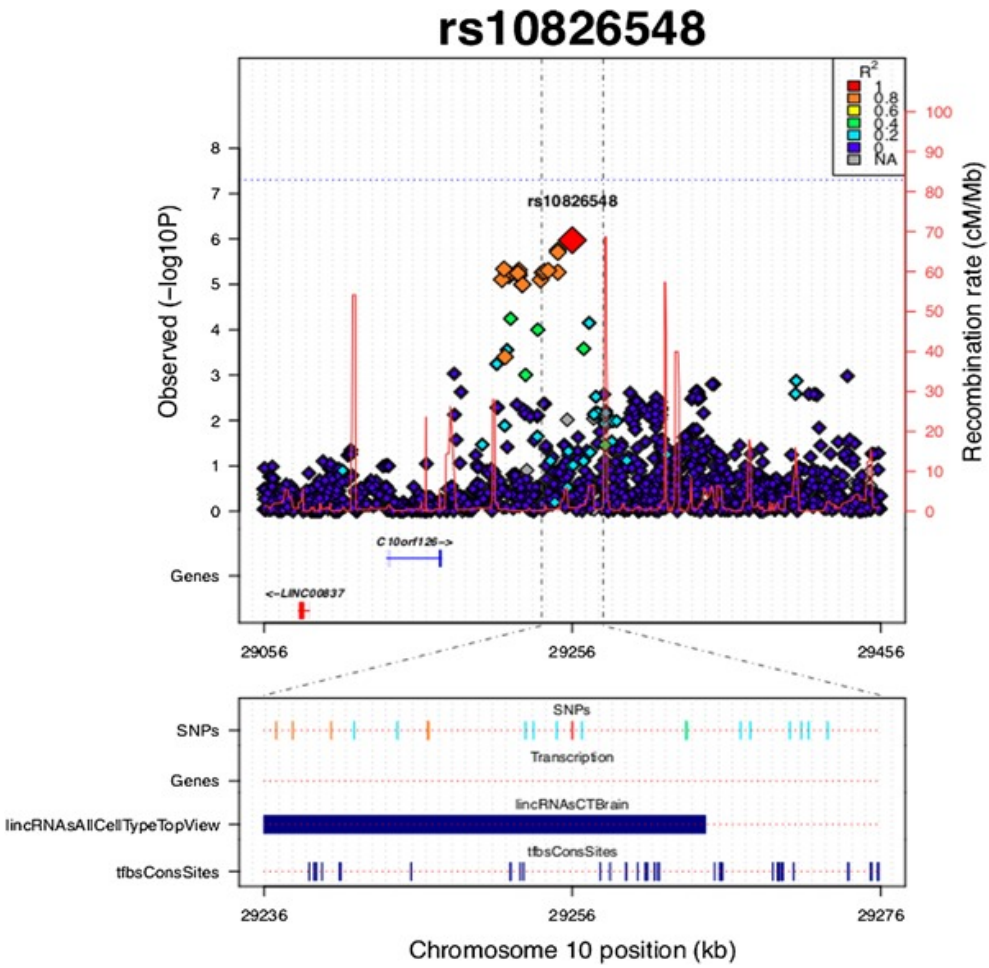


**Figure 1:** Plot of the locus surrounding rs10826548.
SNPs are plotted by position on chromosome 10 against GWA p-values for aggressive behaviour measure in aADHD. Estimated recombination rates from HapMap are plotted in bright red to reflect local LD structure. The SNPs surrounding rs10826548 are color-coded to reflect their LD with it (according to pair-wise r2 values from the HapMap CEU database). SNPs with LD r2≥0.2 are plotted at the bottom of the graph with LD color-coding specified in the top right corner. "Genes" refers to protein-coding genes in the presented region. "lincRNAsAllCellTypeTopView" reflects the data from lncRNA USCS track in brain tissue. "tfbsConsSites" reflects the TFBS UCSC track.

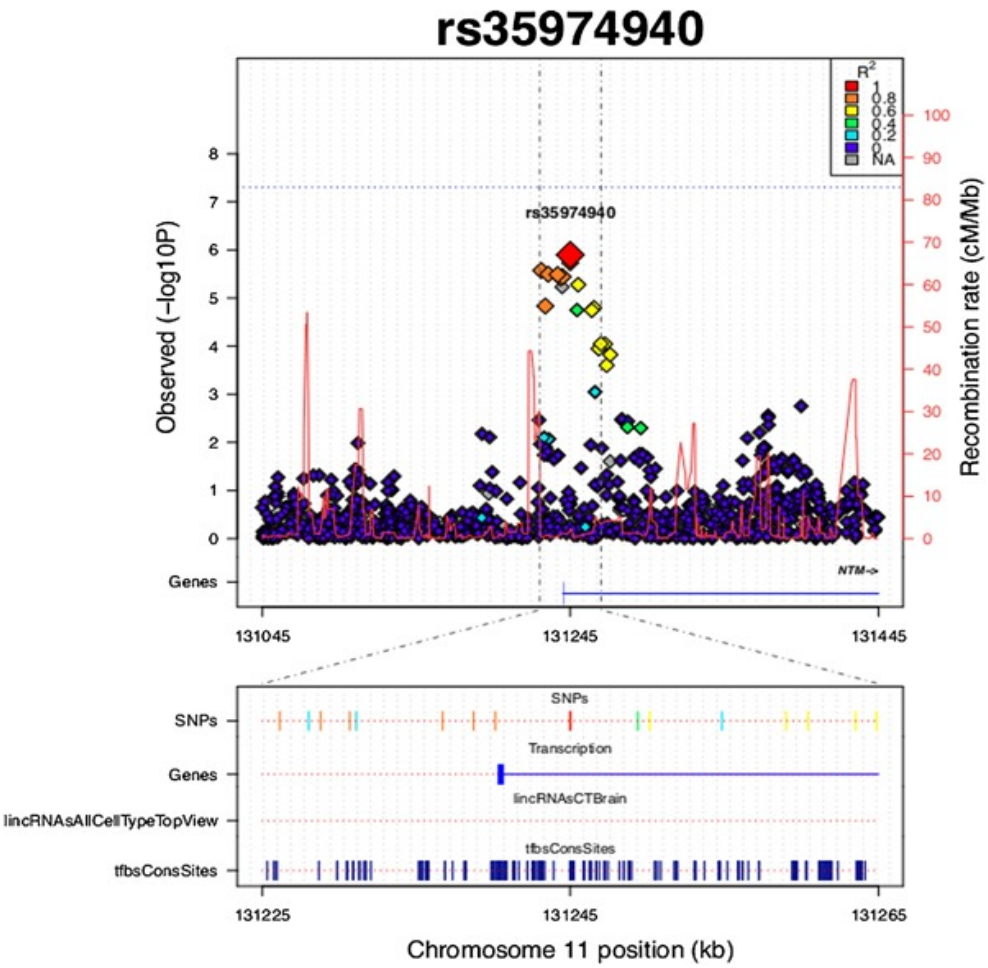


**Figure 2:** Plot of the locus surrounding rs35974940.
SNPs are plotted by position on chromosome 11 against GWA p-values for aggressive behaviour measure in aADHD. Estimated recombination rates from HapMap are plotted in bright red to reflect local LD structure. The SNPs surrounding rs35974940 are color-coded to reflect their LD with it (according to pair-wise r2 values from the HapMap CEU database). SNPs with LD r2≥0.2 are plotted at the bottom of the graph with LD color-coding specified in the top right corner. "Genes" refers to protein-coding genes in the presented region. "lincRNAsAllCellTypeTopView" reflects the data from lncRNA USCS track in brain tissue. "tfbsConsSites" reflects the TFBS UCSC track.

## Gene-based and Gene-Set association of aggressiveness in the aADHD meta-analyzed sample

Among annotated protein-coding genes, 17.595 had more than one SNP present in the aADHD data. The strongest signal was noted for the WD repeat domain 62 (*WDR62*) gene (p-value = 4.84E-05). Supplementary Table 3 summarizes the top protein-coding genes (p≤1.00E-03) observed in aADHD sample. None of the protein-coding gene-based tests survived the correction for multiple testing.

Among lncRNA genes, 22.696 had more than one SNP present in our aADHD data. The strongest association was observed for ENST00000427806 (p-value = 3.04E-05). The top lncRNA genes (p≤1.00E-03) detected in this study are reported in Supplementary Table 4. None of the non-protein-coding gene-based tests survived the correction for multiple testing.

Among GO pathways, 1.945 terms contained SNPs in at least 10 genes per term in the aADHD data. The most prominent association was observed for negative regulation of I-kappaB kinase/NF-kappaB signaling pathway (GO:0043124 term, p-value = 7.26E-04). Supplementary Table 5 reports top GO terms (p≤0.01) recognized in this study. None of the GO pathways survived the correction for multiple testing.



**Genome-wide enrichment analyses between GWA results in aADHD and cADHD samples**

To assess potential genome-wide overlap of association signals between measures of childhood aggressiveness in aADHD and cADHD, we investigated the independent ($r^2$ < 0.2) GWA signals of suggestive significance (p≤1.00E-03) in aADHD for enrichment in GWA signals of either defiant/vindictive or irritable dimensions in cADHD. Given our modest sample size, only those SNPs were considered in cADHD results that revealed a p-value below or equal to 0.05 to avoid the examination of effects with a wide confidence interval. The top GWA SNPs of WURS-derived childhood aggressiveness in aADHD showed significant enrichment of signals from both the defiant/vindictive dimension (Fisher's p-value = 2.28E-06) and the irritable dimension in cADHD GWA analysis (Fisher's p-value = 0.0061) (Figure 3A).

Next, we examined the directionality of effects of variants with association signals in both aADHD and cADHD samples (p≤1.00E-03 in aADHD and p<0.05 in cADHD). Significant correlation between betas was observed in assessment of both oppositional dimensions in cADHD and childhood aggressiveness in aADHD (p=0.0053 and p=0.0045 for defiant/vindictive and irritable dimensions respectively), but the direction of the relationship was negative (Figure 3B and 3C). Supplementary Table 7 summarizes the top hits (p≤1-00E-05) observed in GWA meta-analysis of childhood aggressiveness in aADHD and their corresponding statistics observed in cADHD.

**Examination of previously reported aggressiveness-related candidate genes and GWA loci**

Among previously reported aggressiveness-related GWA loci, several SNPs noted to be associated with anger, conduct disorder and adult anti-social personality disorder revealed p-values below 0.05 in our study (Supplementary Table 8). The strongest signal in the GWA analysis of childhood aggressiveness in aADHD among the aforementioned loci was observed for rs4889240 in the *PKD1L2* (polycystic kidney disease 1-like 2) gene (beta = -0.73, SE =0.25, p-value = 0.0039), previously reported to be associated with CD symptom count in ADHD patients. The same SNP also revealed nominally significant association in the same direction with the defiant/vindictive dimension (beta = -0.54, SE = 0.21, p-value

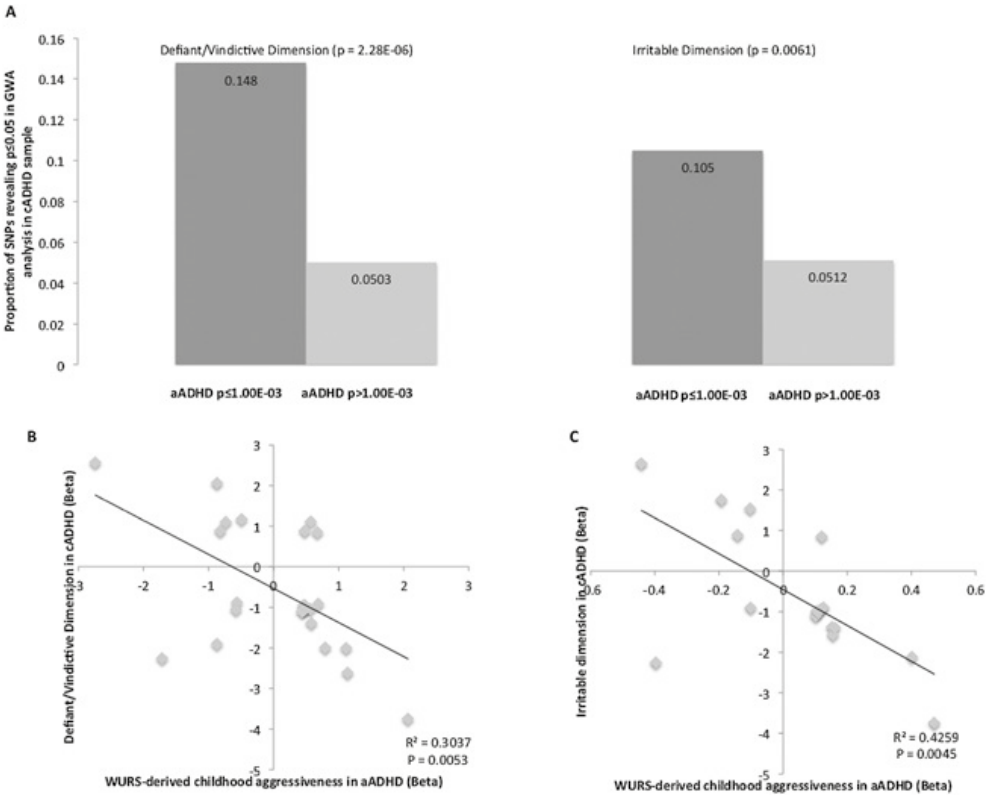


**Figure 3:** Enrichment and direction of effect among GWA signals of oppositional dimensions in cADHD and WURS-derived childhood aggressiveness in aADHD.
Panel A reflects the proportion of SNPs nominally associated (p<0.05) with each examined oppositional dimension in cADHD (defiant/vindictive and irritable) among suggestive signals (p≤1.00E-03) of association with childhood aggressiveness in aADHD. Reported p-values are those of Fisher's exact test.
Panels B and C reflect directions of effect of 24 independent nominally significant loci in GWA analyses of defiant/vindictive dimension in cADHD and childhood aggressiveness in aADHD (panel B) as well as 17 independent nominally significant loci in GWA analyses of irritable dimension in cADHD and childhood aggressiveness in aADHD (Panel C). Linear regression $r^2$ measures and p-values are shown.

= 0.0094), but not with the irritable dimension in cADHD. In this result, one should keep in mind that the cADHD described here is a subsample of the sample in which the original finding for rs4889240 was described (Aebi et al., 2015). Full results of our literature search are presented in Supplementary Table 8.

## DISCUSSION

In this study, we performed a genome-wide exploration of childhood aggressiveness as reported retrospectively by adult patients with ADHD (aADHD), examining both conventional protein-coding and lncRNA genes. We also explored the overlap with parent-

reported oppositional behavior in youth with ADHD (cADHD) and evaluated previously reported aggression-related GWA loci. Given our modest sample size (1060 aADHD patients) and the anticipated small effect of common polymorphisms in complex traits, it is not surprising that we did not observe any genome-wide significant SNPs (p<5.00E-08). Nonetheless, we were able to identify several nominally significant variants (p≤1.00E-05) in biologically interesting genes for follow-up studies of aggressiveness in ADHD, a feature of the disorder that has received little attention so far.

The strongest signal in the performed single-point GWA tests of childhood aggressiveness in aADHD was noted for rs10826548 (beta=-1.16, SE=0.34, p=1.07E-06, Supplementary Table 1). This variant resides in the transcript of a lncRNA with uncertain coding potential (TCONS_00018147) (Figure 1). Non-protein coding RNAs play a critical role in the regulation of gene expression and have been previously associated with neuropsychiatric disorders, including ADHD (Gonzalez-Giraldo et al., 2015; Perkins et al., 2005; Zayats et al., 2015). In addition, it has recently been observed that SNPs previously associated with neurological and psychiatric conditions may be highly concentrated in the regions of long non-protein coding RNA genes (Ning et al., 2014).

The second most significant locus identified in this study is located within the neurotrimin (*NTM*) gene (intronic rs35974940, p=1.26E-06, Supplementary Table 1, Figure 2). *NTM* is a protein-coding gene, encoding a member of glycosylphosphatidylinositol (GPI)-anchored cell adhesion molecules, containing immunoglobulin (Ig) domain. These proteins are predominantly expressed in the central nervous system (CNS) (Struyk et al., 1995).

Among the association signals observed in *NTM* gene, several have the potential to alter its expression. As determined in the TRANSFAC database implemented in the SNPinfo server of the National Institute of Environmental Health Sciences (http://snpinfo.niehs.nih.gov), rs34588147 and rs35665773 (GWA p-values of 3.59E-06 and 3.25E-06 respectively, Supplementary Table 1) are transcription factor binding sites (TFBS) (Figure 2). Moreover, two other SNPs in high linkage disequilibrium with the aforementioned ones (rs12804059 and rs7119590, r2=1 in CEU population) also represent TFBS. Notably, differential expression of *NTM* between two major brain regions linked to aggression subtypes – prefrontal cortex and amygdala – was observed in early prenatal stage of human brain development (p=0.015, http://www.brainspan.org).

Gene expression regulation during neuronal development as one of the possible mechanisms behind aggressiveness in aADHD was further affirmed by our top associated lncRNA gene - ENST00000427806 (p=3.04E-05, Supplementary Table 4). The target gene of this lncRNA has been predicted to be the protein-coding ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 5 (*ST6GALNAC5*) gene (Vucicevic et al., 2015). The protein encoded by *ST6GALNAC5* is a member of sialyltransferases, with reported function in cell adhesion through cell-cell and cell-extracellular matrix interactions (Tsuchida et al., 2003). Intriguingly, *ST6GALNAC5,* similarly to *NTM,* also revealed differential expression in the aggression-related structures of prefrontal cortex and amygdala in early prenatal stages of human brain development (p=0.013, http://www.brainspan.org).

As the adult measure of aggressiveness was derived from self-reported experiences in childhood, we examined the possibility of overlap of its GWA signals with those from GWA analyses of two oppositional dimensions in a cADHD sample. We observed a slight enrichment of association signals between the nominally associated loci in aADHD and those observed in the GWA of both the defiant/vindictive and the irritable ODD dimensions examined in cADHD (Figure 3). However, it is noteworthy that the aADHD and cADHD samples were imputed using different reference panels with disparate genomic coverage.

Surprisingly, the correlation between the direction of effects of the aforementioned SNPs was negative (Figure 3B and 3C). Such an inverse relationship in effect directionality between parent-reported ODD dimensions and adult retrospective report of childhood aggressiveness is most likely a chance finding due to our study being under-powered or it might also be related to phenotypic and genetic heterogeneity of the examined samples. There were considerable differences in the percentage of females between the aADHD and the cADHD samples (Table 1), which could indicate such mechanisms. It has been shown that both age and sex are important factors in genetic influences in ADHD and aggression (Faraone et al., 2015; Lyons et al., 1995; Miles and Carey, 1997; Tuvblad and Baker, 2011). In addition, the aggressiveness in cADHD sample was determined by parent-report, while in the aADHD sample it was based on retrospective self-report. The correlation between parent-report and self-report has been shown to be generally poor (Achenbach et al., 1987), as also discussed in a recent study that found little overlap between samples of cADHD and aADHD (Moffitt et al., 2015). Therefore, the measures of aggressiveness in aADHD and cADHD samples are different. Furthermore, the youth and adult ADHD samples may also be heterogeneous because childhood ADHD does not always persist into adulthood (Faraone et al., 2006; Moffitt et al., 2015). Thus, to gain better understanding of the genetic overlap between childhood aggression in aADHD and oppositional dimensions in cADHD, this relationship should be examined in larger sample using more rigorous statistical methods, such as those developed to test specifically for genetic correlation among various traits (Bulik-Sullivan et al., 2015a; Bulik-Sullivan et al., 2015b; Yang et al., 2011). This was not possible to implement in the current study due to our modest sample size.

Examination of previously reported aggressiveness-related GWA loci revealed modest commonality in genetic architecture between the childhood measures of aggressiveness in both cADHD and aADHD, as well as in CD and anti-social personality disorder (Supplementary Table 8). This observation may be in line with formerly reported phenotypic overlap between these conditions, although to which extent this overlap can be transmitted to various subtypes of aggressiveness remains to be determined (Storebo and Simonsen, 2013).

This study should be viewed in light of its limitations. One explanation for not observing any genome-wide significant loci ($p<5.00E-08$) could be our relatively modest sample size and examination of common variants only (MAF>1%). This study had 63% power to detect common variants with small effect size of explaining 0.5% of variability under an additive model and an alpha level of 0.05 (http://genome.sph.umich.edu/wiki/Power_

Calculations: Quantitative_Traits). This may also be observed in the distribution of the QQ plots (Supplementary Figure 3).

Another explanation for the lack of significant findings may lay in phenotypic variability. Clinical heterogeneity may weaken true association signals due to the use of different assessment protocols or real genetic heterogeneity among subtypes of ADHD (McClellan and King, 2010). There are several methodological caveats to assessing aggressiveness (Moffitt et al., 2015). As our samples consist of outpatients, we investigate a broader and perhaps "softer" aspect of aggressiveness than say, for example, if we were to study prison inmates and/or juvenile offenders. However, this approach provides us with access to the vast majority of aggressive behaviors, which may not come to be written in official records (Moffitt, 2005). Further, we lack assessment of different subtypes of aggressive behaviour that may be related to different genotypes.

Considering the different direction of effects and different measures of aggression in the cADHD and the aADHD samples, analysing the adult samples and the youth sample together could potentially have obscured the genetic association signal. This is why we refrained from performing meta-analysis across all samples. Nonetheless, the WURS includes a host of symptoms related to various elements of aggressiveness, which, based on our factor analysis as well as previous research (Ward et al., 1993) seem to be of key importance to the phenotype of aADHD, and the ODD measures have also been validated in previous studies of cADHD (Aebi et al., 2013; Stringaris et al., 2012). Our approach may add to the discussion of the Negative Valence System in the Research Domain Criteria (RDoC) of the National Institute of Mental Health (NIMH) of how to conceptualize and operationalize aggressiveness as a dimension across different samples and disorders (Verona and Bresin, 2015; Veroude et al., 2015).

We lacked information on current substance abuse in our aADHD sample. Substance abuse is known to be frequently comorbid with ADHD and may confound the relationship between ADHD and current aggressiveness. However, we utilized a retrospective measure of childhood aggressiveness that is likely to reflect behavior over a longer period of time and should, thus, be less affected by volatile environmental influences (Gulberg-Kjär and Johansson, 2009).

Finally, since the genome-wide genotyping arrays consist of SNPs only, we were not able to assess the contribution of previously reported variable tandem repeats (e.g. those in MAOA) that were noted to be associated with aggressive behaviors and/or ADHD.

Taken together with evidence from previous studies, our results implicate mechanisms of cell adhesion as well as regulation of gene expression in the etiology of childhood aggressiveness in ADHD. As there is a substantial degree of overlap in aggressiveness among neuropsychiatric disorders, it could be beneficial to analyse conditions where aggression is present together in order to pinpoint biological processes in dysfunctional forms of aggressiveness. Further studies including samples of both children, adolescents and adults, adopting multimodal measures and longitudinal designs are warranted. Such studies may help our understanding as to which extent various subtypes of aggression are mediated by different mechanisms.

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## SUPPLEMENTARY MATERIAL

**Supplementary Table 1:** Top hits (p≤1-00E-05) observed in GWA analyses of oppositional dimensions in childhood ADHD.

| Defiant/Vindictive Dimension | | | | | | |
|---|---|---|---|---|---|---|
| SNP | CHR | Gene (nearest gene) | Effect Allele | BETA | SE | P-value |
| rs1330598 | 13 | intergenic | C | -1.172 | 0.2402 | 1.29E-06 |
| rs4075163 | 9 | intergenic (C9orf47) | A | 1.065 | 0.2192 | 1.45E-06 |
| rs7043095 | 9 | C9orf47 | T | 1.018 | 0.2156 | 2.82E-06 |
| rs11791652 | 9 | intergenic (C9orf47) | T | 1.017 | 0.2158 | 2.92E-06 |
| rs3849811 | 8 | intergenic (ST18) | C | 1.079 | 0.2291 | 2.95E-06 |
| rs3892512 | 8 | intergenic (ST18) | G | 1.079 | 0.2291 | 2.95E-06 |
| rs1895885 | 8 | intergenic (ST18) | C | 1.079 | 0.2291 | 2.95E-06 |
| rs9463078 | 6 | SUPT3H | A | 0.9733 | 0.2069 | 3.05E-06 |
| rs10483554 | 14 | intergenic (ATP5G2P2) | G | -1.482 | 0.3218 | 4.86E-06 |
| rs9380938 | 6 | intergenic (EDN1) | G | 1.036 | 0.2281 | 6.53E-06 |
| rs137887 | 22 | TTLL8 | G | 0.9683 | 0.2138 | 6.91E-06 |
| rs9367214 | 6 | SUPT3H | G | -0.9467 | 0.2099 | 7.56E-06 |
| **Irritable Dimension** | | | | | | |
| SNP | CHR | Gene (nearest gene) | Effect Allele | BETA | SE | P-value |
| rs10910623 | 1 | MAP10 | A | -0.7022 | 0.1362 | 3.31E-07 |
| rs12042052 | 1 | intergenic (MAP10) | G | -0.7052 | 0.1378 | 3.97E-07 |
| rs12036759 | 1 | intergenic (MAP10) | G | -0.7084 | 0.1392 | 4.65E-07 |
| rs1278352 | 10 | ADAM12 | A | 0.2377 | 0.04806 | 9.40E-07 |
| rs6892228 | 5 | intergenic (XR_925892.1) | C | 0.2236 | 0.04699 | 2.34E-06 |
| rs10075707 | 5 | intergenic (XR_925892.1) | G | 0.2236 | 0.04699 | 2.34E-06 |
| rs13184157 | 5 | intergenic (XR_925892.1) | A | 0.2236 | 0.04699 | 2.34E-06 |
| rs6869294 | 5 | intergenic (XR_925892.1) | A | 0.2236 | 0.04699 | 2.34E-06 |
| rs3787069 | 19 | MLLT1 | G | -0.6959 | 0.1512 | 4.92E-06 |
| rs6127978 | 20 | BMP7 | G | -0.2907 | 0.06339 | 5.29E-06 |
| rs9559203 | 13 | intergenic | C | -0.3784 | 0.08397 | 7.66E-06 |
| rs2447961 | 8 | intergenic | T | 0.3281 | 0.07305 | 8.20E-06 |

Association was evaluated in the form of linear regression. "Beta" refers to effect size of each copy of the specified allele ("Effect allele") on the examined oppositional dimension in childhood ADHD. "SE" refers to standard error. "P-value" reflects the strength of association signal. "CHR" refers to a chromosome where a SNP is located and "Gene" is referring to a gene within which a SNP is located, nearest gene is determined as a gene located within ±20Kbp around a SNP.

**Supplementary Table 2:** Top hits (p≤1-00E-05) observed in genome-wide association meta-analysis of aggressive behavior in adult ADHD.

| SNP | CHR | Gene (nearest gene) | Effect Allele | Beta | SE | P-value | Het P-value |
|---|---|---|---|---|---|---|---|
| rs10826548 | 10 | intergenic (LOC101929236) | A | -1.6612 | 0.3405 | 1.07E-06 | 0.9073 |
| rs35974940 | 11 | NTM | T | 3.2303 | 0.6666 | 1.26E-06 | 0.1794 |
| rs188370812 | 10 | intergenic | A | -1.7787 | 0.3721 | 1.75E-06 | 0.9384 |
| rs34807050 | 11 | NTM | T | -3.176 | 0.6658 | 1.84E-06 | 0.2032 |
| rs78030545 | 4 | intergenic | T | -3.7706 | 0.7918 | 1.91E-06 | 0.6677 |
| rs192649950 | 10 | Intergenic | A | 1.7727 | 0.3727 | 1.97E-06 | 0.9371 |
| rs138850252 | 12 | intergenic (NR_110053.1) | C | -2.9016 | 0.6101 | 1.97E-06 | 0.0579 |
| rs11058510 | 12 | (NR_110053.1) | T | -2.8978 | 0.61 | 2.03E-06 | 0.05755 |
| rs80113388 | 8 | CSMD1 | T | -4.3626 | 0.9284 | 2.61E-06 | 0.3371 |
| rs34547019 | 11 | intergenic (NTM) | T | 3.1449 | 0.6699 | 2.67E-06 | 0.2605 |
| rs76037120 | 5 | intergenic (KRT18P42) | C | 3.2224 | 0.6885 | 2.87E-06 | 0.06077 |
| rs12600304 | 16 | TEPP | T | 1.6231 | 0.3469 | 2.88E-06 | 0.3298 |
| rs35042821 | 11 | intergenic (NTM) | A | -3.1949 | 0.6859 | 3.20E-06 | 0.3432 |
| rs35665773 | 11 | intergenic (NTM) | T | 3.0938 | 0.6647 | 3.25E-06 | 0.2473 |
| rs34588147 | 11 | NTM | A | 3.051 | 0.6584 | 3.59E-06 | 0.2745 |
| rs12804059 | 11 | NTM | T | 3.0437 | 0.6586 | 3.81E-06 | 0.2376 |
| rs77206607 | 12 | intergenic | T | -2.759 | 0.5994 | 4.17E-06 | 0.04155 |
| rs3893429 | 3 | CPNE4 | A | -1.5454 | 0.3361 | 4.26E-06 | 0.665 |
| rs10831734 | 11 | intergenic (MICAL2) | A | -1.336 | 0.2912 | 4.47E-06 | 0.6032 |
| rs7899136 | 10 | LOC101929236 | A | 1.5467 | 0.3375 | 4.59E-06 | 0.7998 |
| rs77277634 | 12 | intergenic (NR_110053.1) | A | -2.8197 | 0.6154 | 4.61E-06 | 0.07106 |
| rs11022174 | 11 | intergenic (MICAL2) | A | -1.3326 | 0.2912 | 4.74E-06 | 0.6097 |
| rs10740790 | 10 | LOC101929236 | A | -1.5482 | 0.3387 | 4.84E-06 | 0.9025 |
| rs7903633 | 10 | Intergenic (LOC101929236) | T | -1.5474 | 0.3388 | 4.96E-06 | 0.9311 |
| rs10826546 | 10 | intergenic | A | 1.5444 | 0.3388 | 5.15E-06 | 0.9306 |
| rs35385808 | 11 | NTM | T | 3.3222 | 0.7296 | 5.28E-06 | 0.202 |
| rs931200 | 8 | CSMD1 | A | -4.1975 | 0.9226 | 5.38E-06 | 0.3132 |
| rs117352156 | 10 | intergenic | T | -1.5494 | 0.3407 | 5.41E-06 | 0.9305 |
| rs4587639 | 10 | LOC101929236 | A | 1.5419 | 0.3391 | 5.43E-06 | 0.8723 |
| rs10826545 | 10 | intergenic | A | -1.5399 | 0.3389 | 5.51E-06 | 0.9282 |
| rs11058518 | 12 | LOC101927464 | T | 2.79 | 0.6146 | 5.65E-06 | 0.078 |
| rs7092115 | 10 | LOC101929236 | T | -1.5371 | 0.3387 | 5.69E-06 | 0.8871 |
| rs10826543 | 10 | LOC101929236 | T | -1.5368 | 0.3387 | 5.69E-06 | 0.8878 |
| rs10508740 | 10 | LOC101929236 | A | 1.5369 | 0.3387 | 5.70E-06 | 0.8872 |
| rs10826542 | 10 | LOC101929236 | A | -1.5365 | 0.3387 | 5.73E-06 | 0.8874 |
| rs12367682 | 12 | NR_110053.1 | A | 2.7087 | 0.5973 | 5.77E-06 | 0.04113 |
| rs17760150 | 10 | LOC101929236 | A | 1.5373 | 0.3392 | 5.83E-06 | 0.8758 |
| rs12411424 | 10 | LOC101929236 | T | -1.5368 | 0.3391 | 5.85E-06 | 0.8791 |
| rs2887379 | 10 | LOC101929236 | T | 1.5365 | 0.3392 | 5.89E-06 | 0.8791 |
| rs72664414 | 1 | intergenic | C | -4.4025 | 0.9718 | 5.89E-06 | 0.7281 |
| rs7119590 | 11 | NTM | C | 2.9673 | 0.655 | 5.89E-06 | 0.3228 |
| rs72796654 | 10 | intergenic | A | 3.157 | 0.6991 | 6.30E-06 | 0.02185 |

| SNP | CHR | Gene (nearest gene) | Effect Allele | Beta | SE | P-value | Het P-value |
|---|---|---|---|---|---|---|---|
| rs117970560 | 12 | LOC101927464 | A | -2.7832 | 0.6167 | 6.38E-06 | 0.0773 |
| rs117169431 | 12 | LOC101927464 | A | 2.7832 | 0.6166 | 6.38E-06 | 0.07712 |
| rs12737863 | 1 | H3F3A | T | 2.362 | 0.5233 | 6.38E-06 | 0.09153 |
| rs10763677 | 10 | LOC101929236 | A | -1.5319 | 0.3394 | 6.39E-06 | 0.896 |
| rs2368966 | 10 | LOC101929236 | A | -1.5352 | 0.3405 | 6.52E-06 | 0.8802 |
| rs11058488 | 12 | LOC105370057 | T | 2.7151 | 0.6023 | 6.56E-06 | 0.03986 |
| rs34674354 | 10 | intergenic | A | -3.1479 | 0.6992 | 6.73E-06 | 0.02519 |
| rs140265971 | 10 | intergenic | A | 3.1539 | 0.7011 | 6.84E-06 | 0.02184 |
| rs12752329 | 1 | Intergenic (ACBD3) | C | 2.3339 | 0.5199 | 7.16E-06 | 0.1768 |
| rs10750165 | 11 | LOC101929156 | A | 1.5241 | 0.34 | 7.38E-06 | 0.5623 |
| rs117063229 | 10 | intergenic (LOC105376469) | T | 3.5443 | 0.7913 | 7.49E-06 | 0.2038 |
| rs10128443 | 10 | intergenic | T | 2.6274 | 0.5874 | 7.72E-06 | 0.1408 |
| rs72752681 | 1 | intergenic (LOC105373223) | A | -3.1736 | 0.7098 | 7.78E-06 | 0.3127 |
| rs9326778 | 5 | intergenic (KRT18P42) | T | 3.6559 | 0.8177 | 7.80E-06 | 0.1268 |
| rs7894571 | 10 | LOC101929236 | A | -1.5119 | 0.3383 | 7.86E-06 | 0.764 |
| rs2195588 | 8 | intergenic | A | 2.7004 | 0.6043 | 7.88E-06 | 0.1006 |
| rs10826544 | 10 | LOC101929236 | A | -1.5224 | 0.3409 | 7.99E-06 | 0.9107 |
| rs114439515 | 4 | SPINK2 | A | -3.8041 | 0.8544 | 8.50E-06 | 0.9683 |
| rs692916 | 18 | PHLPP1 | T | 1.492 | 0.3351 | 8.50E-06 | 0.983 |
| rs61947646 | 13 | UFM1 | T | -3.4562 | 0.7785 | 9.02E-06 | 0.1763 |
| rs11815470 | 10 | intergenic | A | 3.0323 | 0.685 | 9.58E-06 | 0.05543 |
| rs11005885 | 10 | intergenic | C | 2.579 | 0.5833 | 9.81E-06 | 0.1375 |
| rs4387281 | 10 | intergenic | A | -2.5772 | 0.5832 | 9.91E-06 | 0.1374 |

"Beta" refers to the effect size of each copy of the specified allele ("Effect allele") on the childhood aggressiveness in adult ADHD. "SE" refers to standard error. "P-value" reflects the strength of association signal, while "Het p-value" reflects the level of heterogeneity among the examined samples of IMpACT. "CHR" refers to a chromosome where a SNP is located and "Gene" is referring to a gene within which a SNP is located, nearest gene is determined as a gene located within ±20Kbp around a SNP.

**Supplementary Table 3:** Summary of the top protein-coding genes (p≤1.00E-03) from genome-wide gene-based association analysis of aggressiveness in aADHD and cADHD respectively.

| WURS-derived aggressiveness in aADHD | | | |
|---|---|---|---|
| **GENE ID** | **CHR** | **N SNPs** | **P-value** |
| 284403 | 19 | 130 | 4.84E-05 |
| 100526835 | 1 | 591 | 5.95E-05 |
| 10678 | 2 | 41 | 0.00011065 |
| 79025 | 20 | 7 | 0.00017479 |
| 1155 | 19 | 25 | 0.00017511 |
| 115825 | 13 | 209 | 0.00017668 |
| 8790 | 1 | 33 | 0.00019857 |
| 777 | 1 | 761 | 0.00021359 |
| 283554 | 14 | 190 | 0.00021715 |
| 127255 | 1 | 272 | 0.00022337 |
| 197370 | 16 | 94 | 0.00025686 |
| 3762 | 11 | 76 | 0.00032003 |
| 51450 | 9 | 209 | 0.00033461 |
| 10538 | 14 | 58 | 0.00041025 |
| 374739 | 16 | 36 | 0.00043095 |
| 167691 | 6 | 103 | 0.00049349 |
| 109 | 2 | 363 | 0.00051071 |
| 23576 | 1 | 755 | 0.00053187 |
| 5438 | 19 | 4 | 0.00054113 |
| 90417 | 15 | 32 | 0.00055524 |
| 4053 | 14 | 262 | 0.00059176 |
| 2880 | 6 | 3 | 0.00073707 |
| 199745 | 19 | 49 | 0.00079207 |
| 257202 | 6 | 28 | 0.00079486 |
| 238 | 2 | 2464 | 0.00083899 |
| 84984 | 3 | 24 | 0.00084005 |
| 4831 | 17 | 15 | 0.00085045 |
| 112609 | 6 | 141 | 0.00089164 |
| 3777 | 2 | 76 | 0.00089631 |
| 7289 | 12 | 183 | 0.00094173 |
| 745 | 11 | 57 | 0.00095995 |

| Defiant/Vindictive Dimension in cADHD | | | |
|---|---|---|---|
| **GENE** | **CHR** | **NSNPS** | **P** |
| 8464 | 6 | 481 | 0.00018043 |
| 374618 | 15 | 48 | 0.00020873 |
| 5317 | 1 | 38 | 0.00021958 |
| 22846 | 14 | 28 | 0.00028333 |
| 6638 | 15 | 324 | 0.00028779 |
| 55148 | 14 | 12 | 0.00029561 |
| 100132916 | 5 | 12 | 0.00030611 |
| 57325 | 20 | 35 | 0.00039792 |

| Defiant/Vindictive Dimension in cADHD | | | |
|---|---|---|---|
| **GENE** | **CHR** | **NSNPS** | **P** |
| 4897 | 7 | 252 | 0.00051268 |
| 10725 | 16 | 3 | 0.00056183 |
| 79644 | 4 | 13 | 0.00063294 |
| 55329 | 15 | 21 | 0.00066138 |
| 131450 | 3 | 37 | 0.0006674 |
| 170482 | 12 | 8 | 0.00070134 |
| 54490 | 4 | 7 | 0.00071266 |
| 81558 | 17 | 28 | 0.0007647 |
| 51643 | 12 | 20 | 0.00083041 |
| 860 | 6 | 122 | 0.00085638 |
| 83439 | 2 | 88 | 0.00088179 |
| 25871 | 3 | 4 | 0.0009544 |
| 145497 | 14 | 34 | 0.00097612 |
| 245930 | 20 | 5 | 0.00098481 |
| 79603 | 19 | 5 | 0.00099264 |

| Irritable Dimension in cADHD | | | |
|---|---|---|---|
| **GENE** | **CHR** | **NSNPS** | **P** |
| 1004 | 5 | 103 | 1.38E-05 |
| 63976 | 1 | 169 | 2.04E-05 |
| 3617 | 6 | 121 | 2.79E-05 |
| 8038 | 10 | 490 | 8.42E-05 |
| 11076 | 5 | 29 | 0.00013628 |
| 5317 | 1 | 38 | 0.00015208 |
| 83416 | 1 | 19 | 0.00018883 |
| 135228 | 6 | 54 | 0.0002044 |
| 143425 | 11 | 266 | 0.00023102 |
| 115350 | 1 | 18 | 0.0002376 |
| 57165 | 1 | 5 | 0.00026708 |
| 64359 | 17 | 66 | 0.00033676 |
| 5336 | 16 | 354 | 0.00050823 |
| 721 | 6 | 5 | 0.00058998 |
| 23784 | 22 | 25 | 0.00061831 |
| 199223 | 3 | 19 | 0.00077712 |
| 129531 | 2 | 3 | 0.00083791 |
| 53616 | 7 | 144 | 0.00092337 |
| 767 | 8 | 81 | 0.00098055 |

"Gene ID" reflects that of Entrez Gene nomenclature. "CHR" reflects chromosomal location of a gene. "N SNPs" refers to the number of observed SNPs (and, thus, contributing to statistical calculations) in a gene. "P-value" reflects cumulative association of all SNPs per gene.

**Supplementary Table 4:** Summary of the top lncRNA genes (p≤1.00E-03) from genome-wide gene-based association analysis of aggressiveness in aADHD and cADHD respectively.

| WURS-derived aggressiveness in aADHD | | | |
|---|---|---|---|
| **GENE ID** | **CHR** | **N SNPs** | **P-value** |
| ENST00000427806 | 1 | 32 | 3.04E-05 |
| ENST00000439901 | 9 | 308 | 7.50E-05 |
| ENST00000566854 | 16 | 35 | 0.0001133 |
| ENST00000550680 | 14 | 61 | 0.00014633 |
| ENST00000565060 | 16 | 24 | 0.0001954 |
| ENST00000548631 | 14 | 38 | 0.00025783 |
| ENST00000445660 | 5 | 6 | 0.00027217 |
| ENST00000417409 | 1 | 41 | 0.00031884 |
| ENST00000512838 | 5 | 853 | 0.00035131 |
| ENST00000499939 | 16 | 9 | 0.00038143 |
| ENST00000554814 | 14 | 917 | 0.00042467 |
| ENST00000472514 | 3 | 85 | 0.00048725 |
| ENST00000563475 | 16 | 41 | 0.00056988 |
| ENST00000565633 | 16 | 37 | 0.00057884 |
| ENST00000414538 | 2 | 107 | 0.00058443 |
| ENST00000569710 | 7 | 4 | 0.00062894 |
| ENST00000453798 | 7 | 6 | 0.00063255 |
| ENST00000524252 | 8 | 49 | 0.0006423 |
| ENST00000566535 | 16 | 8 | 0.0006477 |
| ENST00000550470 | 12 | 135 | 0.00069247 |
| ENST00000476130 | 11 | 22 | 0.00074027 |
| ENST00000586962 | 19 | 71 | 0.00074715 |
| ENST00000550118 | 14 | 54 | 0.00080919 |
| ENST00000573772 | 17 | 90 | 0.00091225 |
| ENST00000606866 | 20 | 2 | 0.00092522 |
| ENST00000569786 | 16 | 32 | 0.00093974 |
| ENST00000504773 | 1 | 9 | 0.0009623 |
| ENST00000434635 | 2 | 59 | 0.00097046 |

| Defiant/Vindictive Dimension in cADHD | | | |
|---|---|---|---|
| **GENE ID** | **CHR** | **N SNPs** | **P-value** |
| ENST00000422117 | 15 | 83 | 6.93E-05 |
| ENST00000428142 | 17 | 5 | 0.00017532 |
| ENST00000433110 | 10 | 3 | 0.00025754 |
| ENST00000455238 | 1 | 2 | 0.00060977 |
| ENST00000452460 | 21 | 23 | 0.00064512 |
| ENST00000424138 | 1 | 4 | 0.00084691 |
| ENST00000357401 | 21 | 148 | 0.00087883 |

| Irritable Dimension in cADHD | | | |
|---|---|---|---|
| **GENE ID** | **CHR** | **NSNPs** | **P-value** |
| ENST00000433110 | 10 | 3 | 5.15E-06 |
| ENST00000422117 | 15 | 83 | 5.88E-05 |
| ENST00000502568 | 5 | 3 | 0.00029639 |
| ENST00000526206 | 11 | 9 | 0.00034421 |
| ENST00000420845 | 10 | 4 | 0.00051141 |
| ENST00000523584 | 5 | 190 | 0.00052843 |
| ENST00000440090 | 6 | 23 | 0.00054093 |
| ENST00000458633 | 6 | 5 | 0.00058998 |
| ENST00000435946 | 6 | 356 | 0.00063865 |
| ENST00000431422 | 6 | 32 | 0.00068667 |
| ENST00000425914 | 1 | 15 | 0.00071815 |
| ENST00000592607 | 1 | 15 | 0.00071815 |
| ENST00000585367 | 1 | 15 | 0.00071815 |
| ENST00000587839 | 1 | 15 | 0.00071815 |
| ENST00000547794 | 12 | 7 | 0.00079658 |
| ENST00000552469 | 12 | 7 | 0.00079658 |
| ENST00000510225 | 4 | 7 | 0.00086799 |
| ENST00000631169 | 1 | 16 | 0.00090993 |
| ENST00000631064 | 1 | 16 | 0.00090993 |

"Gene ID" reflects that of Ensembl transcript nomenclature. "CHR" reflects chromosomal location of a gene. "N SNPs" refers to the number of observed SNPs in a gene (and, thus, contributing to statistical calculations). "P-value" reflects cumulative association of all SNPs per gene.

**Supplementary Table 5:** Summary of the top GO terms (p≤0.01) from pathway analysis of aggressiveness in aADHD.

| WURS-derived aggressiveness in aADHD | | |
|---|---|---|
| **SET** | **N GENEs** | **P-value** |
| GO:0043124 | 12 | 0.00072631 |
| GO:0016571 | 12 | 0.0013408 |
| GO:0005520 | 19 | 0.0021063 |
| GO:0021987 | 30 | 0.0025693 |
| GO:0043046 | 11 | 0.0027753 |
| GO:0005905 | 44 | 0.0037567 |
| GO:0000786 | 40 | 0.003955 |
| GO:0048255 | 13 | 0.0056072 |
| GO:0008344 | 27 | 0.006711 |
| GO:0030195 | 10 | 0.006885 |
| GO:0050840 | 10 | 0.0070362 |
| GO:0007283 | 257 | 0.0074138 |
| GO:0042953 | 10 | 0.0080466 |
| GO:0005537 | 16 | 0.0081564 |
| GO:0043691 | 17 | 0.0083633 |
| GO:0001975 | 20 | 0.0085483 |

| | | |
|---|---|---|
| GO:0007155 | 511 | 0.0085612 |
| GO:0000299 | 17 | 0.0098109 |
| GO:0005901 | 49 | 0.0099714 |

| Defiant/Vindictive Dimension in cADHD | | |
|---|---|---|
| **SET** | **N GENEs** | **P-value** |
| GO:0005109 | 10 | 0.0026287 |
| GO:0045454 | 51 | 0.0033997 |
| GO:0051403 | 41 | 0.003553 |
| GO:0008305 | 26 | 0.0038064 |
| GO:0018298 | 14 | 0.0038912 |
| GO:0014070 | 77 | 0.0045464 |
| GO:0001657 | 34 | 0.0056237 |
| GO:0070469 | 33 | 0.0057444 |
| GO:0008137 | 32 | 0.0058441 |
| GO:0048661 | 27 | 0.0074798 |
| GO:0006120 | 31 | 0.0079751 |
| GO:0005452 | 16 | 0.0079799 |
| GO:0050680 | 23 | 0.008541 |
| GO:0045987 | 12 | 0.0089587 |
| GO:0007204 | 72 | 0.0091489 |
| GO:0005747 | 33 | 0.0091869 |

| Irritable Dimension in cADHD | | |
|---|---|---|
| **SET** | **N GENEs** | **P-value** |
| GO:0015250 | 11 | 0.00087038 |
| GO:0043034 | 10 | 0.0010436 |
| GO:0017046 | 19 | 0.0012288 |
| GO:0001568 | 27 | 0.0013583 |
| GO:0045859 | 10 | 0.0022 |
| GO:0045669 | 23 | 0.0026856 |
| GO:0001569 | 17 | 0.0032703 |
| GO:0004860 | 18 | 0.0032923 |
| GO:0005743 | 197 | 0.0048677 |
| GO:0015631 | 19 | 0.0051031 |
| GO:0001508 | 14 | 0.0059176 |
| GO:0045597 | 11 | 0.0059883 |
| GO:0071564 | 10 | 0.0060913 |
| GO:0007588 | 34 | 0.0068517 |
| GO:0005488 | 399 | 0.007008 |
| GO:0031124 | 26 | 0.0071667 |
| GO:0016192 | 155 | 0.0076797 |
| GO:0005921 | 14 | 0.0077692 |
| GO:0007126 | 53 | 0.0083275 |

"SET" reflects the name of the GO term (http://geneontology.org). "N GENEs" refers to the number genes in a term with observed SNP data (and, thus, contributing to statistical calculations). "P-value" reflects cumulative association of all genes per term after adjustment for genome-wide linkage disequilibrium, gene size and gene density per term.

**Supplementary Table 6:** Most Significant SNPs reaching p≤1.00E-03 in GWA analysis of WURS-derived childhood aggressiveness in aADHD and p<0.05 in GWA analyses of oppositional dimensions in cADHD.

| | Defiant/Vindictive Dimension in cADHD | | | | WURS-derived childhood aggressiveness in aADHD | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SNP | Effect Allele | BETA | SE | P-value | Effect Allele | Beta | SE | P-value | HetPVal |
| rs7229460 | C | -0.819 | 0.2189 | 0.0001969 | T | 0.8889 | 0.267 | 0.000872 | 0.3573 |
| rs7093307 | T | -2.738 | 0.7449 | 0.0002556 | T | 2.5782 | 0.7582 | 0.000672 | 0.4968 |
| rs1053561 | A | 0.6693 | 0.2246 | 0.00298 | A | 0.8478 | 0.253 | 0.000807 | 0.01241 |
| rs889061 | T | 0.6828 | 0.2367 | 0.004032 | T | -0.9092 | 0.2662 | 0.000635 | 0.0088 |
| rs11776310 | C | -1.715 | 0.6387 | 0.007414 | T | -2.2554 | 0.6855 | 0.001 | 0.1026 |
| rs6980667 | T | 2.064 | 0.7882 | 0.00901 | A | -3.7482 | 1.045 | 0.000334 | 0.08916 |
| rs6903040 | C | -0.7367 | 0.2854 | 0.01003 | T | 1.1071 | 0.3333 | 0.000894 | 0.437 |
| rs17073719 | C | 1.111 | 0.4355 | 0.01094 | T | -1.9961 | 0.5601 | 0.000365 | 0.7926 |
| rs1352368 | A | 0.5662 | 0.2222 | 0.01105 | A | -1.0398 | 0.2808 | 0.000212 | 0.01185 |
| rs12614111 | G | -0.5629 | 0.2263 | 0.0131 | C | -0.8823 | 0.2675 | 0.000974 | 0.1418 |
| rs17593647 | T | 1.137 | 0.4761 | 0.0172 | T | -2.6038 | 0.7267 | 0.000339 | 0.6409 |
| rs2115436 | T | -0.5809 | 0.2485 | 0.01968 | A | -1.0502 | 0.3137 | 0.000814 | 0.7213 |
| rs11546303 | G | -0.4922 | 0.2186 | 0.02464 | T | 1.1777 | 0.3537 | 0.000870 | 0.6717 |
| rs11716177 | T | -0.8679 | 0.3998 | 0.03025 | T | -1.8976 | 0.5746 | 0.000958 | 0.2187 |
| rs4077679 | C | 0.4783 | 0.2211 | 0.03082 | T | 0.8838 | 0.2679 | 0.000972 | 0.349 |
| rs4954579 | A | 0.577 | 0.2672 | 0.03111 | A | -1.3845 | 0.3974 | 0.000493 | 0.996 |
| rs2636639 | A | 0.4785 | 0.2226 | 0.03191 | A | -0.9456 | 0.2642 | 0.000344 | 0.1768 |
| rs17129503 | G | -0.8676 | 0.4039 | 0.03202 | A | 2.0724 | 0.613 | 0.000722 | 0.4513 |
| rs2141484 | G | 0.4335 | 0.2106 | 0.03993 | T | -1.108 | 0.3333 | 0.000887 | 0.6132 |
| rs6694431 | T | 0.7897 | 0.3869 | 0.04162 | T | -1.9851 | 0.4792 | 3.44E-05 | 0.8448 |
| rs7130044 | C | 0.4571 | 0.2262 | 0.04368 | T | -0.9435 | 0.2665 | 0.0004 | 0.2033 |
| rs2722013 | G | 0.4693 | 0.2345 | 0.04571 | A | -1.0683 | 0.3078 | 0.000519 | 0.8164 |
| rs12827088 | T | 0.5743 | 0.2871 | 0.04588 | T | 1.12 | 0.3341 | 0.000801 | 0.9039 |
| rs9347083 | A | 0.4698 | 0.2379 | 0.04866 | A | -1.0248 | 0.3038 | 0.000743 | 0.5482 |

| | Irritable dimension in cADHD | | | | WURS-derived childhood aggressiveness in aADHD | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SNP | Effect Allele | BETA | SE | P-value | Effect Allele | Beta | SE | P-value | HetPVal |
| rs7229460 | C | -0.1414 | 0.05015 | 0.004931 | T | 0.8889 | 0.267 | 0.000872 | 0.3573 |
| rs11776310 | C | -0.396 | 0.1459 | 0.006812 | T | -2.2554 | 0.6855 | 0.001 | 0.1026 |
| rs6980667 | T | 0.4707 | 0.1801 | 0.009167 | A | -3.7482 | 1.045 | 0.000334 | 0.08916 |
| rs4954579 | A | 0.152 | 0.06085 | 0.01269 | A | -1.3845 | 0.3974 | 0.000493 | 0.996 |
| rs10761445 | G | 0.1544 | 0.06285 | 0.01423 | A | -1.5704 | 0.4241 | 0.000213 | 0.4467 |
| rs1053561 | A | 0.1205 | 0.05166 | 0.01989 | A | 0.8478 | 0.253 | 0.000807 | 0.01241 |
| rs7115584 | C | 0.1266 | 0.05437 | 0.02013 | C | -0.9034 | 0.2718 | 0.000887 | 0.09245 |
| rs2636639 | A | 0.116 | 0.05067 | 0.02231 | A | -0.9456 | 0.2642 | 0.000344 | 0.1768 |
| rs4389996 | C | -0.4417 | 0.2065 | 0.03278 | T | 2.6511 | 0.7911 | 0.000805 | 0.08657 |
| rs2141484 | G | 0.1017 | 0.04809 | 0.0347 | T | -1.108 | 0.3333 | 0.000887 | 0.6132 |
| rs16923279 | G | -0.1929 | 0.09214 | 0.03661 | T | 1.763 | 0.4891 | 0.000312 | 0.1707 |
| rs3893429 | G | -0.1024 | 0.04908 | 0.03719 | A | 1.5454 | 0.3361 | 4.26E-06 | 0.665 |
| rs6049571 | T | 0.4013 | 0.193 | 0.03788 | A | -2.1148 | 0.5412 | 9.32E-05 | 0.3955 |
| rs655145 | G | -0.1007 | 0.04936 | 0.04167 | A | -0.8996 | 0.2598 | 0.000535 | 0.07896 |
| rs12284766 | G | 0.16 | 0.07873 | 0.04244 | C | -1.393 | 0.4047 | 0.000578 | 0.05734 |
| rs9347083 | A | 0.1092 | 0.05434 | 0.04484 | A | -1.0248 | 0.3038 | 0.000743 | 0.5482 |
| rs6959073 | G | 0.1043 | 0.05221 | 0.04608 | T | -0.9876 | 0.2698 | 0.000252 | 0.2959 |

"Beta" refers to effect size of each copy of the specified allele ("Effect allele") on the measure of aggressiveness in ADHD. "SE" refers to standard error. "P-value" reflects the strength of association signal. "Het p-value" reflects the level of heterogeneity among the examined aADHD samples of IMpACT.

**Supplementary Table 7:** Top hits (p≤1-00E-05) observed in genome-wide association meta-analysis of childhood aggressiveness in aADHD and their corresponding statistics observed in cADHD.

*Supplementary Table 7 is available upon request (61 pages).*

"Beta" refers to the effect size of each copy of the specified allele ("Effect allele") on the measure of aggressiveness in aADHD or cADHD. "SE" refers to standard error. "P-value" reflects the strength of association signal. "Het p-value" reflects the level of heterogeneity among the examined aADHD samples of IMpACT. "CHR" refers to a chromosome where a SNP is located and "Gene" is referring to a gene within which a SNP is located, nearest gene is determined as a gene located within ±20Kbp around a SNP.

**Supplementary Table 8:** Summary of aggressiveness-related GWA loci from previous studies (p≤1.00E-05) and presented analysis in aADHD and cADHD respectively.

| Candidate loci from GWA studies | | | | | | Present study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Previous studies | | | | WURS-derived childhood aggressiveness in aADHD | | | Defiant/Vindictive dimension in cADHD | | | Irritable dimension in cADHD | | |
| Study (reference) | Phenotype (sample size) | SNP | Gene (nearest gene) | Effect Size (effect allele) | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value |
| Genome-Wide Association Study of Proneness to Anger (Mick et al., 2014) | Anger measured by Spielberger State-Trait Anger Scale (8,747 individuals of European descent) | rs12249434 | intergenic (PNLIP) | 0.22 (A) | 4.3E-06 | T | -0.1340 | 0.7672 | T | 0.011 | 0.979 | T | -0.126 | 0.176 |
| | | **rs1299926** | **ABAT** | **0.26 (T)** | **7.8E-06** | **A** | **1.1855** | **0.03011** | NA | NA | NA | NA | NA | NA |
| | | **rs16840114** | **FMN2** | **0.25 (C)** | **9.1E-06** | A | 0.8544 | 0.1131 | G | -0.596 | 0.139 | **G** | **-0.192** | **0.036** |
| | | rs16924133 | HIPK2 | 0.38 (A) | 1.7E-06 | A | -0.0152 | 0.9853 | T | 0.558 | 0.343 | T | 0.099 | 0.461 |
| | | rs2148710 | FYN | 0.22 (T) | 2.9E-08 | T | 0.1576 | 0.6377 | T | -0.24 | 0.421 | T | -0.099 | 0.148 |
| | | rs2844775 | TRIM26 | 0.15 (A) | 8.3E-07 | A | 0.3027 | 0.2446 | A | 0.019 | 0.943 | A | -0.007 | 0.905 |
| | | rs3752433 | PHEX | 0.15 (A) | 2.5E-07 | A | -0.0550 | 0.8333 | NA | NA | NA | NA | NA | NA |
| | | rs555017 | MBOAT1 | 0.15 (T) | 4.0E-06 | C | 0.3494 | 0.1649 | A | 0.009 | 0.966 | A | 0.078 | 0.120 |
| | | rs6012564 | ARFGEF2 | 0.12 (G) | 6.7E-06 | T | 0.2309 | 0.4718 | G | -0.27 | 0.215 | G | -0.06 | 0.203 |
| | | rs670292 | IYD | 0.13 (C) | 1.6E-06 | T | 0.0492 | 0.9013 | NA | NA | NA | NA | NA | NA |
| | | rs6834498 | intergenic | 0.16 (A) | 4.9E-06 | A | -0.1718 | 0.642 | T | -0.049 | 0.857 | T | 0.015 | 0.802 |
| | | **rs6954895** | **intergenic** | **0.16 (G)** | **2.1E-07** | T | 0.0702 | 0.9261 | C | 0.33 | 0.168 | **C** | **0.178** | **0.0011** |
| | | rs7578047 | intergenic (LOC102724389 and XR_245020.2) | 0.22 (C) | 4.4E-06 | A | -0.6085 | 0.3307 | G | -0.125 | 0.698 | G | 0.059 | 0.419 |
| | | rs8102754 | intergenic (NA5SP470) | 0.12 (A) | 8.3E-06 | A | 0.3488 | 0.1891 | T | -0.358 | 0.084 | T | -0.077 | 0.104 |
| Genome-wide association study of conduct disorder symptomatology. (Dick et al., 2011) | Conduct Disorder (872 substance dependence cases; 3,091 controls, all of European, African American and other ancestry controls) | rs11838918 | LINC00331 | 0.28 (C) | 1.3E-08 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | | rs12302829 | intergenic (LOC390282) | 0.35 (G) | 8E-06 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | | rs1256531 | LOC105370536 | 0.09 (G) | 4E-06 | A | -0.5719 | 0.3082 | G | 0.27 | 0.442 | G | 0.054 | 0.499 |
| | | rs16831128 | intergenic (GPR39) | 0.08 (G) | 5E-06 | A | -0.4063 | 0.3366 | G | -0.11 | 0.654 | G | -0.034 | 0.545 |
| | | rs16891867 | C1QTNF7 | 0.14(G) | 3.27E-09 | A | -0.0983 | 0.8846 | NA | NA | NA | NA | NA | NA |
| | | **rs2122554** | **intergenic** | **0.10 (A)** | **3E-06** | T | 0.4339 | 0.5558 | **T** | **1.53** | **0.023** | T | 0.11 | 0.481 |
| | | rs3136202 | ERCC4 | 0.07 (A) | 6E-06 | A | -0.6670 | 0.05199 | A | 0.313 | 0.157 | T | 0.035 | 0.501 |
| | | **rs4434872** | **LOC105371448** | **0.08 (T)** | **8E-06** | T | 0.2595 | 0.5606 | **T** | **-0.79** | **0.0056** | T | -0.03 | 0.651 |

| Candidate loci from GWA studies | | | | | | Present study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Previous studies | | | | WURS-derived childhood aggressiveness in aADHD | | | Defiant/Vindictive dimension in cADHD | | | Irritable dimension in cADHD | | |
| Study (reference) | Phenotype (sample size) | SNP | Gene (nearest gene) | Effect Size (effect allele) | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value |
| | | rs4792394 | LOC100506974 | 0.06 (C) | 9E-06 | A | 0.4440 | 0.2237 | C | -0.255 | 0.237 | C | -0.086 | 0.081 |
| | | rs6031252 | TOX2 | 0.11 (A) | 6E-06 | A | 0.7128 | 0.5876 | NA | NA | NA | NA | NA | NA |
| | | rs6750486 | intergenic | 0.10 (T) | 6E-06 | T | -0.6701 | 0.3578 | T | -0.47 | 0.37 | T | 0.017 | 0.884 |
| | | rs7762160 | PDE10A | 0.07 (C) | 1E-06 | T | -0.4080 | 0.2457 | C | 0.075 | 0.738 | C | -0.058 | 0.253 |
| | | rs7950811 | intergenic (LOC642791) | 0.16 (A) | 1.29E-08 | A | -0.1437 | 0.8111 | A | -0.366 | 0.489 | A | -0.11 | 0.340 |
| | | rs8179116 | SELPLG LOC105369968 | 0.23 (A) | 3E-06 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Genome-wide association data suggest ABCB1 and immune-related gene sets may be involved in adult antisocial behavior (Salvatore et al., 2015) | Adult antisocial behavior (1379 participants from a case–control study in which the cases met criteria for alcohol dependence, all participants were of European ancestry) | rs1016793 | ABCB1 | 0.37 (G) | 5.91E-07 | A | -0.1282 | 0.6185 | NA | NA | NA | NA | NA | NA |
| | | rs1016794 | ABCB1 | 0.35 (G) | 1.05E-06 | A | -0.0664 | 0.7956 | NA | NA | NA | NA | NA | NA |
| | | rs10234411 | ABCB1 | -0.34 (T) | 1.94E-06 | A | 0.1919 | 0.4469 | T | -0.057 | 0.798 | T | -0.006 | 0.912 |
| | | rs10276036 | ABCB1 | -0.36 (C) | 6.22E-07 | T | -0.1004 | 0.7681 | C | -0.036 | 0.871 | C | -0.012 | 0.806 |
| | | rs10808072 | ABCB1 | -0.33 (A) | 2.69E-06 | A | -0.3970 | 0.1114 | A | -0.217 | 0.324 | A | -0.027 | 0.598 |
| | | rs10985798 | RC3H2 | 0.66 (G) | 3.88E-06 | C | -0.3848 | 0.4855 | NA | NA | NA | NA | NA | NA |
| | | rs10985807 | RC3H2 | 0.66 (C) | 3.88E-06 | T | -0.3851 | 0.4851 | NA | NA | NA | NA | NA | NA |
| | | rs10985892 | STRBP | 0.67 (T) | 2.35E-06 | T | 0.5667 | 0.299 | C | 0.135 | 0.754 | C | 0.016 | 0.866 |
| | | rs10985900 | STRBP | 0.67 (T) | 2.34E-06 | T | 0.4665 | 0.3948 | C | 0.123 | 0.774 | C | 0.017 | 0.858 |
| | | rs10985908 | STRBP | 0.67 (C) | 2.35E-06 | T | -0.4675 | 0.3939 | T | -0.04 | 0.928 | T | -0.006 | 0.948 |
| | | rs10985911 | STRBP | 0.68 (T) | 2.04E-06 | T | 0.5154 | 0.3424 | NA | NA | NA | NA | NA | NA |
| | | rs10985923 | intergenic (STRBP) | 0.71 (G) | 9.10E-07 | T | -0.5772 | 0.3021 | NA | NA | NA | NA | NA | NA |
| | | rs1128503 | ABCB1 | -0.36 (A) | 5.97E-07 | A | -0.0543 | 0.8314 | A | -0.036 | 0.871 | A | -0.012 | 0.806 |
| | | rs11763872 | ABCB1 | 0.33 (T) | 4.40E-06 | T | -0.2598 | 0.456 | NA | NA | NA | NA | NA | NA |
| | | rs11975994 | ABCB1 | -0.35 (G) | 1.05E-06 | A | 0.0643 | 0.8019 | NA | NA | NA | NA | NA | NA |
| | | rs12002466 | RABGAP1 | 0.65 (T) | 3.40E-06 | T | 0.9509 | 0.1555 | NA | NA | NA | NA | NA | NA |
| | | rs1202165 | ABCB1 | 0.35 (G) | 3.00E-06 | A | -0.0975 | 0.7077 | C | 0.038 | 0.861 | C | -0.053 | 0.284 |
| | | rs1202167 | ABCB1 | 0.35 (C) | 1.37E-06 | T | -0.0591 | 0.818 | NA | NA | NA | NA | NA | NA |
| | | rs1202168 | ABCB1 | 0.35 (G) | 1.53E-06 | A | -0.0605 | 0.8135 | NA | NA | NA | NA | NA | NA |

| Candidate loci from GWA studies | | | | | | Present study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Previous studies | | | | WURS-derived childhood aggressiveness in aADHD | | | Defiant/Vindictive dimension in cADHD | | | Irritable dimension in cADHD | | |
| Study (reference) | Phenotype (sample size) | SNP | Gene (nearest gene) | Effect Size (effect allele) | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value |
| | | rs1202169 | ABCB1 | 0.35 (T) | 1.55E-06 | T | 0.0616 | 0.8104 | NA | NA | NA | NA | NA | NA |
| | | rs12378760 | RC3H2 | 0.65 (T) | 4.46E-06 | T | 0.3226 | 0.5574 | C | -0.032 | 0.940 | C | -0.005 | 0.962 |
| | | rs12514901 | intergenic (XR_948719.1) | -0.54 (T) | 4.84E-06 | T | 0.4580 | 0.278 | NA | NA | NA | NA | NA | NA |
| | | rs12539098 | ABCB1 | 0.35 (T) | 1.04E-06 | T | 0.0547 | 0.8311 | NA | NA | NA | NA | NA | NA |
| | | rs12704364 | ABCB1 | -0.34 (C) | 1.83E-06 | T | 0.3926 | 0.1151 | C | -0.225 | 0.306 | C | -0.02 | 0.646 |
| | | **rs12979706** | **CDF15** | **-0.44 (G)** | **4.75E-06** | **A** | **-0.8899** | **0.00959** | NA | NA | NA | NA | NA | NA |
| | | rs142285425 | STRBP | 0.67 (A) | 2.34E-06 | A | 0.4664 | 0.3948 | NA | NA | NA | NA | NA | NA |
| | | rs17149293 | intergenic (XR_948719.1) | -0.55 (G) | 4.53E-06 | T | -0.4678 | 0.2711 | T | -0.669 | 0.074 | **T** | **-0.179** | **0.037** |
| | | rs180996880 | GREB1 | -0.65 (C) | 3.41E-06 | A | 0.1568 | 0.7557 | NA | NA | NA | NA | NA | NA |
| | | rs2032582 | ABCB1 | -0.35 (A) | 1.23E-06 | A | -0.0080 | 0.981 | A | -0.04 | 0.856 | A | -0.002 | 0.964 |
| | | rs2235013 | ABCB1 | -0.34 (C) | 1.83E-06 | T | 0.4062 | 0.1034 | C | -0.225 | 0.306 | C | -0.02 | 0.646 |
| | | rs2235020 | ABCB1 | 0.36 (T) | 7.30E-07 | A | -0.0639 | 0.8034 | NA | NA | NA | NA | NA | NA |
| | | rs2235021 | ABCB1 | 0.36 (C) | 7.15E-07 | A | -0.0646 | 0.8014 | NA | NA | NA | NA | NA | NA |
| | | rs2235026 | ABCB1 | -0.36 (T) | 6.32E-07 | T | -0.0407 | 0.8732 | NA | NA | NA | NA | NA | NA |
| | | rs2235027 | ABCB1 | -0.34 (G) | 1.91E-06 | T | 0.3938 | 0.1139 | NA | NA | NA | NA | NA | NA |
| | | rs2235033 | ABCB1 | -0.34 (A) | 1.83E-06 | A | -0.2424 | 0.462 | A | -0.226 | 0.306 | A | -0.02 | 0.646 |
| | | rs2235046 | ABCB1 | -0.35 (T) | 8.20E-07 | T | -0.1309 | 0.6061 | T | -0.121 | 0.581 | T | -0.029 | 0.565 |
| | | rs2373586 | ABCB1 | -0.36 (A) | 9.85E-07 | A | -0.2262 | 0.3754 | A | -0.04 | 0.856 | A | -0.002 | 0.964 |
| | | rs2520464 | ABCB1 | 0.35 (C) | 1.06E-06 | T | -0.0546 | 0.8314 | NA | NA | NA | NA | NA | NA |
| | | rs28368138 | IFNA10 | -0.82 (C) | 2.97E-06 | C | -0.6651 | 0.2782 | NA | NA | NA | NA | NA | NA |
| | | rs3789244 | ABCB1 | -0.36 (G) | 6.22E-07 | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| | | rs41277128 | intergenic (RC3H2) | 0.66 (G) | 3.85E-06 | A | -0.3186 | 0.5627 | NA | NA | NA | NA | NA | NA |
| | | rs4148738 | ABCB1 | -0.34 (C) | 1.96E-06 | T | 0.2034 | 0.4201 | C | -0.057 | 0.798 | C | -0.0057 | 0.912 |
| | | rs4728700 | ABCB1 | -0.36 (T) | 6.08E-07 | T | -0.1226 | 0.6295 | T | -0.087 | 0.692 | T | -0.022 | 0.666 |
| | | rs4728702 | ABCB1 | -0.36 (A) | 5.77E-07 | A | -0.0464 | 0.8557 | A | -0.036 | 0.871 | A | -0.012 | 0.806 |

| Candidate loci from GWA studies | | | | | | Present study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Previous studies | | | | WURS-derived childhood aggressiveness in aADHD | | | Defiant/Vindictive dimension in cADHD | | | Irritable dimension in cADHD | | |
| Study (reference) | Phenotype (sample size) | SNP | Gene (nearest gene) | Effect Size (effect allele) | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value |
| | | rs4810138 | APCDD1L-AS1 | 0.38 (G) | 1.90E-06 | C | 0.3575 | 0.2123 | NA | NA | NA | NA | NA | NA |
| | | rs5020877 | GREB1 | -0.67 (A) | 2.12E-06 | A | -0.1313 | 0.7933 | NA | NA | NA | NA | NA | NA |
| | | rs55919124 | LOC105379151 | 0.56 (T) | 4.05E-06 | A | -0.4574 | 0.2892 | NA | NA | NA | NA | NA | NA |
| | | rs55980995 | intergenic (XR_948719.1) | -0.52 (A) | 2.84E-06 | A | 0.2261 | 0.579 | NA | NA | NA | NA | NA | NA |
| | | rs58738000 | GREB1 | -0.66 (C) | 1.87E-06 | C | -0.0438 | 0.9316 | NA | NA | NA | NA | NA | NA |
| | | rs62578960 | intergenic (RABGAP1, STRBP, NR_026677.1) | 0.66 (G) | 3.97E-06 | A | -0.5183 | 0.3433 | NA | NA | NA | NA | NA | NA |
| | | rs62578996 | STRBP | 0.66 (G) | 2.56E-06 | A | -0.6141 | 0.2565 | NA | NA | NA | NA | NA | NA |
| | | rs62579000 | STRBP | 0.67 (C) | 2.34E-06 | T | -0.4664 | 0.3949 | NA | NA | NA | NA | NA | NA |
| | | rs62580884 | RC3H2 | 0.66 (C) | 3.88E-06 | A | -0.3848 | 0.4855 | NA | NA | NA | NA | NA | NA |
| | | rs62580922 | RC3H2 | 0.66 (T) | 3.88E-06 | T | 0.3849 | 0.4855 | NA | NA | NA | NA | NA | NA |
| | | rs62580923 | RC3H2 | 0.65 (A) | 4.13E-06 | A | 0.3564 | 0.5176 | NA | NA | NA | NA | NA | NA |
| | | rs62817660 | ABCB1 | 0.35 (G) | 1.03E-06 | A | 0.0824 | 0.8085 | NA | NA | NA | NA | NA | NA |
| | | **rs6413435** | **GDF15** | **-0.44 (G)** | **4.18E-06** | **A** | **-0.8893** | **0.00963** | NA | NA | NA | NA | NA | NA |
| | | rs6504898 | intergenic | -0.36 (G) | 1.42E-06 | T | 0.1836 | 0.4724 | NA | NA | NA | NA | NA | NA |
| | | rs6504902 | intergenic | -0.35 (T) | 1.88E-06 | T | -0.1510 | 0.5558 | C | -0.173 | 0.419 | C | 0.013 | 0.784 |
| | | rs67242082 | intergenic | -0.48 (A) | 3.66E-06 | A | -0.0969 | 0.798 | NA | NA | NA | NA | NA | NA |
| | | rs68152859 | GREB1 | -0.67 (G) | 2.02E-06 | A | 0.1308 | 0.794 | NA | NA | NA | NA | NA | NA |
| | | **rs6893509** | **intergenic** | **-0.55 (T)** | **2.92E-06** | T | 0.6448 | 0.118 | G | -0.669 | 0.074 | **G** | **-0.179** | **0.037** |
| | | rs6948766 | ABCB1 | -0.33 (G) | 4.32E-06 | A | 0.4745 | 0.05914 | NA | NA | NA | NA | NA | NA |
| | | rs6959435 | ABCB1 | -0.36 (G) | 9.82E-07 | T | 0.2174 | 0.3936 | NA | NA | NA | NA | NA | NA |
| | | rs6961665 | ABCB1 | -0.34 (C) | 1.83E-06 | A | 0.3926 | 0.1151 | C | -0.225 | 0.306 | C | -0.023 | 0.646 |
| | | rs7033878 | RC3H2 | 0.66 (T) | 3.88E-06 | T | 0.4535 | 0.4097 | NA | NA | NA | NA | NA | NA |
| | | rs7034822 | RC3H2 | 0.65 (G) | 4.46E-06 | A | -0.3848 | 0.4856 | A | -0.032 | 0.940 | A | -0.005 | 0.962 |
| | | rs74602468 | intergenic (XR_948719.1) | -0.56 (C) | 3.48E-06 | T | -0.4622 | 0.2773 | NA | NA | NA | NA | NA | NA |

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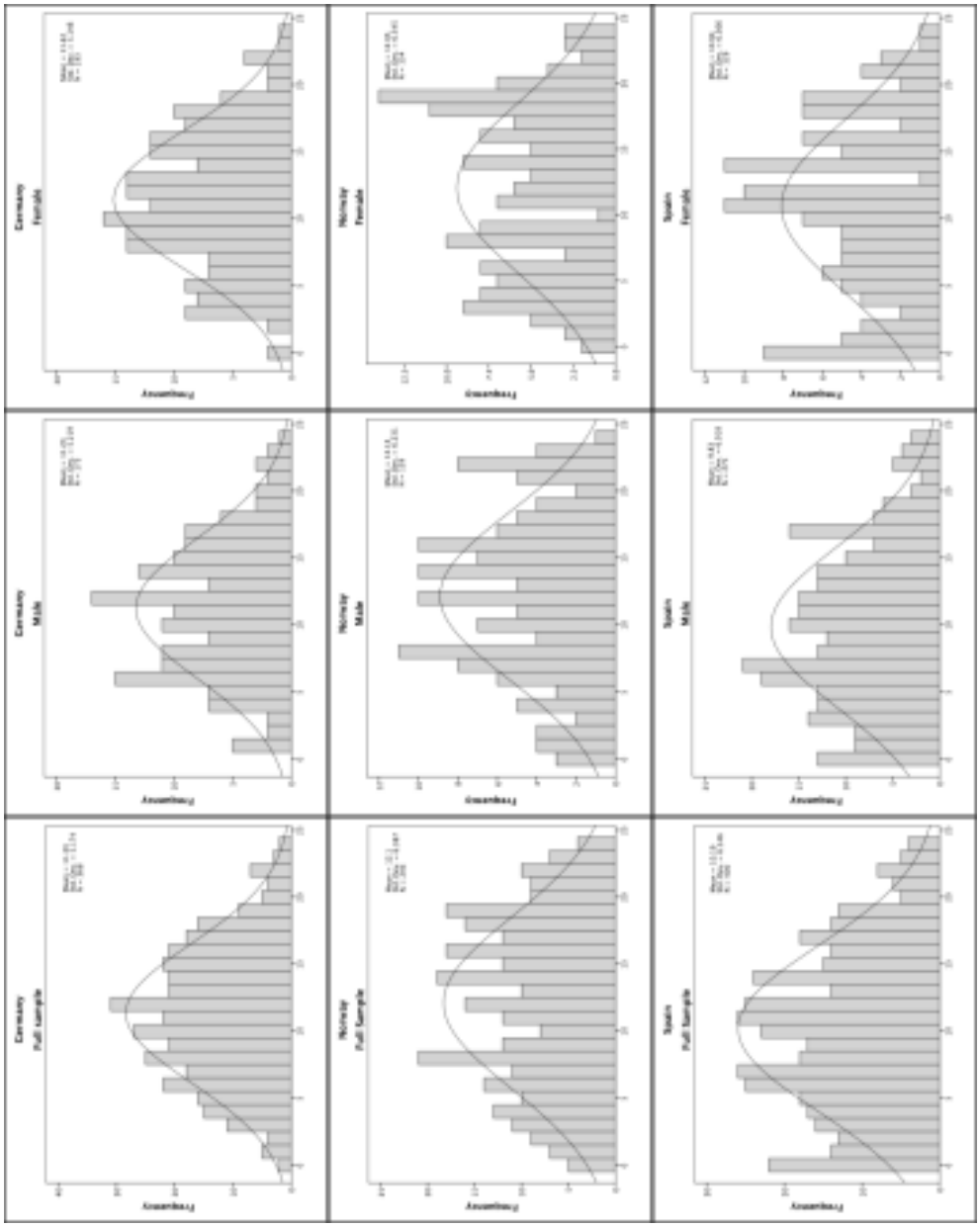
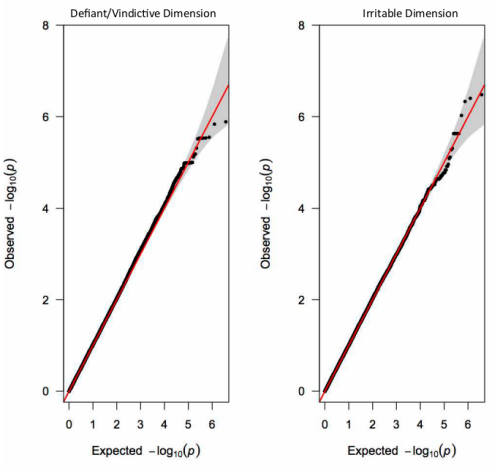
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Previous studies | | | | WURS-derived childhood aggressiveness in aADHD | | | Defiant/Vindictive dimension in cADHD | | | Irritable dimension in cADHD | | |
| Study (reference) | Phenotype (sample size) | SNP | Gene (nearest gene) | Effect Size (effect allele) | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value |
| | | rs76585835 | intergenic (XR_948719.1) | -0.56 (G) | 3.83E-06 | A | -0.4742 | 0.2652 | NA | NA | NA | NA | NA | NA |
| | | rs76809806 | intergenic (XR_948719.1) | -0.55 (G) | 4.66E-06 | A | -0.4523 | 0.2887 | NA | NA | NA | NA | NA | NA |
| | | rs78303085 | intergenic (RABGAP1, STRBP, NR_026677.1) | 0.66 (T) | 3.96E-06 | A | -0.5182 | 0.3435 | NA | NA | NA | NA | NA | NA |
| | | rs78479583 | intergenic (XR_948719.1) | -0.55 (G) | 4.96E-06 | A | -0.4675 | 0.2724 | NA | NA | NA | NA | NA | NA |
| | | rs7870519 | STRBP | 0.67 (A) | 2.34E-06 | A | 0.5319 | 0.3284 | T | 0.135 | 0.754 | T | 0.016 | 0.866 |
| | | rs80233205 | intergenic (XR_948719.1) | -0.56 (G) | 4.03E-06 | A | -0.4596 | 0.2806 | NA | NA | NA | NA | NA | NA |
| | | rs868755 | ABCB1 | -0.37 (T) | 6.15E-07 | T | -0.1125 | 0.6606 | T | 0.018 | 0.935 | T | -0.0009 | 0.968 |
| Conduct disorder and ADHD: evaluation of conduct problems as a categorical and quantitative trait in the international multicentre ADHD genetics study. (Anney et al., 2008) | Attention deficit hyperactivity disorder and conduct disorder (938 cases; European ancestry trios) | rs10229603 | LOC101988318 | (C) | 5E-06 | T | 0.0677 | 0.8543 | C | 0.250 | 0.273 | C | 0.087 | 0.093 |
| | | **rs10492664** | **intergenic** | **(C)** | **1E-06** | **T** | **0.9760** | **0.03761** | T | 0.448 | 0.123 | T | 0.02 | 0.762 |
| | | **rs10797919** | **RGL1** | **(G)** | **9E-06** | C | -0.3999 | 0.1226 | **C** | **0.439** | **0.041** | C | 0.09 | 0.066 |
| | | rs10815798 | intergenic | (A) | 6E-06 | A | 0.5269 | 0.1292 | A | -0.103 | 0.622 | A | -0.054 | 0.260 |
| | | **rs10831284** | **intergenic** | **(G)** | **2E-06** | A | 0.7036 | 0.137 | **G** | **0.86** | **0.005** | **G** | **0.181** | **0.009** |
| | | rs12921846 | RBFOX1 | (A) | 9E-06 | A | -0.0608 | 0.8521 | NA | NA | NA | NA | NA | NA |
| | | rs1381102 | intergenic | (A) | 6E-06 | A | 0.0032 | 0.9928 | A | -0.015 | 0.944 | A | -0.025 | 0.617 |
| | | rs1521882 | KIAA2012 | (A) | 8E-06 | A | -0.0577 | 0.8945 | G | 0.383 | 0.157 | G | 0.01 | 0.866 |
| | | **rs1557488** | **KIRREL3** | **(T)** | **5E-06** | **T** | **0.9271** | **0.02646** | T | -0.199 | 0.478 | T | 0.049 | 0.447 |
| | | rs1644305 | intergenic | (A) | 8E-06 | A | -0.1609 | 0.5265 | A | 0.267 | 0.213 | A | 0.061 | 0.209 |
| | | rs16973500 | PKD1L3 | (C) | 7E-06 | T | 0.0077 | 0.9879 | T | -0.345 | 0.257 | T | -0.102 | 0.141 |
| | | rs1951082 | LOC101927062 | (T) | 5E-06 | A | -0.3618 | 0.1674 | T | 0.349 | 0.109 | **T** | **0.122** | **0.014** |
| | | **rs2180233** | **intergenic** | **(C)** | **9E-06** | T | -0.5724 | 0.1189 | **C** | **0.714** | **0.0018** | **C** | **0.153** | **0.0036** |
| | | **rs2764980** | **LOC105376353** | **(A)** | **9E-06** | A | 0.2470 | 0.3265 | G | -0.357 | 0.09 | **G** | **-0.11** | **0.023** |
| | | rs2826340 | intergenic | (T) | 2E-06 | A | -0.0935 | 0.7893 | T | 0.238 | 0.406 | T | 0.021 | 0.749 |

| Candidate loci from GWA studies | | | | | | Present study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Previous studies | | | | WURS-derived childhood aggressiveness in aADHD | | | Defiant/Vindictive dimension in cADHD | | | Irritable dimension in cADHD | | |
| Study (reference) | Phenotype (sample size) | SNP | Gene (nearest gene) | Effect Size (effect allele) | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value | Effect Allele | Effect Size | p-value |
| | | rs4533251 | intergenic (XR_932671.1) | (T) | 4E-06 | T | 0.3951 | 0.4112 | T | 0.360 | 0.227 | T | 0.111 | 0.102 |
| | | **rs4889240** | **PKD1L2** | **(T)** | **7E-06** | **T** | **-0.7279** | **0.00387** | **T** | **-0.537** | **0.009** | T | -0.054 | 0.247 |
| | | rs6427356 | intergenic | (G) | 8E-06 | A | 0.2595 | 0.5117 | G | 0.413 | 0.079 | **G** | **0.129** | **0.016** |
| | | rs6733379 | LINC01317 | (G) | 4E-06 | T | -0.5850 | 0.1284 | G | 0.124 | 0.603 | G | 0.023 | 0.669 |
| | | rs701157 | Intergenic (XR_949260.1) | (C) | 4E-06 | T | -0.3190 | 0.2222 | C | -0.282 | 0.181 | C | -0.066 | 0.173 |
| | | rs7236632 | ATP8B1 | (A) | 6E-06 | A | -0.6451 | 0.1768 | G | -0.388 | 0.197 | G | -0.096 | 0.163 |
| | | rs7297018 | PAWR | (A) | 4E-06 | A | -0.9981 | 0.02346 | A | -0.012 | 0.963 | A | -0.061 | 0.326 |
| | | rs7595103 | LOC101927967 | (A) | 7E-06 | A | 0.5078 | 0.148 | G | 0.130 | 0.563 | G | 0.071 | 0.162 |
| | | **rs789560** | **MYRFL** | **(G)** | **7E-06** | T | 0.8335 | 0.09736 | **T** | **-0.641** | **0.05** | T | -0.114 | 0.124 |
| | | rs931812 | YWHAZ | (C) | 5E-06 | T | -0.3968 | 0.1668 | T | -0.008 | 0.972 | T | 0.0015 | 0.976 |
| | | rs9512900 | PDX1-AS1 | (C) | 9E-06 | T | 0.0393 | 0.8843 | C | -0.218 | 0.316 | T | -0.0007 | 0.988 |

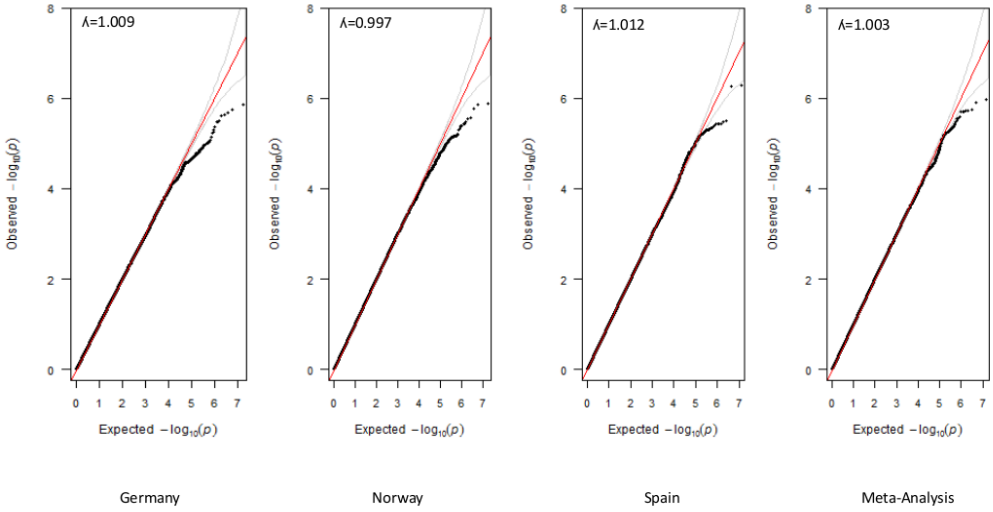
All effect sizes per SNP are reported as beta value of linear regression. "NA" refers to non-applicable and reflects instances when we observed no SNP data in our sample. SNPs reaching p-value below 0.05 in our sample are highlighted in bold. "Gene" is referring to a gene within which a SNP is located, nearest gene is determined as a gene located within ±20Kbp around a SNP.



**Supplementary Figure 1:** Distribution of WURS derived aggression measure in the examined datasets.




**Supplementary Figure 2:** QQ plots of GWA analysis of oppositional dimensions in cADHD.




**Supplementary Figure 3:** QQ plots of the GWA analysis of aggressiveness in aADHD.

# Chapter 4

## Monoamine and neuroendocrine gene-sets associate with frustration-based aggression in a gender-specific manner

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## ABSTRACT

Investigating phenotypic heterogeneity in aggression and understanding the molecular biological basis of aggression subtypes may lead to new prevention and treatment options. In the current study, we evaluated the taxonomy of aggression and examined specific genetic mechanisms underlying aggression subtypes in healthy males and females. Confirmatory Factor Analysis (CFA) was used to replicate a recently reported three-factor model of the Reactive Proactive Questionnaire (RPQ) in healthy adults (n=661; median age 24.0 years; 41% male). Gene-set association analysis, aggregating common genetic variants within (a combination of) three molecular pathways previously implicated in aggression, i.e. serotonergic, dopaminergic, and neuroendocrine signaling, was conducted with MAGMA software in males and females separately (total n=395) for aggression subtypes. We replicate the three-factor CFA model of the RPQ, and found males to score significantly higher on one of these factors compared to females: proactive aggression. The genetic association analysis showed a female-specific association of genetic variation in the combined gene-set with a different factor of the RPQ; reactive aggression due to internal frustration. Both the neuroendocrine and serotonergic gene-sets contributed significantly to this association. Our genetic findings are subtype- and sex-specific, stressing the value of efforts to reduce heterogeneity in research of aggression etiology. Importantly, subtype- and sex-differences in the underlying pathophysiology of aggression suggest that optimal treatment options will have to be tailored to the individual patient. Male and female needs of intervention might differ, stressing the need for sex-specific further research of aggression. Our work highlights opportunities for sample size maximization offered by population-based studies of aggression.

## INTRODUCTION

Aggression has been defined as any behavior directed toward the goal of causing harm or injury to others (Baron and Richardson, 1994). From an evolutionary perspective, aggressive behaviors can be adaptive and have an important role in survival and competition for resources (Georgiev et al., 2013). In modern societies, aggression often is maladaptive and associated with negative consequences, causing psychological and somatic burden to victims as well as to aggressive individuals themselves (Fergusson et al., 2005; Reef et al., 2010). Aggression poses a substantial financial burden on society, for example caused by increased legal costs and work absence (WHO, 2007). A better understanding of the subtypes and etiology of aggression is needed to facilitate prevention and to improve treatment options (Fergusson et al., 2005). Given that about half of the variance in aggressive behaviors may be explained by genetic influences (Tuvblad and Baker, 2011; Veroude et al., 2016), studying the molecular genetics underlying these behaviors can provide important mechanistic insights. Research into aggression etiology is, however, complicated by several factors, including considerable phenotypic as well as genetic heterogeneity and the existence of sex differences in aggressive behaviors (Baker et al., 2008; Georgiev et al., 2013).



### Subtypes of aggression

Heterogeneity in the etiology of aggression may be parsed by considering subtypes. Different classification systems have been proposed; one based on biological hypotheses is the distinction of proactive and reactive aggression (Dodge and Coie, 1987). Proactive aggression, also referred to as instrumental aggression, is goal-oriented, organized behavior often associated with low autonomic arousal and affect. Reactive aggression on the other hand, is also known as impulsive or affective aggression, and occurs in response to provocation or a negative emotional state (Raine et al., 2006; Stanford et al., 2003). Importantly, the subtypes have been associated with distinct behavioral, neurocognitive, and neural characteristics. For example, proactive aggression has been related to psychopathic traits and delinquent behavior (Cima and Raine, 2009; Cima et al., 2013), while the reactive subtype of aggression has been associated with impulsivity, anxiety, and hostile interpretation bias (Brugman et al., 2015; Bubier and Drabick, 2009). Twin studies showed slightly higher heritability estimates for proactive than reactive aggression (Baker et al., 2008; Brendgen et al., 2005; Tuvblad et al., 2009). The two aggression subtypes may have partially distinct genetic contributions. Serotonergic and dopaminergic neurotransmission may regulate both reactive and proactive aggression, whereas endocrine signaling seems to be more involved in the regulation of reactive aggression, e.g. through modulation of impulsivity and the stress response (Waltes et al., 2015). Recently, a further subdivision of reactive aggression has been proposed based on an exploratory factor analysis of the Reactive Proactive Questionnaire (RPQ). This analysis was conducted in a sample of adolescents (71.6% male), who were referred to clinical services for externalizing behavior problems (Smeets et al., 2016). Besides a proactive factor, reactive aggression was further subdivided into a subtype associated with external provocation or threat and another one associated with internal frustration. Improved fit indices for this three-factor model compared to the original two-factor model were also

reported based on an adult, males-only sample recruited partly in forensic psychiatric in- and outpatient clinics and partly from the general population. (Brugman et al., 2016). The reactive subtypes differed in their associated behavioral correlates, which suggests that the three-factor model may further reduce phenotypic heterogeneity and facilitate the search for genes involved in the etiology of aggression.

**Sex differences in aggression**

The most convincing observation supporting the existence of sex differences in aggression is the difference in crime rate statistics between males and females. Females are vastly less likely to commit serious offenses than males, and males are more likely to display antisocial behavior than females (Stephenson et al., 2014). Males are also overrepresented in aggression-related disorders such as conduct disorder (CD), where the gender ratio is approximately 2.5 (Hill, 2002). Importantly, sex differences are also found in the type of aggressive behavior displayed (Collett et al., 2003). The clear gender-specificity of aggression is thought to have evolved by sexual selection, and to reflect differences in optimal strategies in the competition for resources for males and females (Georgiev et al., 2013). Sex differences in heritability estimates have been observed in some but not all of the aggression twin studies conducted to date, with higher heritability estimates for boys than girls, when self-report measures were assessed (Baker et al., 2008; Wang et al., 2013). Incorporation of sex in aggression studies may be essential to identify the underlying biological mechanisms of aggressive behaviors.

**Biological systems**

The biological systems most investigated in the context of aggression phenotypes (as well as related traits such as mood disturbances and impulsivity) are the monoaminergic neurotransmitter systems related to serotonin and dopamine and the neuroendocrine system. Multiple reviews to date discuss these systems in the context of aggression and list the candidate genes that have been investigated for association with aggressive behaviors (Pavlov et al., 2012; Veroude et al., 2016; Waltes et al., 2015).

The serotonergic system is hypothesized to play a key role in aggression due to its influence on functions including social cognition, emotional regulation, and cognitive control (Lesch et al., 2012). Both human and animal studies link genes within these systems to aggressive behavior. For example, the serotonin transporter gene (*SLC6A4)* is one of the most investigated candidate genes for aggression. Variation in the serotonin receptor 2B gene (*5-HT2B*) has been associated with violent impulsivity in a Finnish population, and 5-HT2B and 5-HT1B knockout studies in mice implicate these genes in aggression and/or impulsivity (Bevilacqua et al., 2010; Nautiyal et al., 2015). While candidate genetic association studies have often produced equivocal results, investigations measuring levels of the serotonin metabolite 5-HIAA in cerebrospinal fluid, e.g. (Brown et al., 1979; Coccaro and Lee, 2010), or manipulating central serotonin function through tryptophan depletion/loading, e.g. (Bjork, 2000), have revealed a highly significant relationship between serotonin availability and aggression (Rosell and Siever, 2015). Dopamine is relevant for understanding aggression because of its effects on reward, motivated behavior, and decision

making (Costa et al., 2012). While studies of dopamine manipulation have mostly been conducted in animals, the involvement of dopamine in aggression is also evidenced by the fact that in humans, D2-receptor antagonists have been used effectively to treat aggressive behavior (Nelson and Trainor, 2007). Additional evidence linking the serotonergic and dopaminergic neurotransmitter systems comes from genetic association studies of the *MAOA* gene. This X-linked gene encodes the enzyme monoamine oxidase A, which breaks down both serotonin and dopamine, and has been robustly associated with aggression, especially in the context of stress and maltreatment (Brunner et al., 1993; Caspi et al., 2002; Byrd and Manuck, 2014). The third system implicated in aggression is the neuroendocrine system, including both stress-related hypothalamic-pituitary-adrenal (HPA) axis signaling and sex-hormone-related hypothalamo-pituitary-gonadal (HPG) axis signaling. As early life stress is known to increase risk for the development of mood and aggression-related disorders (Agid et al., 1999; Éthier et al., 2004; Fonagy, 2006; Heim et al., 2001), the neuroendocrine stress response with its genetic components is a major candidate system for the development of aggressive behaviors. The relation of the HPA axis to aggression has been well established, especially through animal studies (Veenema, 2009). Also in humans, cortisol levels have been related to aggression repeatedly (Alink et al., 2012; Loney et al., 2006; Popma et al., 2007; Shirtcliff et al., 2005; van Bokhoven et al., 2004). The HPG axis involves signaling between hypothalamus, pituitary, and the gonadal glands, which produce estrogen and testosterone. Testosterone levels have been related to human aggression (Book et al., 2001; Brown et al., 2008; Chichinadze et al., 2010; Yu and Shi, 2009) and it has been hypothesized that especially the interplay between cortisol and sex steroids is important in determining aggression liability (Pavlov et al., 2012; Terburg et al., 2009).
Extensive reviews of aggression candidate gene studies have recently been published (Fernandez-Castillo and Cormand, 2016; Pavlov et al., 2012; Veroude et al., 2016; Waltes et al., 2015). Although a moderate number of studies has been conducted, a meta-analysis of individual candidate variants did not reveal any significant associations with aggressive behavior (Vassos et al., 2014). One reason for this may be the complex genetic background of aggression in most people. While a few monogenic aggression disorders caused by rare genetic variations with a high effect size exist (Brunner et al., 1993; Zhang-James et al., 2016), aggression in the population has a complex and polygenic genetic background, which can be aggravated by environmental factors (Veroude et al., 2016).

In the current study, we assessed the genetic mechanisms underlying aggression subtypes in the general population. Firstly, we aimed to verify the existence of three aggression subtypes in adult males and females from the general population based on the RPQ. Second, we aimed to assess the association of common genetic variants in the three biological systems with most evidence for a role in aggression, i.e. the serotonergic system, the dopaminergic system, and the neuroendocrine system with the different subtypes. We aimed to maximize power for finding genetic associations by (1) parsing phenotypic heterogeneity through differentiating between subtypes, (2) by assessing males and females separately, and (3) by combining genetic variants in a gene-set analysis (Bralten et al., 2011; Bralten et al., 2013; Naaijen et al., 2017).

## METHODS

### Sample

The investigated sample consisted of participants of the Brain Imaging Genetics (BIG) study conducted at the Donders Institute for Brain, Cognition and Behaviour (Franke et al., 2010). The BIG study consists of self-reported healthy adults, who participated in smaller-scale imaging studies at the institute and gave consent to be included in the BIG study. Saliva samples for genetic testing were collected, and an internet-based test-battery of questionnaires was applied. The Reactive Proactive Questionnaire (RPQ; Raine et al., 2006) was available for 661 participants (age range 18-45 years). Of those, 395 participants had genome-wide genotyping data available.

All participants were of Caucasian descent and were screened using a self-report questionnaire for the following exclusion criteria before study participation: a history of somatic disease potentially affecting the brain, current or past psychiatric or neurological disorder, medication (except hormonal contraceptives) or illicit drug use during the past 6 months, history of substance abuse, current or past alcohol dependence, pregnancy, lactation, menopause, and magnetic resonance imaging contraindications (Gerritsen et al., 2012). All participants gave written informed consent, and the study was approved by the regional ethics committee.

### Aggression Questionnaire

The Reactive Proactive Questionnaire (RPQ) was used to assess subtypes of aggression (Raine et al., 2006). The RPQ is a self-report questionnaire consisting of 23 items. For each item, subjects are asked to indicate, how often they have engaged in a given type of behavior. Items are rated on a three-point Likert scale ('never' =0, 'sometimes' =1, 'often' =2). Responses were summed to yield the three factors that best described the RPQ in an earlier exploratory factor analysis (Smeets et al., 2016): 'proactive aggression', 'reactive aggression due to internal frustration', and 'reactive aggression due to external provocation'. Items relating to each subtype can be found in Supplementary Table 1.

### Factor analysis

Confirmatory factor analysis (CFA) was conducted using Mplus (version 6.11; https://www.statmodel.com/). Results were considered acceptable, when both the Comparative Fit Index (CFI) and the Tucker-Lewis Index (TLI) exceeded .90 (with values closer to 1 indicating better fit), and the Root Mean Squared Error of Approximation (RMSEA) was below .06 (with values closer to 0 indicating better fit) (Hu and Bentler, 1999; Smeets et al., 2016).

### Genotyping and imputation

Genetic analyses were carried out at the Department of Human Genetics of the Radboud University Medical Center. Saliva samples were collected using Oragene kits (DNA Genotek, Kanata, Canada), and genomic DNA was extracted as specified by the manufacturer. Genome-wide genotyping was performed on two different platforms, Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix Inc., Santa Clara, CA, USA) (n=243)

and the Infinium PsychArray-24 v1.1 BeadChip (http://www.illumina.com/products/psycharray.html) (n=152). Genotype calling and quality control steps are described in the Supplementary Information. MACH software was used for haplotype phasing and minimac for the final imputation (Howie et al., 2012; Li et al., 2010), with 1000 Genomes Phase 1.v3 reference data (Abecasis et al., 2012).

**Gene-set selection and construction**

Gene selection for aggression candidate gene-sets involved in neuroendocrine signaling, dopamine neurotransmission, and serotonin neurotransmission was performed using the Ingenuity Pathway Analysis (IPA) software (http://www.ingenuity.com). Ingenuity draws on the Ingenuity Knowledge Base which is based on information from published literature as well as on various other sources including gene expression and gene annotation databases. The serotonergic gene-set contained genes involved in serotonergic receptor signaling and de dopaminergic gene-set contained genes involved in dopaminergic receptor signaling. The neuroendocrine gene-set contained genes involved in corticotropin-releasing hormone, glucocorticoid, androgen, and estrogen signaling. An overview of selected genes can be found in Table 1. All single nucleotide polymorphisms (SNPs) in or within 100kb flanking regions of the genes (also capturing regulatory sequences; Veyrieras et al., 2008) were selected for analysis.

**Table 1:** Selected genes for each of the three gene-sets (serotonergic, dopaminergic, neuroendocrine).

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Serotonergic gene-set (n=43 genes)* | | | | | | | | | | |
| *5HT1A* | *5HT1B* | *5HT1D* | *5HT1E* | *5HT4* | *5HT6* | *5HT7* | *ADCY1* | *ADCY10* | *ADCY2* | *ADCY3* |
| *ADCY4* | *ADCY5* | *ADCY6* | *ADCY7* | *ADCY8* | *ADCY9* | *DDC* | *GCH1* | *GNAS* | *HTR2A* | *HTR2B* |
| ***HTR2C*** | *HTR3A* | *HTR3B* | *HTR3C* | *HTR3D* | *HTR3E* | *HTR5A* | *IL4I1* | ***MAOA*** | ***MAOB*** | *PCBD* |
| *PTS* | *QDPR* | *SERT* | *SLC18A1* | *SLC18A2* | *SLC18A3* | *SMOX* | *SPR* | *TPH1* | *TPH2* | |
| *Dopaminergic gene-set (n=77 genes)* | | | | | | | | | | |
| *ADCY1* | *ADCY10* | *ADCY2* | *ADCY3* | *ADCY4* | *ADCY5* | *ADCY6* | *ADCY7* | *ADCY8* | *ADCY9* | *CALY* |
| *COMT* | *DAT* | *DDC* | *DRD1* | *DRD2* | *DRD3* | *DRD4* | *DRD5* | *GCH1* | *GNAS* | *IL4I1* |
| ***MAOA*** | ***MAOB*** | *NCS1* | ***PCBD*** | *PPM1J* | *PPM1L* | *PPP1CA* | *PPP1CB* | *PPP1CC* | *PPP1R1B* | PPP1R10 |
| *PPP1R11* | *PPP1R12A* | *PPP1R14A* | *PPP1R14B* | *PPP1R14C* | *PPP1R14D* | *PPP1R3A* | *PPP1R3C* | *PPP1R3D* | *PPP1R7* | *PPP2CA* |
| *PPP2CB* | *PPP2R1A* | *PPP2R1B* | *PPP2R2A* | *PPP2R2B* | *PPP2R2C* | *PPP2R3A* | ***PPP2R3B*** | *PPP2R4* | *PPP2R5A* | *PPP2R5B* |
| *PPP2R5C* | *PPP2R5D* | *PPP2R5E* | *PRKACA* | *PRKACB* | *PRKACG* | *PRKAG1* | *PRKAG2* | *PRKAR1A* | *PRKAR1B* | *PRKAR2A* |
| *PRKAR2B* | *PRL* | *PTH* | *PTS* | *QDPR* | *SLC18A1* | *SLC18A2* | *SLC18A3* | *SMOX* | *SPR* | *TH* |
| *Neuroendocrine gene-set (n=426 genes)* | | | | | | | | | | |
| ***A2M*** | *CB1* | *EP300* | *GNB5* | *HSP40* | *KRAS* | *MEKK1* | *PIK3C2A* | *PRKAA2* | *SMAD2* | *TAF7* |
| *ACTB* | *CBP* | *ER* | *GNG10* | *HSP90AA1* | *KRT1* | *MKP1* | *PIK3C2B* | *PRKAB1* | *SMAD3* | ***TAF7L*** |
| *ACTL6B* | *CC10* | *ERCC2* | *GNG11* | *HSP90AB1* | *KRT32* | *MMP1* | *PIK3C2G* | *PRKAB2* | *SMAD4* | *TAF9* |
| *ADCY1* | *CCL11* | *ERCC3* | *GNG12* | *HSP90B1* | *KRT35* | *MNAT1* | *PIK3C3* | *PRKACA* | *SMARCA2* | ***TAF9B*** |
| *ADCY2* | *CCL13* | *ERβ* | *GNG13* | *HSPA14* | *KRT36* | *MR* | *PIK3CA* | *PRKACB* | *SMARCA4* | *TAK1* |
| *ADCY3* | *CCL2* | *FASLG* | *GNG2* | *HSPA1A* | *MAP2K1* | *MRAS* | *PIK3CB* | *PRKACG* | *SMARCB1* | *TAT* |
| *ADCY5* | *CCL3* | *FCGR1* | *GNG3* | *HSPA1B* | *MAP2K2* | *NCOA2* | *PIK3CD* | *PRKAG1* | *SMARCC1* | *TBP* |
| *ADCY6* | *CCL5* | *FGG* | *GNG4* | *HSPA1L* | *MAP2K4* | *NCOA3* | *PIK3CG* | *PRKAG2* | *SMARCC2* | *TEBP* |
| *ADCY7* | *CCNC* | *FKBP51* | *GNG5* | *HSPA2* | *MAP2K7* | *NCOR1* | *PIK3R1* | *PRKAR1A* | *SMARCD1* | *TFIIB* |
| *ADCY8* | *CCND1* | *FKBP52* | *GNG7* | *HSPA4* | *MAPK1* | *NCOR2* | *PIK3R2* | *PRKAR1B* | *SMARCD2* | *TGFB1* |
| *ADCY9* | *CCNH* | *FOS* | *GR* | *HSPA5* | *MAPK10* | *NFAT5* | *PIK3R3* | *PRKAR2A* | *SMARCD3* | *TGFB2* |
| *ADRB2* | ***CD163*** | *FOXO3A* | *GRB2* | *HSPA6* | *MAPK11* | *NFATC1* | *PIK3R4* | *PRKAR2B* | *SMARCE1* | *TGFB3* |
| *AGT* | *CD247* | *G6PC* | *GTF2A1* | *HSPA8* | *MAPK12* | *NFATC2* | *PIK3R5* | *PRKCA* | *SMILE* | *TGFBR1* |
| *AKT1* | *CD3D* | *G6PC2* | *GTF2A2* | *HSPA9* | *MAPK13* | *NFATC3* | *PIK3R6* | *PRKCB* | *SOS1* | *TGFBR2* |
| *AKT2* | *CD3E* | *G6PC3* | *GTF2E1* | *ICAM1* | *MAPK14* | *NFATC4* | *PLAU* | *PRKCD* | *SOS2* | *THRAP3* |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *AKT3* | *CD3G* | ***GILZ*** | *GTF2E2* | *IFNG* | *MAPK3* | *NFKB1* | *PLCG1* | *PRKCE* | *SRA1* | *TNF* |
| *ANF* | *CDK7* | *GLI1* | *GTF2F1* | *IGFBP1* | *MAPK8* | *NFKB2* | *PLCG2* | *PRKCG* | *SRC* | *TRA* |
| *ANXA1* | *CDK8* | *GLI2* | *GTF2F2* | *IKBKB* | *MAPK9* | *NFKBIA* | *POLR2A* | *PRKCH* | *SRC-1* | *TRAF2* |
| ***AR*** | *CDKN1A* | *GLI3* | *GTF2H1* | *IKBKE* | *MED10* | *NFKBIB* | *POLR2B* | *PRKCI* | ***SRY*** | *TRAF6* |
| *ARA55* | *CDKN1C* | *GNA11* | *GTF2H2* | ***IKBKG*** | ***MED12*** | *NFKBIE* | *POLR2C* | *PRKCQ* | *STAT1* | *TRB* |
| *ARA70* | *CEBPα* | *GNA12* | *GTF2H3* | *Il10* | *MED12L* | *NIK* | *POLR2D* | *PRKCZ* | *STAT3* | *TRRAP* |
| *ARID1A* | *CEBPβ* | *GNA13* | ***GTF2H4*** | *IL13* | *MED13* | *NOS1* | *POLR2E* | *PRKD1* | *STAT5A* | *TSG101* |
| *ARID2* | *CHP1* | *GNA14* | *GTF2H5* | *IL1B* | *MED13L* | *NOS2* | *POLR2F* | *PRKD3* | *STAT5B* | *UBC9* |
| *ATF2* | *CHUK* | *GNA15* | *GUCY1A2* | *IL1R2* | *MED15* | *NOS3* | *POLR2G* | *PRKDC* | *SUMO1* | *VCAM1* |
| *ATF4* | *COX2* | *GNAI1* | *GUCY1A3* | *IL1RA* | *MED16* | *NPR1* | *POLR2H* | *PRL* | *TAB1* | *VIPR1* |
| *ATM* | *CREB* | *GNAI2* | *GUCY1B3* | *IL2* | *MED17* | *NPR2* | *POLR2I* | *RAC1* | ***TAF1*** | *YWHAH* |
| *BAG1* | *CRH* | *GNAI3* | *GUCY2C* | *IL3* | *MED18* | *NR0B2* | *POLR2J* | *RAF1* | *TAF10* | |
| *BCL2* | *CRHR1* | *GNAL* | *GUCY2D* | *IL4* | *MED20* | *NR4A1* | *POLR2J2* | *REA* | *TAF11* | |
| *BCL2L1* | *CRHR2* | *GNAO1* | ***GUCY2F*** | *IL5* | *MED21* | *NRAS* | *POLR2J3* | *RELA* | *TAF12* | |
| *BDNF* | *CSF2* | *GNAQ* | *H3F3A* | *IL6* | *MED23* | *OPN1SW* | *POLR2K* | *RIP140* | *TAF13* | |
| *BGLAP* | *CSN2* | *GNAS* | *H3F3B* | *IL8* | *MED24* | *PAI1* | *POLR2L* | *RRAS* | *TAF15* | |
| *BRAF* | *CTBP1* | *GNAT1* | *HBO1* | *ITPR1* | *MED27* | *PBRM1* | *POMC* | *RRAS2* | *TAF1L* | |
| *BRD7* | *CTBP2* | *GNAT2* | *HDAC3* | *ITPR2* | *MED30* | *PBX* | *POU2F1* | *RTA* | *TAF2* | |
| *CALM1* | ***CXCL2*** | *GNAZ* | *HIST1H3C* | *ITPR3* | *MED31* | *PCAF* | *POU2F2* | *RUNX2* | *TAF3* | |
| *CALM2* | ***DAX1*** | *GNB1* | *HIST2H3C* | *IVL* | *MED4* | *PCK1* | *PPP3CA* | *SELE* | *TAF4* | |
| *CALM3* | *DDX5* | *GNB1L* | *HIST3H3* | *JAK1* | *MED6* | *PCK2* | *PPP3CB* | *SGK1* | *TAF4B* | |
| *CALML5* | *DPF1* | *GNB2* | *HLTF* | *JAK2* | *MEF2A* | *PELP1* | *PPP3CC* | *SHARP* | *TAF5* | |
| *CALR* | ***DRIP150*** | *GNB2L1* | *HMG-1* | *JAK3* | *MEF2B* | *PGC-1* | *PPP3R1* | *SHBG* | *TAF5L* | |
| *CAMK4* | *DRIP205* | *GNB3* | *HNRNPD* | *JUN* | *MEF2C* | *PGR* | *PPP3R2* | *SHC* | *TAF6* | |
| *CARM1* | ***ELK1*** | *GNB4* | *HRAS* | *JUND* | *MEF2D* | *PHF10* | *PRKAA1* | *SLPI* | *TAF6L* | |

**Bold**: Located on the X- or Y-chromosome or not captured by the genotyping array. The selected dopamine and serotonin pathway-sets overlap in 24 genes. The neuroendocrine set overlaps with the serotonin-set in 9 genes and with the dopamine-set in 19 genes.

**Gene-set analyses**

Genome-wide association analyses for the three subtypes of aggression were performed using Mach2qtl/Mach2dat (Li et al., 2010), adjusting for age, age$^2$, and four population components derived from multidimensional scaling analysis. For RPQ proactive aggression scores only, scores were dichotomized into high- and low-scoring (score ≥ 2 and score ≤ 1, respectively), because of a highly positively skewed distribution (Supplementary Table 2). Separate analyses were run for males and females, and for subjects genotyped on the two different genotyping arrays. SNPs with low imputation quality ($R^2$ <0.6) and minor allele frequency of less than 1% were filtered out. Resulting SNP p-values for each of the traits were used to run gene-set analysis using MAGMA v1.04 (de Leeuw et al., 2015). SNPs were mapped onto genes using 1000 Genomes Phase 1.v3 reference data followed by computation of gene p-values. Fixed-effects meta-analysis of the output of the two genotyping arrays was run using the weighted Stouffer's Z method as implemented in MAGMA. We first assessed association of all three gene-sets combined on the three aggression subtypes. The MAGMA competitive gene-set analysis was used to assess association, which will correct for confounding due to gene-size, gene density, differential sample size and the log of those values. Results of the self-contained test option in MAGMA, which tests whether a signal is present in the aggregated set of SNPs compared with a signal being present by random chance, are also reported for comparability with previously used methods in literature. This association method does not take into account gene-size and gene density, or whether the association of the gene-set is greater than that of other genes. Results were considered significant if they reached the Bonferroni-corrected *P*-value-threshold for testing of three aggression subtypes and two sexes (*P*-value threshold=0.05/6 =0.0088). For significant associations observed in the competitive test, we performed post-hoc tests to localize effects amongst the three separate gene-sets and individual genes within the sets. An additional post-hoc analysis assessed association of all three gene-sets combined using the two-factor classification of reactive and proactive aggression (Supplementary Information).

## RESULTS

The general characteristics of our sample of 661 participants and the genotyped sample of n=395 are shown in Table 2. The tree factor model of the RPQ, consisting of a proactive factor, a reactive factor due to internal frustration, and a reactive factor due to external provocation or threat, showed a good model fit in the healthy adults (RMSEA 90% CI: .041-.051, RMSEA: .046, CFI: .915, TLI: .905), Cronbach's alpha = 0.687 (proactive), 0.663 (reactive internal frustration), 0.684 (reactive external provocation). An overview of fit-measures for one-, two-, and three-factor models are provided in Supplementary Table 3. In line with earlier studies, inter-correlations between the three investigated aggression subtypes were moderate and significant in our investigated sample (.436 ≥ *r* ≤ .574), marking them as distinguishing but correlated dimensions of aggression.

**Table 2:** Sample characteristics for phenotypic and genetic analyses.

| | Phenotypic sample (n=661) | Phenotyped females (n=391) | Phenotyped males (n=270) | Genotyped sample (n=395) | Genotyped females (n=227) | Genotyped males (n=168) |
|---|---|---|---|---|---|---|
| Sex (% male) | 41% | - | - | 43% | - | - |
| Mean age (SD) | 25.45 (4.56) | 25.60 (4.88) | 25.24 (4.07) | 25.60 (4.70) | 25.85 (5.08) | 25.30 (4.11) |
| Mean proactive score (SD; range) | 1.38 (1.81;0-12) | 1.00 (1.38;0-8) | 1.92 (2.19;0-12) | 1.45 (1.91;0-12) | 0.97* (1.40;0-8) | 2.09* (2.3;0-12) |
| Mean reactive internal frustration score (SD; range) | 3.01 (1.76;0-9) | 2.96 (1.75;0-9) | 3.10 (1.79;0-9) | 3.02 (1.82; 0-9) | 2.89 (1.78;0-9) | 3.20 (1.85;0-9) |
| Mean reactive external provocation score (SD; range) | 2.39 (1.89;0-11) | 2.29 (1.86;0-11) | 2.55 (1.92;0-9) | 2.37 (1.91;0-11) | 2.24 (1.86;0-11) | 2.54 (1.98;0-9) |

* RPQ proactive aggression scores were dichotomized into high- and low-scoring (score ≥ 2 and score ≤ 1, respectively), because of a highly positively skewed distribution in both males and females.

Gene-set association analysis with aggression subtypes was conducted in the 395 subjects with genotyping information available. Males scored significantly higher on proactive aggression than females in the genotyped ($t(393) = 5.97$, $P < 0.001$) as well as the phenotyped cohort ($t(659) = 6.59$, $P < 0.001$). A total of 483 unique autosomal genes were selected for the combined dopaminergic, serotonergic, and neuroendocrine gene-set. Twenty additional genes, either located on the X- and Y-chromosome or not captured by the array, could not be included in the analysis (Table 1).

Association analysis of all three gene-sets combined with each of the three aggression subtypes was performed for males and females separately (Table 3). In females, the combined gene-set was significantly associated with frustration-based reactive aggression, but not with reactive aggression due to external provocation/threat or with proactive aggression scores. The significant association of the combined set with reactive aggression due to internal frustration as measured by competitive testing was observed for both genotyping arrays ($P_{Affymetrix\_competitive} = 1.397e\text{-}03$ and $P_{Infinium\_competitive} = 2.175e\text{-}04$, respectively), showing replicability of the finding. In males, the combined gene-set was not associated with any of

the aggression subtypes using competitive tests. Post-hoc analysis results, comparing our main association results with associations based on the two-factor model of reactive and proactive aggression, can be found in the Supplementary Information. Self-contained test results were highly significant for proactive aggression scores in both males and females.

**Table 3:** Results for the association of the serotonergic, dopaminergic and neuroendocrine gene-sets combined with three aggression subtypes.

| | **Females** | | **Males** | |
|---|---|---|---|---|
| | $P_{competitive}$ | $P_{self\text{-}contained}$ | $P_{competitive}$ | $P_{self\text{-}contained}$ |
| Proactive aggression | 0.316 | 1.12E-17 | 0.043 | 3.20E-28 |
| Reactive internal frustration | 2.275E-5* | 5.51E-07 | 0.337 | 0.525 |
| Reactive external provocation | 0.438 | 0.014 | 0.273 | 0.159 |

* Indicates significance after Bonferroni correction for testing 3 subtypes and 2 sexes (Pthreshold = 0.0088).

For the significant finding for reactive aggression due to internal frustration in females, we subsequently explored contributions of the three separate gene-sets and of individual genes within these sets. As shown in Table 4, these post-hoc analyses showed that the neuroendocrine and the serotonergic gene-set were independently contributing to the association. Separate tests of each of the subsets of the neuroendocrine pathway (corticotropin-releasing hormone, glucocorticoid, estrogen, and androgen signaling cascades) provided evidence for contributions of each of these cascades to the association, with lowest p-values for glucocorticoid and androgen signaling (Table 4). No single genes showed significant associations after Bonferroni correction for 40 (serotonin), 73 (dopamine) and 411 (neuroendocrine) genes tested (Supplementary Table 4). The gene with the strongest association in the serotonergic set was the serotonin transporter (*SLC6A4*, P =0.0098), and the gene with the strongest association in the neuroendocrine set was Cyclin-Dependent Kinase-Activating Kinase Complex Subunit (*CCNH*, P =0.0004).

**Table 4:** Results for the association of the serotonergic, dopaminergic, and neuroendocrine gene-sets with reactive aggression due to internal frustration in females.

| | **Ngenes** | **Pcompetitive** |
|---|---|---|
| Serotonin | 40 | 0.016* |
| Dopamine | 73 | 0.059 |
| Neuroendocrine | 411 | 1.147E-4* |
| *Glucocorticoid* | *264* | *8.49E-04* |
| *Corticotropin-releasing hormone* | *107* | *0.012* |
| *Androgen* | *110* | *6.46E-03* |
| *Estrogen* | *123* | *0.023* |

* Indicates significance after Bonferroni correction for multiple testing (3 sets; Pthreshold = 0.0167).

## DISCUSSION

In the current study, we investigated genetic mechanisms underlying aggression subtypes in the healthy population. Factor analysis confirmed that three correlated but separate dimensions of aggression can be distinguished in healthy adults, using the self-report scale RPQ ('proactive aggression', 'reactive aggression due to internal frustration', and 'reactive aggression due to external provocation'). Aggregated analysis of common variants within monoaminergic and neuroendocrine systems confirmed association of these systems with reactive aggression due to internal frustration in females.

Our results confirming the existence of three distinguishable dimensions of aggression in healthy adults are in line with the previous study investigating alternative factor solutions for the RPQ in adults (Brugman et al., 2016). These authors reported improved fit-indices in exploratory factor analysis for the three-factor model compared to the original two-factor model in a males-only sample, recruited partly in forensic psychiatric in- and outpatient clinics and partly from the general population. The first study to find the three-factor structure of the RPQ investigated a younger sample of adolescents, all from clinical samples (Smeets et al., 2016). The current study extends these findings further by showing them to be valid in a highly educated healthy population sample. The specificity of our genetic finding for one of the subtypes, underscores the biological meaningfulness of the observed three-factor structure.

The scores for both reactive subtypes showed a normal distribution in our general population sample; proactive aggression scores were heavily skewed towards the lower end, reflecting the fact that proactive aggression includes more severe behaviors less prevalent in the general population. Proactive aggression scores were significantly higher for males compared to females in our sample of healthy adults. In general, males and females have been shown to differ markedly, both in terms of prevalence and type of aggression displayed. Males are at increased risk of showing overt/physical aggression (Baillargeon et al., 2007; Côté, 2007; Hill et al., 2006), while females may show slightly more indirect aggression (also termed social aggression, relational aggression) compared to males (Card et al., 2008). As proactive aggression is often displayed in a covert manner, and reactive aggressive behavior is more overt, it has been suggested that girls show more proactive aggression and boys show more reactive aggression (Kempes et al., 2005). However, prior studies that have investigated gender-differences in rates of proactive and reactive aggression in children do not confirm this idea. A study of the prevalence of proactive and reactive aggression in a sample of clinically referred children and adolescents did not find gender differences for either of the subtypes (Connor et al., 2003). Studies in non-referred children did find differences, and reported higher rates of both reactive and proactive aggression in boys (Salmivalli & Nieminen, 2002; Baker et al., 2008). It has been suggested that gender-differences may be more pronounced in non-clinical samples (Connor et al., 2003). In our current study of healthy adults, we only find higher proactive (not reactive) aggression scores in males, suggesting that an age effect may also be at play. It has been hypothesized that proactive

aggression may become more pronounced at a later age, when cognitive abilities are fully developed and aggressive behaviour may become more calculative in nature (Kempes et al., 2005), a hypothesis that warrants further investigation in future studies.

Our identified association of candidate genetic systems with reactive aggression due to internal frustration in females was driven by variation in serotonergic and neuroendocrine signaling. This finding is in line with literature describing specific effects of serotonin, cortisol, and the sex steroids on aggressive behavior. Indeed, the reported associations of these molecules with aggression often differ as a function of sex and type of aggression studied (reviewed in Rosell and Siever, 2015). For example, higher cortisol reactivity was reported for reactive aggression compared to proactive aggression (Lopez-Duran et al., 2008). One influential theory hypothesizes that a high testosterone/cortisol ratio predisposes to increased aggression, with serotonin modulating the balance between impulsive and instrumental aggression. Specifically, the high testosterone/cortisol ratio is thought to facilitate the fight-flight response by acting on the amygdala-hypothalamus-periaqueductal gray network, while low serotonin reduces inhibitory control by the prefrontal cortex, together leading to increased impulsive, reactive aggression (Montoya et al., 2012). It is interesting to mention that, although not significant after correcting for the number of genes tested, the gene with the strongest association in our serotonergic set was the serotonin transporter (*SLC6A4).* This is one of the most investigated candidate genes for aggression (Veroude et al., 2016) and has been associated with antisocial behavior in meta-analysis (Ficks and Waldman, 2014).

Our finding for neuroendocrine and serotonergic signaling was specific to one of the two reactive aggression subtypes, i.e. the frustration-based reactive subtype. Although gene-set association of reactive aggression as defined by the two-factor classification was also significant (Supplementary Information), providing evidence for the usefulness of the two-factor model in research of aggression etiology, our analysis using three subtypes shows that the association was strongly driven by frustration-based reactive aggression and not by threat-based reactive aggression, underscoring the biological meaningfulness of the three-factor structure. This highlights the value of the further reduction of phenotypic heterogeneity for the identification of underlying biological mechanisms of aggression. One of the characteristics of the frustration-based subtype is thought to be an inflexibility to changes in the environment (Smeets et al., 2016). Our specific finding of strong association of frustration-based reactive aggression with neuroendocrine and serotonergic genes may thus arise (partly) from the function of these genes in stress modulation. However, more research is needed to assess the complex interactions and mechanisms through which the investigated systems lead to aggression-related phenotypes. In this context it will be useful to investigate the effects of early environment on the epigenome and the genetic factors moderating these effects (Provencal et al., 2015). Additionally, imaging genetics studies will be instrumental in investigating the modulation of aggression brain circuitry by aggression risk genes (Bogdan et al., 2017; Thompson et al., 2014).

Our findings were female-specific, a possible explanation for which lies in the idea that the signaling and interaction of the endocrine HPA and HPG axes is different between the sexes. For example, the two axes contribute to androgen production in different proportions in the different sexes (Burger, 2002; Montoya et al., 2012). In general, males and females probably developed different aggression strategies during evolution as a result of sex-specific sex hormone signaling (Georgiev et al., 2013). When using self-contained tests, we found a highly significant association of the gene-set with proactive aggression scores in both sexes. While no biological inferences can be made regarding the tested systems based on self-contained tests, nominally significant competitive association results for proactive aggression in males might nevertheless potentially point towards a role of the investigated systems in proactive aggression risk in males. The sex-specificity of at least some of our findings forms an important starting point into genetic differences in aggressive behavior between males and females. With most studies to date including male subjects only, the aggression phenotype in females specifically has been understudied and deserves more attention.



This study provides new information on the underlying mechanisms of aggression, thereby facilitating the search for diagnostic, preventive, and treatment options based on understanding biology. Importantly, from a clinical perspective, the sex and subtype specificity of our findings emphasizes the need for individually tailored treatment options. For example, our genetic association results suggest there is a biological aspect to sexual dimorphism. Fundamental differences in underlying pathophysiology may have important consequences for therapeutic interventions, suggesting that male and female needs for intervention might differ markedly.

Our study should be viewed in the context of specific strengths and limitations. One strength of the current study is the large sample size used to verify the factor structure of the RPQ. Moreover, the study addresses three different types of heterogeneity, tackling issues with phenotypic, sex-related, and allelic heterogeneity. By aggregating the effect of multiple genetic variants relating to the biological processes implicated in aggressive behavior, we were able to boost statistical power for finding genetic association (Naaijen et al., 2017). Nevertheless, power of the study provided limited opportunity for an expansion of the number of variables investigated. Future studies should further investigate correlates of female reactive aggression that could serve to explain our main association results. Possible variables of interest are provided by a study by Connor and coworkers (2003), who specifically investigated the correlates of proactive and reactive aggression in males and females separately. They showed that while a large amount of variance in male reactive aggression was mediated by hyperactive/impulsive behaviors, a large amount of explained variance in female reactive aggression was mediated by early traumatic stress (Connor et al., 2003). X- and Y-linked genetic variation could not be taken into account in our study, and we were thus unable to include genetic variation in the well-known *MAOA* gene in the analysis. Including this variation may further improve power of genetic studies, however, the assumed underlying polygenic risk model (many genetic variants, each with small effect

size, are assumed to contribute to the phenotype) was sufficiently captured in the current analysis. Our study of aggression was performed in healthy individuals. In doing so, we assumed a model in which patients diagnosed with aggression disorders can be seen as the extremes in a distribution of aggressive traits. Several lines of research have already shown that this model is relevant in other psychiatric traits such as attention-deficit/hyperactivity disorder and autism spectrum disorders (Martin et al., 2014; Middeldorp et al., 2016; Riglin et al., 2016; Robinson et al., 2016). We selected genes based on their implication in aggression disorders, and indeed, were able to find association with aggressive traits in the general population. Showing that common genetic variants underlying aggression phenotypes are similar in typical and psychiatric populations, this offers many possibilities for future research. While recruitment of large clinical cohorts often proves challenging, large population-based samples are much easier to investigate, offering important opportunities for sample size maximization.

We provide evidence for the existence of three correlated but separate dimensions of aggression in healthy adults, and identify variation in neuroendocrine and serotonergic signaling as a biological risk factor involved in the etiology of frustration-based reactive aggression in females. To our knowledge, this is the first study investigating the combined effect of common genetic variants related to monoaminergic and neuroendocrine signaling on aggression subtypes. The findings stress the value of reducing phenotypic and sex-related heterogeneity in research of aggression etiology, and the opportunities offered by population-based studies of aggression.

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## SUPPLEMENTARY MATERIAL

### Genotyping and quality control

Genotyping and imputation for the Affymetrix Genome-Wide Human SNP Array 6.0 are described in Guadalupe et al. (Guadalupe et al., 2015). Shortly, genotype calls were made using the Birdseed algorithm. Samples with call rates lower than 90% and/or deviant values of genome-wide heterozygosity (Purcell et al., 2007) were excluded, as well as SNPs with a minor allele frequency below 1% or that failed the Hardy–Weinberg equilibrium test at a threshold of $p \leq 10{-}6$.

For the Infinium PsychArray-24 v1.1 data, genotypes were called using Illumina GenomeStudio software. Samples with a call rate <0.994 were excluded. Clustering was performed using GeneTrain 2.0 (no-call threshold 0.15) after which samples with call rate <0.98 were excluded. Quality control steps performed prior to imputation included removal of SNPs with a call rate below 98%, removal of SNPs with a minor allele frequency of less than 1% or failing the Hardy-Weinberg equilibrium test at a threshold of p≤10−6, and removal of individuals with a call rate below 98% or heterozygosity rate of more than 3 standard deviations from the mean. For both platforms, MACH software was used for haplotype phasing and minimac for the final imputation (Howie et al., 2012; Li et al., 2010), with 1000 Genomes Phase 1.v3 reference data (Abecasis et al., 2012).

### Post-hoc association analysis based on the two-factor model

To compare our main gene-set association result based on the (best fit) Reactive Proactive Questionnaire three-factor model ('proactive aggression', 'reactive aggression due to internal frustration', and 'reactive aggression due to external provocation') with the two-factor classification of aggression ('proactive aggression', 'reactive aggression'), we assessed association of the combined dopaminergic, serotonergic, and neuroendocrine gene-sets with reactive aggression as defined by the two-factor model. As our main results (i.e. using the three-factor model), showed significant association of the combined gene-set with frustration-based reactive aggression in females only, this post-hoc analysis was conducted in females. Responses of the Reactive Proactive Questionnaire were summed to yield the reactive aggression score based on the two-factor model (mean reactive score (SD) =5.13 (3.25); range 0-20; n=227). The combined gene-set showed association significance with reactive aggression as defined by the two-factor classification in females (p=0.01). Although this provides evidence for the usefulness of the two-factor model, our analysis using three subtypes shows that the association was strongly driven by frustration-based (P =2.27E-5) and not threat-based (P =0.438) reactive aggression, underscoring the biological meaningfulness of the three-factor structure.

**Supplementary Table 1:** Reactive Proactive Questionnaire items relating to each of the aggression subtypes.

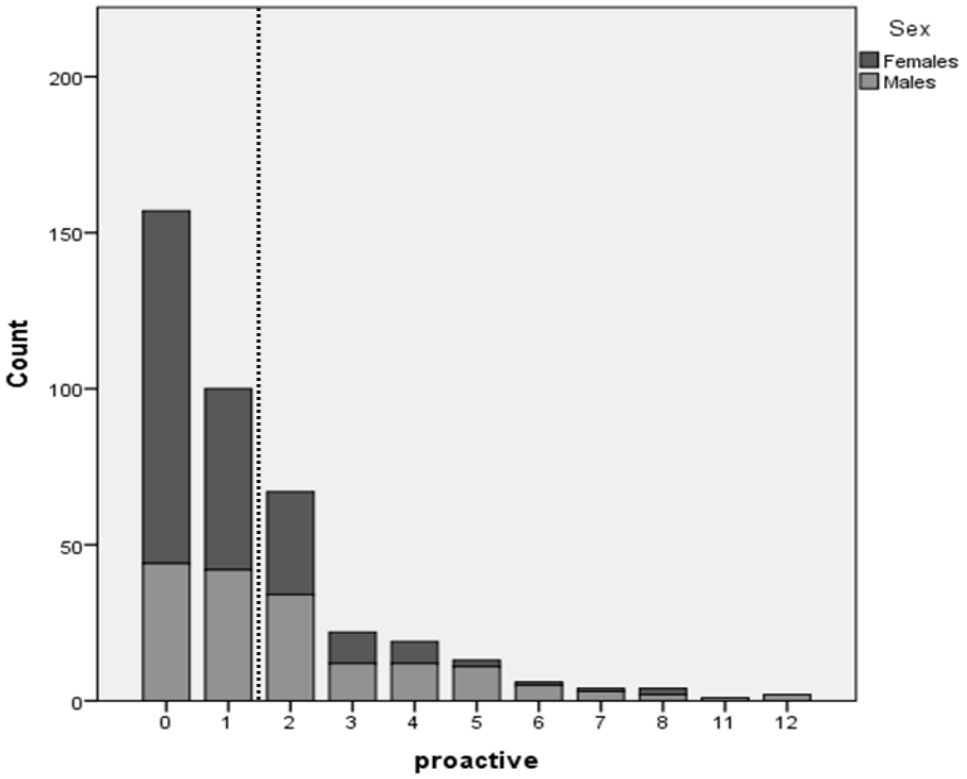
**Proactive aggression**
2: Had fights with others to show who was on top
4: Taken things from other students
6: Vandalized something for fun
9: Had a gang fight to be cool
10: Hurt others to win a game
12: Used physical force to get others to do what you want
15: Used force to obtain money or things from others
17: Threatened and bullied someone
18: Made obscene phone calls for fun
20: Gotten others to gang up on someone else
21: Carried a weapon to use in a fight
23: Yelled at others so they would do things for you
Reactive internal frustration
1: Yelled at others when they have annoyed you
5: Gotten angry when frustrated
8: Damaged things because you felt mad
11: Become angry or mad when you do not get your way
13: Gotten angry or mad when you lost a game
**Reactive external provocation**
3: Reacted angrily when provoked by others
7: Had temper tantrums
14: Gotten angry when others threatened you
16: Felt better after hitting or yelling at someone
19: Hit others to defend yourself
22: Gotten angry or mad or hit others when teased

**Supplementary Table 2:** Confirmatory Factor Analysis results for the Reactive Proactive Questionnaire; overview of fit-measures for different models.

| Model | RMSEA estimate | RMSEA 90% CI | CFI | TLI |
|---|---|---|---|---|
| Single-factor | .053 | .048 - .057 | .888 | .877 |
| Two-factor | .048 | .043 - .053 | .908 | .899 |
| Three-factor | .046 | .041 - .051 | .915 | .905 |

**Supplementary Table 3:** Association results for individual genes with aggression due to internal frustration in females.

*Supplementary Table 3 is available upon request (11 pages).*




**Supplementary Figure 1:** Distribution of Reactive Proactive Questionnaire proactive aggression scores in males and females. Dotted line represents the cut-off for dichotomized high- and low-scores.

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# Part 2

## Imaging genetics in neurodevelopmental psychopathology and aggression

# Chapter 5

## Imaging genetics in neurodevelopmental psychopathology

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## ABSTRACT

Neurodevelopmental disorders are defined by highly heritable problems during development and brain growth. Attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASDs), oppositional defiant disorder (ODD), conduct disorder (CD), and intellectual disability (ID) are frequent neurodevelopmental disorders, with common comorbidity among them. Imaging genetics studies on the role of disease-linked genetic variants on brain structure and function have been performed to unravel the etiology of these disorders. Here, we reviewed imaging genetics literature on these disorders attempting to understand the mechanisms of individual disorders and their clinical overlap. For ADHD, ASD, and ODD/CD, we selected replicated candidate genes implicated through common genetic variants. For ID, which is mainly caused by rare variants, we included genes for relatively frequent forms of ID occurring comorbid with ADHD, ASD, or ODD/CD. We reviewed case-control studies and studies of risk variants in healthy individuals. Imaging genetics studies for ADHD were retrieved for *SLC6A3/DAT1*, *DRD2*, *DRD4*, *NOS1,* and *SLC6A4/5HTT*. For ASD, studies on *CNTNAP2*, *MET*, *OXTR*, and *SLC6A4/5HTT* were found. While no reports on case-control imaging genetics studies were retrieved for ODD/CD, studies of the effects of well-known risk variants from aggression literature, in the *MAOA, AVPR1A,* and *SLC6A4/5HTT* genes*,* on the brain of healthy individuals are reviewed here. For ID, we reviewed the genes *FMR1*, *TSC1 and TSC2*, *NF1*, and *MECP2*. Alterations in brain volume, activity, and connectivity were observed. Several findings were consistent across studies, implicating e.g. *SLC6A4/5HTT* in brain activation and functional connectivity related to emotion regulation. However, many studies had small sample sizes, and hypothesis-based, brain region-specific studies were common. Results from available studies confirm that imaging genetics can provide insight into the link between genes, disease-related behavior, and the brain. However, the field is still in its early stages, and conclusions about shared mechanisms cannot yet be drawn.

## INTRODUCTION

Neurodevelopmental disorders are broadly defined as disorders in the development and growth of the brain (Goldstein and Reynolds, 1999), but this term is largely used to describe neurological and psychiatric disorders that have their onset prior to adulthood. Most neurodevelopmental disorders are highly heritable, either caused by single genetic defects, like many of the intellectual disability (ID) disorders (Deciphering Developmental Disorders Study, 2015), or with a more multifactorial background, in which several to multiple less penetrant genetic variants cause the disease in combination with environmental factors, like in many cases of autism spectrum disorders (ASDs; (Gaugler et al., 2014; Iossifov et al., 2014), as well as in attention-deficit/hyperactivity disorder (ADHD; (Faraone et al., 2015; Franke et al., 2012), oppositional defiant disorder, and conduct disorder (Salvatore and Dick, 2016).

While technological advances in the last decade, especially genome-wide association studies (GWASs) and next generation sequencing, have enabled the identification of many genetic factors involved, the biological mechanisms contributing to the neurodevelopmental disorders are still largely unknown. It is thought that gene variation/mutation will alter molecular and cellular processes, which leads to altered brain development, be it structurally and/or functionally, and subsequently to altered behavior and disease symptoms (Franke et al., 2009). Measures that mediate the effects of genes on behavioral/disease phenotypes have been termed endophenotypes or intermediate phenotypes (Gottesman and Gould, 2003; Kendler and Neale, 2010).

Much research into the consequences of gene aberrations is performed in animal models. However, brain imaging methods like magnetic resonance imaging (MRI), electroencephalography (EEG), and magnetoencephalography (MEG) offer excellent ways to investigate the effects of genetic variation on brain structure, function, and connectivity directly in humans *in vivo*. Such 'imaging genetics' approaches can unveil the brain-biological consequences of molecular changes induced by genetic variants – both common and rare – linked to neurodevelopmental disorders. In that way they can help to understand the mechanisms through which differences in behavior arise. It has been argued that the effects of disease-linked (common) genetic variation on the brain would be larger than those on behavior and clinical phenotypes (Gottesman and Gould, 2003; Rose and Donohoe, 2013)), although more recent work using hypothesis-free imaging genetics approaches argues against this – at least for brain structural phenotypes (Franke et al., 2016).

Different neuroimaging methods can be used in imaging genetics studies, including different forms of structural and functional MRI as well as EEG and MEG. They have complementary characteristics enabling information to be gathered on different aspects of (gene effects on) brain anatomy and function, like location (especially MRI-based methods) and timing (especially EEG and MEG). In this review, we concentrated on those methods that have most frequently been used in imaging genetics studies of neurodevelopmental disorders, i.e. MRI-based methods evaluating gene effects on brain structure, function, and connectivity.

With structural magnetic resonance imaging (sMRI) it is possible to noninvasively characterize the structure of the human brain. Thereby, the different magnetic properties of brain tissues are used to map the spatial distribution of these structural properties of the brain. In this way, the different brain tissues (grey and white matter) and cortical and subcortical structures of the brain can be mapped. By adapting scanning parameters, different weighting techniques of the signal can be used, such as T1-weighted imaging (used to visualise anatomy) and T2-weighted imaging (which is useful for demonstrating lesions and pathology). Different aspects of brain structure can be used for quantitative analyses. To investigate whether volumetric differences are global or regional, specific brain regions of interest (ROIs) can be selected *a priori* and studied individually. In contrast, global changes in grey or white matter intensity can be detected by using voxel-based morphometry (VBM) analyses. Next to volumetric differences observed in grey matter, structural differences of white matter connectivity can also be quantified. With the help of diffusion tensor imaging (DTI), it is possible to non-invasively investigate the macrostructural integrity and orientation of white matter fibre bundles. Thereby, the directional diffusion of water molecules along neuronal membranes is measured, allowing to map white matter connection within the brain. Multiple measures can be derived from DTI. A frequently measured parameter is fractional anisotropy (FA). Basically, anisotropy indicates that diffusion takes place in a directional manner, whereas isotropy indicates diffusion in all directions. Additional DTI-derived parameters include mean diffusivity (MD; average of axial diffusivity (AD) and perpendicular diffusivities), and radial diffusivity (RD; average of perpendicular diffusivities), the mode of anisotropy (sensitive to crossing fibres), and the apparent diffusion coefficient (indicating the magnitude of diffusion) (Le Bihan, 2003; Le Bihan et al., 2001; Yoncheva et al., 2016).
Resting state functional MRI (rs-fMRI), allows to analyse the temporal correlations of neural activity across anatomically disparate brain regions and thereby to examine the functional connectivity based on spontaneous brain activity, neural organization, and circuit architecture.
To investigate potential changes in brain activity, functional magnetic resonance imaging (fMRI) can be used. Since fMRI is sensitive to the oxygenation of the blood, the so-called blood-oxygen-level-dependent (BOLD) signal can be measured. Thereby brain function is measured, based on the premise that active cells consume oxygen, thus causing changes in blood oxygenation, and subsequently leading to increased blood flow. However, the exact link between cell activation, oxygen saturation, and cerebral blood flow changes is debatable (Hillman, 2014). Generally in fMRI, alterations in blood flow after e.g. a task-induced stimulus or during a resting condition are measured.

Here, we systematically reviewed the imaging genetics literature for five frequent neurodevelopmental disorders, ADHD, ASDs, ODD and CD, and selected intellectual disability (ID) disorders. The choice for those neurodevelopmental disorders was based on their frequent comorbidity (Bailey et al., 2008; Connor and Doerfler, 2008; Hill et al., 2014; Vorstman and Ophoff, 2013) and robustly established associations with specific genetic variants. The aim of this work was to extract core brain mechanisms affected by

disease-linked genetic factors related to the individual disorders as well as their clinical overlap.

ADHD is one of the most common neurodevelopmental disorders, with a prevalence of 5-6% in childhood (American Psychiatric Association, 2013; Polanczyk et al., 2007). ADHD can be clinically characterized by two core symptom domains: inattention and hyperactivity/impulsivity (American Psychiatric Association, 2013; Faraone et al., 2015). Up to 60% of all patients diagnosed in childhood show ADHD symptoms and/or meet formal diagnostic criteria for the disorder in adulthood, and prevalence rates of persistent ADHD in adults range between 2.5 and 4.9% (Simon et al., 2009). ASD affects approximately 0.6% to 1% of the children, making it one of the most prevalent disorders in childhood (Elsabbagh et al., 2012). Although there are some important differences in core symptom definition, the co-occurrence between ADHD and ASD is supported by clinical (Craig et al., 2015), common biological (Rommelse et al., 2010), and non-biological risk factors (Kroger et al., 2011). Moreover, several studies identified that symptoms of autism or autistic traits appear in 20% to 30% of children with ADHD (Grzadzinski et al., 2011; Kochhar et al., 2011). Additionally, ADHD is a common comorbid disorder in children with ID, and the risk increases with increasing severity of ID (Voigt et al., 2006). Studies of children with mild and borderline ID have identified ADHD in 8% to 39% of the cases (Baker et al., 2010; Dekker and Koot, 2003; Emerson, 2003). ADHD is highly heritable (heritability 70-80%) (Burt, 2009; Faraone et al., 2005). However, identification of ADHD risk genes has been difficult (Franke et al., 2009; Gizer et al., 2009), mainly due to ADHD's complex genetic background (Faraone et al., 2015; Franke et al., 2012). Mostly genetic variants, which occur quite frequent in the population and have generally small effects on disease risk have been investigated for their role in ADHD until today, either through candidate gene studies or hypothesis-free GWASs. Only a few of the candidate genes have been confirmed through meta-analysis (Gizer et al., 2009). However, none of the eleven GWAS (Hinney et al., 2011; Lasky-Su et al., 2008a; Lasky-Su et al., 2008b; Lesch et al., 2008; Mick et al., 2010; Neale et al., 2008; Neale et al., 2010a; Sanchez-Mora et al., 2014; Sonuga-Barke et al., 2008; Stergiakouli et al., 2012; Yang et al., 2013) nor a meta-analysis of many of them (Neale et al., 2010b) published to date, reported any genome-wide significant risk variant.

ASDs refer to a heterogeneous group of neurodevelopmental disorders diagnosed in approximately 1 of 88 children (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Incestigators and Centers for Disease Control and Prevention, 2012). It is characterized by deficits in social behavior and language development, as well as restricted or stereotypic interests (American Psychiatric Association, 2013). About 70% of individuals with ASDs have some level of ID while the remaining 30% have some disability (speech, behavior) other than cognitive dysfunction (Mefford et al., 2012). Whereas early reports estimated ASD heritability to be higher than 90% (Bailey et al., 1995; Folstein and Rutter, 1977; Ritvo et al., 1985; Steffenburg et al., 1989), recent population-based studies provided an estimate of ~50% heritability (Gaugler et al., 2014; Sandin et al., 2014). ASDs are genetically highly complex, as part of the cases has oligogenic or even monogenic causes (with an important role for *de novo* mutations (Iossifov et al., 2014)), whereas the concerted action of common genetic variants of individually small

effect sizes and environmental factors is likely to cause most of the disease burden of ASDs (Iossifov et al., 2014) (Gaugler et al., 2014; Zhao et al., 2007). Several of those common variants contributing to ASD risk have been identified through hypothesis-driven studies. Until now, three GWASs have been performed for ASDs (Anney et al., 2010; Wang et al., 2009; Weiss et al., 2009), which identified a single locus on chromosome 5p14, in-between *CDH10* and *CDH9* (Wang et al., 2009). Association with this locus might be driven by markers located within the *MSNP1AS* pseudogene (Ma et al., 2009).

CD and ODD are disruptive behavior disorders that tend to co-occur during development (Rowe et al, 2002). CD is defined as a repetitive and persistent pattern of behavior, in which the basic rights of others or major age-appropriate societal norms or rules are violated (Hill, 2002). ODD is defined by a pattern of angry and irritable mood, argumentative and defiant behavior, or vindictiveness (APA, 2013). As ODD and CD are the two most typical categorically defined aggression disorders, we discuss their overlap with the other disorders investigated in this paper both disorder specifically and in the general context of aggressive behavior. Genetics of these disorders are also discussed in the broader context of aggressive behavior. Co-morbid presence of ODD/CD is a clinically important dimension of ADHD heterogeneity, with ODD occuring in up to 60% of individuals with ADHD (Connor and Doerfler, 2008). Individuals with ADHD, who show such comorbid aggression-related problems, have a considerably worse prognosis than individuals without them. A defiant/vindictive behavioral pattern is associated with an increased risk for criminal outcomes later in life (Aebi et al., 2013), and irritable mood is thought to underlie the developmental link between ODD and later affective disorders (Loeber et al., 2000; Stringaris et al., 2009). Aggressive behavior – not necessarily in the context of ODD or CD - is also highly prevalent in ASDs. Children with ASD are at higher risk for displaying aggressive and oppositional behavior compared to other populations, with prevalence estimates of up to 68% (Hill et al., 2014). The same is true for IDs. For example, for one of the most common forms of inherited ID, Fragile X syndrome (Hagerman and Hagerman, 2002; Loesch et al., 2004), it has been estimated that 38% of males and 14% of females engage in aggressive behaviors (Bailey et al., 2008). Like in ADHD, disruptive behaviors in ASD and ID are risk factors for later poor outcomes, family stress and greater functional impairment (Lecavalier, 2006). This emphasizes the need to identify risk factors for comorbid aggression in these disorders. Little is known yet about the etiological basis of the described comorbidities, but shared and unique genetic influences between disorders have been postulated to play a role (Dick et al., 2005; Faraone et al., 1991). Heritability of aggression-related disorders, including ODD and CD, has been estimated at about 50% (Veroude et al., 2016). However, like in ADHD, identification of risk genes has been difficult, because of the complex genetic background of aggression. Many common genetic variants with small effect sizes as well as the environment are assumed to contribute to disease etiology (Tielbeek et al., 2017; Veroude et al., 2016). However, rare genetic variations identified in Mendelien disorders with documented aggressive symptoms, often in the context of intellectual disability, also point at genes involved in aggression etiology (Zhang-James and Faraone, 2016). Only two of the common candidate variants for aggression investigated to date have been confirmed through meta-analysis (Ficks and Waldman, 2014). GWAS have been small, with the

exception of a recent GWAS by the early genetics and lifecourse epidemiology (EAGLE) consortium, which included a large cohort of children and adolescents (N=18,988) from the general population. Aggressive behavior was assessed in nine population-based studies using parent-report questionnaires and combined in meta-analysis. The GWAS meta-analysis of the total cohort identified a region reaching near genome-wide significance. A candidate gene-based association test using the summary statistics of the total sample showed association of the *AVPR1A* gene with childhood aggression after correcting for 21 candidate genes tested .

ID refers to a highly heterogeneous group of disorders characterized by below average intellectual functioning (IQ < 70) in conjunction with significant limitations in adaptive functioning with onset during development. ID may occur as an isolated phenomenon or accompanied with malformations, neurological signs, impairment of the special senses, seizures and behavioral disturbances (van Bokhoven, 2011). ID has an estimated prevalence of approximately 2% to 3%, and approximately 0.3% to 0.5% of the population is severely handicapped (Perou et al., 2013). Comorbidity with ADHD and ASDs is frequently observed (Vorstman and Ophoff, 2013). Disease etiology of ID is thought to be largely monogenic, but with many different genetic anomalies implicated (van Bokhoven, 2011). Genetic causes of ID range from large cytogenetically visible chromosomal aberrations, such as trisomy 21, to translocations, subchromosomal abnormalities (such as Prader-Willi syndrome (15q11.2-q13)), copy number variations, and to single gene defects. We concentrated only on the latter in our review, based on the assumption that we can learn most from understanding effects of specific genes/variants on brain structure, function and connectivity. While in many ID disorders, a defect in a single gene can be identified as the cause of the disorder, only a few genes are hit more frequently and cause relatively common ID disorders. To prevent bias of our review by single case reports, we concentrated on those common forms of ID, especially selecting those, in which comorbidity with ADHD, ASD, and aggression is common. This resulted in five ID disorders included in this review: fragile X syndrome, tuberous sclerosis, neurofibromatosis type 1, Rett syndrome, and Timothy syndrome. Fragile X syndrome (FXS), caused by genetic defects in the *FMR1* gene, is associated with a variable clinical phenotype, including intellectual disabilities with a broad range of severities. IQ is 40 on average for affected men (Merenstein et al., 1996) and normal or borderline in females (de Vries et al., 1996), who show a milder phenotype because the disorder is X-chromosome-linked. High rates of autism and autistic behaviors are seen in individuals with FXS (Hagerman et al., 2009), and 59% of FXS subjects shows ADHD symptoms (Sullivan et al., 2006). It has been estimated that 38% of males and 14% of females with the full mutation engage in aggressive behavior (Bailey et al., 2008). Neurofibromatosis type 1 (NF1), caused by mutations in *NF1*, is associated with the presence of usually benign neurofibromas. While IQ in general is average to low average, up to 8% of children with NF1 have an IQ below 70. Learning difficulties, internalizing and externalizing behavior problems, and neuropsychological deficits are common. The core cognitive impairments are in visual spatial function, attention, executive function, and language skills. About 38% of children with NF1 meet diagnostic criteria for ADHD, and a substantial proportion of subjects show social deficits related to ASD (Hyman et al., 2005;

Walsh et al., 2013). Tuberous sclerosis complex (TSC) is caused primarily by mutations in the genes *TSC1* and *TSC2* and is characterized by benign hamartomas in multiple organ systems, including the brain. Intellectual ability in TSC ranges from normal to profoundly impaired, and neurobehavioral abnormalities and epilepsy are common. ASD, ADHD, as well as aggression are all reported in about 50% of individuals with TSC, with an even higher number of diagnoses in intellectually impaired individuals (Eden et al., 2014; Prather and de Vries, 2004). Rett syndrome, caused by mutations in the *MECP2* gene, primarily affects females. Language problems and cognitive and motor deficits start to become obvious around the age of 6 months in the patients. Testing of cognitive dysfunction is difficult because of a characteristic absence of speech, but ASD-related features, such as avoidance of eye contact, are common (Armstrong, 2005). Timothy syndrome is a multisystem disorder caused by missense mutations in the *CACNA1C* gene. Neurodevelopmental features include global developmental delays and ASDs. Average age of death is 2.5 years, usually caused by ventricular tachyarrhythmia, infection, or complications of hypoglycemia (Splawski et al., 1993).

With this review, we aimed at providing a comprehensive overview on the imaging genetics literature for the neurodevelopmental disorders. To prevent bias, we excluded reports including less than 10 cases and focused on specific genetic variants, which for ADHD, aggression and ASDs resulted in a focus on genes/loci implicated through variants that are common in the population, and for ID, we restricted the review to the genes causing the single-gene ID disorders described above. While imaging genetics studies have been performed in patients, the underlying candidate genes and their common genetic variants are also frequently studied in healthy individuals. This allows analysis of effects of common genetic variation in candidate genes on imaging correlates in the general population and offers the opportunity to study brains not influenced by chronic disease and medication. Previous studies showed that neuroimaging correlates of common genetic variants are likely to be similar in typical and psychiatric populations (Hibar et al., 2015b). As such studies of healthy individuals may also be informative regarding the biological mechanisms leading to the diseases of interest, they were also included in this review.

## METHODS

### Search terms

Pubmed was searched for research articles describing imaging genetics studies (April, 14th, 2015; http://www.ncbi.nlm.nih.gov/pubmed). Only studies using magnetic resonance imaging (MRI) were reviewed, specifically structural MRI (sMRI), functional MRI (fMRI), resting-state functional MRI (rs-fMRI), and diffusion tensor imaging (DTI). A general search term was created and was extended by adding the disorder (for ADHD, ASD, and ODD/CD (investigated and described combined)) or syndrome name and gene (for ID) of interest. The following search term shows an example for ADHD (for [Title/Abstract]): ((((ADHD OR Attention-Deficit Hyperactivity Disorder) AND (gene* OR genetic* OR imaging genetic OR imaging genetics OR genotype OR polymorphism OR

SNP OR single nucleotide polymorphism OR meta-analysis OR genome wide association OR GWA OR GWAS)) AND (structural magnetic resonance imaging OR volume OR sMRI OR voxel-based morphometry OR brain morphometry OR brain volumetry OR VBM OR functional magnetic resonance imaging OR fMRI OR diffusion tensor imaging OR diffusion imaging OR connectivity OR tractography OR DTI OR resting-state functional magnetic resonance imaging OR voxel-wise analysis OR rsfMRI)) NOT "review"[Publication Type]). For ID syndromes, the search term did not include (gene* OR genetic* OR imaging genetic OR imaging genetics OR genotype OR polymorphism OR SNP OR single nucleotide polymorphism OR meta-analysis OR genome wide association OR GWA OR GWAS), as the genes of interest were added specifically. Titles and abstracts of the retrieved records were evaluated for relevant publications. Case-reports and reports describing less than 10 cases were excluded to prevent bias, and review articles, medical hypotheses, non-English articles, and studies on animal models were not considered (for a graphical summary of the selection procedure, please see **Figure 1**).

**Candidate gene selection for ADHD, ASD, ODD, CD and ID studies**

Taking into account the differences in the genetic architecture of the neurodevelopmental disorders of interest, we defined selection criteria for the genes to be included in this review as similar as possible. The restriction to studies with 10 or more cases and single genetic variants/single-gene mutations largely defined our search strategy, which resulted in a focus on common genetic variants for ADHD, aggression and ASDs (minor allele frequency ≥ 1%); for ID disorders, this lead to the selection of relatively common forms of the disorder. For ADHD, aggression and ASDs, we selected the most promising genes containing common variants associated with the disorder based on meta-analyses, successful replication studies, and/or significant findings from hypothesis-free (genome-wide) studies.

For ADHD, we included all genes and genetic variants mentioned in Table 1 of the meta-analytic study by Gizer and coworkers (2009) that had reached a significant result at $P \leq 0.05$ for association with ADHD. In addition to this, we also included genes with reported and replicated evidence for association with ADHD from more recent studies. These included two meta-analytic studies (Pan et al., 2015; Wu et al., 2012), a research article (Ribases et al., 2011), and the more recently observed replicated candidate genes *NOS1* and *SLC9A9* (Stergiakouli et al., 2012; Weber et al., 2015) (total number of candidate genes = 10; **Table I**). A recent overview of these ADHD candidate genes has been published by Hawi and colleagues (2015).

For the ASD genes, we based our selection on the review of the most consistently replicated genes harboring common variants associated with autism by Persico and Napolioni (2013). Additionally, the *CDH9/CDH10* locus was included, because it has shown genome-wide significant association with ASD (Prandini et al., 2012; Wang et al., 2009). Selection of the candidate polymorphisms in the selected genes was based on recent research articles, as meta-analyses were only available for the *OXTR* and *RELN* gene (total number of candidate genes = 11; **Table II**).

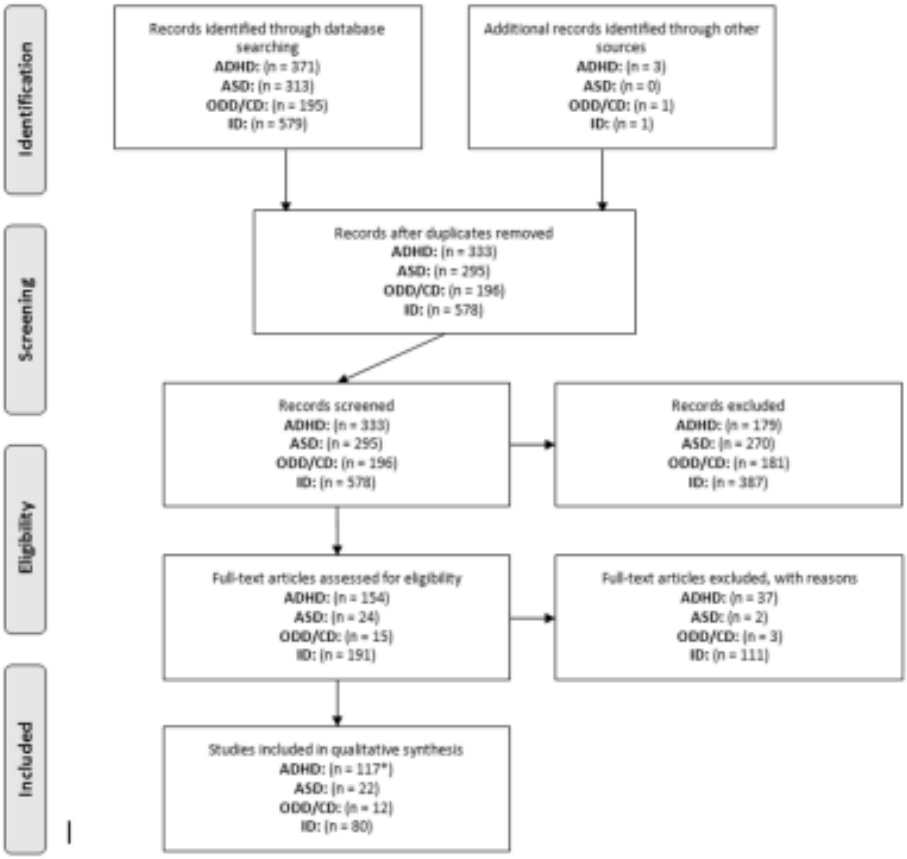


**Figure 1:** Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) flowchart of the literature search and study selection for qualitative analysis.

Note: see http://www.prismastatement.org/ for more information on this reporting system. ADHD = Attention-Deficit/Hyperactivity Disorder, ASD = Autism Spectrum Disorder, ID = Intellectual Disability. Records excluded for ID contain unrelated records identified by screening as well as records describing non-ID samples. *The number of studies for ADHD candidate genes also include the records up to April 2015 for *SLC6A4* (5-HTTLPR), which is also a candidate gene for ODD/CD and ASD.

For ODD/CD, we included the genes that have been most consistently associated with aggression-related phenotypes, thus using a braoder phenotypic definition for gene selection. The two most investigated genes in this context are the serotonin related genes *SLC6A4/5HTT* and *MAOA.* While a first meta-analysis, taking into account 12 polymorphisms in aggression candidate genes, did not provide any meta-analytic evidence for association (Vassos et al., 2014), a later meta-analysis of *5HTTLPR* and *MAOA-uVNTR* variants specifically (described in more detail below) confirmed association with antisocial behaviour (Ficks and Waldman, 2014). Hence, these polymorphisms were selected for the current study. Additionally, we included the *AVPR1A* gene, which showed gene-wide

association significance in the largest aggression GWAS to date, after correcting for 21 candidate gene tests (Pappa et al., 2015). Selection of risk polymorphisms in this gene was based on earlier candidate studies for aggression-related phenotypes (anger and altruistic tendency) (**Table III**; total number of ODD/CD candidate genes = 3).
For the ID, the restrictions to relatively common forms of the disorder resulting from single gene mutations (as opposed to structural genetic variants involving several to many genes) as well as our aim to study potential brain mechanisms contributing to comorbidity among the disorders lead to the inclusion of the following 5 syndromes: fragile X syndrome (*FMR1*), tuberous sclerosis (*TSC1* and *TSC2*), neurofibromatosis type 1 (*NF1*), Rett syndrome (*MECP2*), and Timothy syndrome (*CACNA1C*) (**Table IV**). For our selection, we used Table 1 from Vorstman and Ophoff (2013), describing genetic anomalies associated with ID. We included all disorders with known genetic cause including a single gene (*FMR1, TSC1* and *TSC2, NF1, and CACNA1C*). Patients with these disorders also show a high rate of ASD, aggressive behaviors, and/or ADHD phenotypes (Bailey et al., 2008; Connor and Doerfler, 2008; Hill et al., 2014; Vorstman and Ophoff, 2013). Additionally, we included the Rett syndrome (*MECP2*), because of its known ASD- and ADHD-related features (Armstrong, 2005; Rose et al., 2016; Suter et al., 2014).

## RESULTS

### Imaging genetics of ADHD candidate genes

A total of 76 records were retrieved for the ADHD search term, and a total of 16 research articles describing case-control studies were eligible for review according to our criteria. To those, we added three more recent papers from our own group ((Onnink et al., 2016; Sokolova et al., 2015; van der Meer et al., 2015); **Figure 1**). Most of the studies investigated a single gene (all in Caucasians), and three studies investigated multiple genes (2 in Caucasians, 1 in Asians). In addition, we obtained 295 records for the ADHD candidate gene studies in healthy population samples, of which 98 were eligible (**Figure 1**). Of those, 73 studies investigated a single gene (68 in Caucasians, 5 in Asians), and 25 studies tested more than one gene (1 Asian). The ADHD case-control samples consisted of both childhood/adolescent and adult samples, whereas the studies in the healthy population were largely restricted to samples of (young) adults. Single-gene findings of ADHD case-control studies and studies in the healthy population of both Caucasian and Asian ethnicities can be found in **Table V,** multi-locus studies are shown in **Table VII**. Most of the genes investigated in brain imaging genetics studies in ADHD are from the dopaminergic and serotonergic neurotransmitter systems (*SLC6A3/DAT1*, *DRD2*, *DRD4*, *SLC6A4/5-HTT/SERT*). *SNAP25, DRD5, HTR1B,* and *LPHN3* had also been selected for this study, but for these genes no imaging genetics studies using MRI were found with our search terms.
The **dopamine transporter gene** *DAT1* (official name *SLC6A3*) codes for a solute carrier protein, responsible for the reuptake of dopamine from the synaptic cleft into the presynaptic neuron, representing a primary mechanism of dopamine regulation in the striatum (Ciliax et al., 1999). The most widely studied polymorphism in *SLC6A3/DAT1*

is a variable number of tandem repeat (VNTR) sequence in the 3' untranslated region (3'UTR) that is 40 base pairs (bp) in length. Most common alleles are those with 9 and 10 repeats. Additionally, a 30 bp VNTR in intron 8 of the gene (most common alleles with 5 and 6 repeats), is sometimes studied together with the 3'UTR VNTR as a haplotype. The 10R/10R genotype of the 3'UTR VNTR and the 10-6 haplotype of the two VNTRs are thought to be risk factors for ADHD in children (Asherson et al., 2007; Brookes et al., 2006; Faraone et al., 2005). In contrast, the 9R/9R genotype and the 9-6 haplotype are associated with persistent ADHD (Franke et al., 2010). The sMRI and fMRI studies for *SLC6A3/DAT1*, the latter investigating several cognitive domains known to be impaired in ADHD, i.e. reward processing, working memory, and response inhibition, are summarized in **Table V** and **VII**. The main focus of the studies for this gene has clearly been on the striatum, which shows highest gene expression.

The two sMRI case-control studies were performed in children, and both reported a smaller volume of the caudate nucleus in homozygotes for the 10R allele as compared to children with the 9R/10R genotype (Durston et al., 2005; Shook et al., 2011). A third study, including a large sample of children and adults with and without ADHD, showed that only in the adult ADHD case-control cohort, carriers of the *DAT1* adult ADHD risk haplotype 9-6 had a 5.9% larger striatum volume relative to participants not carrying this haplotype. The effect was depended on diagnostic status, since the risk haplotype affected striatal volume only in patients with ADHD (Onnink et al., 2016).

Two fMRI studies in case-control design investigated the *SLC6A3/DAT1* haplotype using reward paradigms. Independent of the genotype, a recent meta-analysis has shown that in reward-processing paradigms, most studies report lower activation of the ventral striatum in patients with ADHD in anticipation of reward than controls (Plichta and Scheres, 2014). Consistent with this, a study in adolescents (including only males) found the activation of the caudate nucleus to be reduced in the ADHD group as the number of 10-6-haplotype copies increased (Paloyelis et al., 2012). The other study, in adult ADHD cases and controls (in whom the 9-6 allele is the ADHD risk allele), found no effect of *DAT1* haplotype on striatal activity (Hoogman et al., 2013). Studies in healthy adult individuals point in different directions. One found higher activation during reward anticipation in 9R-carriers (Dreher et al., 2009). Another also found increased striatal activation in 9R-carriers in a rewarded task-switching task, especially in high reward conditions (Aarts et al., 2010). A third study in healthy adults suggested that a link between reward sensitivity and striatal activation during reward anticipation is only present in 10R/10R individuals, and is lost in 9R-carriers (Hahn et al., 2011). In studies of response inhibition in children/adolescents, the 10R/10R genotype was found linked to lower (Durston et al., 2008) but also higher (Bedard et al., 2010) striatal activation. Methylphenidate was able to increase activity in the caudate nucleus (as well as a thalamocortical network and inferior frontal gyrus) during successful inhibition in healthy adult male 9R-carriers, but decreased activity in 10R/10R individuals (Kasparbauer et al., 2015). A working memory task in healthy adults elicited more activation in fronto-striatal-parietal regions in 9R/10R individuals under high memory load (Stollstorff et al., 2010). Additionally, a resting-state fMRI study in healthy adults showed stronger connectivity between midbrain (mainly striatal) and prefrontal

regions in 9R/10R heterozygotes compared with 10R/10R homozygotes (Gordon et al., 2015).

Beyond striatum, *SLC6A3/DAT1* genotype effects have also been observed in fMRI studies of cortical regions, especially (pre)frontal, medial (pre-SMA, dorsal ACC), and (temporo) parietal regions (Bedard et al., 2010; Braet et al., 2011) (**Table V and VII**). As expression of DAT is limited outside of striatum and cerebellum, these effects are likely due to direct or indirect connections between the regions of gene expression and the rest of the brain. This is in line with the fact that no effect of *SLC6A3/DAT1* genotype on cortical development has been observed in a longitudinal study (Shaw et al., 2007). Of particular interest might be studies showing effects of *SLC6A3/DAT1* genotype on amygdala reactivity upon exposure to threatening faces (Bergman et al., 2014) as well as on cerebellar activation during response inhibition (Durston et al., 2008). These regions are currently understudied in ADHD. A first study using DTI did not suggest a strong effect of *SLC6A3/DAT1* genotype on structural brain connectivity (Hong et al., 2015) (**Table V**).

In summary, although *SLC6A3/DAT1* is one of the best-studied genes in imaging genetics literature covered in this review, existing studies do not yet clarify sufficiently the role of ADHD-linked genetic variation in brain activity and connectivity related to symptoms/cognitive deficits or their structural brain correlates. A complicating matter for this gene is the switch in ADHD risk allele from childhood to adulthood. Furthermore, interactions between genotype and diagnosis are observed in some studies, which suggest that studying effects of *SLC6A3/DAT1* in healthy individuals will not suffice to fully understand the brain mechanisms linking this gene to ADHD.

The **dopamine D2 receptor** gene (*DRD2*) codes for a G protein-coupled receptor, which inhibits adenylate cyclase (Andersen et al., 1990). Consistent with its broad expression in the brain being highest in striatum, *DRD2* plays a key role in regulating mesolimbic reward processing pathways (Usiello et al., 2000) and is also implicated in other cognitive domains, such as cognitive flexibility and learning (Puig et al., 2014). The gene has been implicated in many different psychiatric disorders, including schizophrenia and substance use disorders (Patriquin et al., 2015; Schizophrenia Working Group of the Psychiatric Genomics, 2014) and is the target of several antipsychotics (Moore et al., 2014). The risk factor for ADHD is the most frequently investigated common genetic variant of *DRD2* rs1800497 (also known as Taq1A restriction fragment length polymorphism). This SNP actually lies downstream of *DRD2* in an exon of a neighboring gene, *ANKK1* (Neville et al., 2004). It affects dopamine D2 receptor expression and striatal dopamine metabolism, with the A1-allele (the ADHD risk allele) reducing the number of *DRD2* receptors (Laakso et al., 2005). No studies in ADHD case-control design are yet available for *DRD2.* The risk SNP has, however, been investigated in healthy individuals using structural and functional MRI covering the cognitive domains of reward processing, task-switching and reversal learning, working memory, emotion recognition, and language (**Table V** and **VII**).

Structural MRI showed that the SNP affects the volume of midbrain structures, with A1-allele carriers having smaller volumes of substantia nigra (Cerasa et al., 2009), cerebellum (Wiener et al., 2014), and ACC (in interaction with *BDNF*; (Montag et al., 2010)).

Functional MRI during reversal learning tasks revealed that A1-allele carriers showed

reduced response of the rostral cingulate to negative feedback and had a reduced recruitment of the right ventral striatum and right lateral occipital frontal cortex (OFC) during reversals (Jocham et al., 2009). Pharmacological fMRI in a reversal learning task showed that cabergoline (D2 receptor agonist) administration induced an allele-specific response, where A1-allele carriers showed increased neural reward responses in medial OFC, cingulate cortex, and striatum (consistent with increased D2-mediated dopamine signaling); this was coupled, however, to worse task performance and lower fronto-striatal functional connectivity (Cohen et al., 2007). The reward-related paradigms showed that A1-allele carriers exhibited increased anterior insula (Richter et al., 2013) and increased nucleus accumbens activation, the latter observed only in a three-way interaction analysis looking for differences between a placebo and bromocriptine (D2 receptor agonist) administration condition (Kirsch et al., 2006). Two multi-locus studies including the *DRD2* Taq1A variant suggested higher activation during reward anticipation, but blunted activity during reward receipt with increasing number of risk factors (**Table VII**).

In summary, the effects of the ADHD risk factor in *DRD2* in fMRI appear to be relatively consistent across most of the studies currently available, with stronger brain activity in parts of the wider reward processing and memory/learning circuits. It seems that this stronger activity is linked to worse functional connectivity and/or performance, thus potentially reflecting compensatory processes. Currently, no data from patients with ADHD are available.

The **dopamine D4 receptor** (encoded by the *DRD4* gene) is another G protein-coupled receptor and belongs to the dopamine D2-like receptor family (Oldenhof et al., 1998). The most widely studied *DRD4* polymorphism in ADHD has been the 48 bp VNTR in exon 3, with the 2-, 4-, and 7-repeat alleles being the most common alleles. Allele frequencies vary significantly across ethnic groups (Chang et al., 1996; Van Tol et al., 1992), and the ADHD risk allele in the Caucasian population (7R) seems to be a different one from that in Asians (Nikolaidis and Gray, 2010; Wang et al., 2004).

Structural MRI suggested that patients with ADHD carrying the 7R-allele have smaller volumes of the superior frontal and cerebellar cortex (Monuteaux et al., 2008), while no differences were found in another study (Castellanos et al., 1998) (**Table V**). Interestingly, carriership of the *DRD4* 7R-allele seemed to affect cortical development in a longitudinal study, with 7R-carriers showing thinner prefrontal and parietal cortex and ADHD patients with this allele having a distinct trajectory of cortical development characterized by normalization of parietal cortical regions (Shaw et al., 2007) (**Table VII**). Structural connectivity was investigated in two studies in Asians using DTI, and while one did not find effects for 4R homozygotes (Hong et al., 2015), a very large recent study reported widespread increases in mean diffusivity in 5R-carriers (Takeuchi et al., 2015) (**Table V**).

With the role of the D4 dopamine receptor in cognition not sufficiently characterized yet, and *DRD4* being expressed in large parts of the cortex (predominantly in frontal lobe regions, such as the OFC and ACC (Floresco and Tse, 2007; Noain et al., 2006)), fMRI studies have investigated the *DRD4* gene in healthy Caucasians covering different cognitive domains, i.e. emotion processing, response inhibition, reward, stimulus-response incompatibility, and time discrimination tasks, as summarized in **Table V**. Depending on

the type of paradigm used in the fMRI studies, *DRD4* genotype was found to modulate brain activity in prefrontal and temporal, but also in striatal and cerebellar brain regions in the healthy adults (**Table V**).

Thus, though existing evidence does not support firm conclusions, *DRD4* may mark a particular developmental trajectory in cortical brain structure related to adult outcome of ADHD, and plays a role in structural connectivity. With only one fMRI study per cognitive domain published to date, no clear picture of *DRD4* action on brain activity emerges, but those studies do clearly indicate that *DRD4* (like *DAT1*) influences brain activity beyond its regions of expression, possibly due to its effects on white matter connectivity (Takeuchi et al., 2015).

The **serotonin transporter** gene (*SLC6A4*, *5HTT, SERT*) codes for a solute carrier protein responsible for the reuptake of serotonin from the synaptic cleft back into the presynaptic neuron, which is the primary mechanism for regulation of serotonergic activity in the brain (Lesch et al., 1996). A functional polymorphism in the promoter region of the gene (referred to as 5HTTLPR) is a 44-bp insertion/deletion yielding short (S) and long (L) alleles. The long variant is associated with more rapid serotonin reuptake, resulting in lower levels of active serotonin (Lesch et al., 1996). However, allele frequencies vary across different ethnic groups (Haberstick et al., 2015). A SNP in the long allele, rs25531, can modify the activity of this allele (Lesch et al., 1996). *SLC6A4/5HTT* has been implicated in emotion regulation as well as (emotional) memory and learning processes (Araragi and Lesch, 2013; Barzman et al., 2015; Meneses and Liy-Salmeron, 2012). Expression of the transporter is observed in regions implicated in attention, memory, and motor activities, such as the amygdala, hippocampus, thalamus, putamen, and ACC (Frankle et al., 2004; Oquendo et al., 2007). Only one recent imaging genetics study in patients with ADHD has been performed for the 5HTTLPR, showing that stress exposure, which is associated with increased ADHD severity in S-allele carriers, was associated with reduced cortical gray matter volume in precentral gyrus, middle and superior frontal gyri, frontal pole, and cingulate gyrus in these individuals. Interestingly, this paper showed that only some of these regions, the frontal pole and the ACC, actually mediated the effects of the gene-environment interaction on ADHD severity. In sMRI studies in healthy individuals, the 5HTTLPR has been associated with volume of the ACC and amygdala as well as hippocampus, though the direction of effect seemed to differ with gender and/or in interaction with environmental factors (**Table V**). Few studies have looked at effects of the 5HTTLPR on structural connectivity (**Table V**). A large study observed reduced connectivity of amygdala with PFC in S-allele carriers (Long et al., 2013), while another reported increased hippocampus-putamen connectivity for this genotype group (Favaro et al., 2014).

Brain activation patterns in task-based fMRI have been studied extensively for the 5HTTLPR following hallmark studies by the Weinberger lab (Hariri et al., 2005; Hariri et al., 2002). They were the first to report increased activation of the amygdala in S-allele carriers in response to negative-emotional faces. Since then, increased amygdala activation has been observed in S-allele carriers in many tasks activating the amygdala (**Table V** and **VII**). In 2013, 34 studies investigating effects of the 5HTTLPR on amygdala activation were meta-analyzed, confirming the increased activation in S-allele carriers (although only

borderline significant) (Murphy et al., 2013). However, this meta-analysis also showed strong heterogeneity between studies and a potential publication bias (towards studies reporting significant associations). Linked to the increased activation seems to be a reduced functional connectivity of the amygdala, as first observed by Pezawas and colleagues (2005) and subsequently also seen in additional studies (**Table V**). Not only the amygdala, but also other cortical and subcortical brain regions (forming the 'threat circuit') seem to be influenced by 5HTTLPR genotype. A recent, replicated fMRI study, for example, also showed stronger activity in dorsomedial prefrontal cortex (dmPFC), insula, thalamus, and regions of the midbrain, in reaction to threat in S-allele carriers (Klumpers et al., 2014); interestingly, also in this study (like in the one by van der Meer and coworkers (2015)) only some of the activated regions actually mediated the genotype effects on psychophysiological responsivity to pending threats (in this case the dmPFC activation, **Table V**).

Increasing evidence suggests that S-allele carriers are hypervigilant to environmental stimuli (Homberg and Lesch, 2011). Potential sustained effects of environmental factors have not sufficiently been addressed in imaging genetics studies published to date. Several studies have taken stressful life events into account, and these studies suggested effects on both brain volume and activation. Only one study to date has directly looked at methylation of the promoter of the *SLC6A4/5HTT* gene, and found correlations with the volume of several regions in the 'threat circuit' of the brain, though these appeared genotype-independent (Dannlowski et al., 2014). Also a combined PET, sMRI plus fMRI study indicated that 5HTTLPR genotype did not influence current (midbrain) serotonin transporter availability (Kobiella et al., 2011), suggesting that other factors (like environmental ones) might overrule this effect. Taking into account epigenetic effects on the *SLC6A4/5HTT* gene might thus help explain the strong heterogeneity observed in the meta-analysis of amygdala reactivity studies (Murphy et al., 2013).

In summary, functional genetic variation in the *SLC6A4/5HTT* gene is clearly linked to emotion regulation through effects on brain activation in the amygdala and the wider 'threat circuit', with those carrying the risk factor for emotional dysregulation showing increased activation in tasks related to emotion processing and learning. Those experiments link reduced availability of the transporter (at some point in development) - and thus increased serotonin signaling capacity - to increased brain activation. This increased activation seems to be linked to functional dysconnectivity, however. Whether brain volume and structural integrity are influenced by the 5HTTLPR, remains to be clarified. Importantly, genotype effects are likely to be sensitive to environmental factors.

The **nitric oxide synthase 1** (encoded by the *NOS1* gene) is an enzyme which synthesizes nitric oxide from L-arginine. Nitric oxide is a reactive free radical, which acts as a biological mediator in several processes, including dopaminergic and serotonergic neurotransmission (Kiss and Vizi, 2001). The *NOS1* gene has a complex structure, including 12 alternative untranslated first exons (exon 1a-1l). In exon 1f, a functional VNTR that affects gene expression has been linked to hyperactive and impulsive behavior in humans (Reif et al., 2009; Weber et al., 2015), with the short allele being the risk factor for ADHD. In addition, a recent *Nos1* knock-out mouse model showed dysregulation of rhythmic activities mimicking ADHD-like behaviors (Gao and Heldt, 2015).

So far, only one case-control study investigated the effect of the VNTR polymorphism on the brain, in his case on reward-related ventral striatal activity (Hoogman et al., 2011) (**Table V**). The study revealed that homozygous carriers of the short allele of *NOS1* demonstrated higher ventral striatal activity than carriers of the other *NOS1* VNTR genotypes (Hoogman et al., 2011). This effect was comparable for both patients and healthy individuals. Similar effects of the genotype were also observed for behavioral impulsivity, with those carrying the ADHD risk factor acting more impulsive than other participants.

**Imaging genetics of candidate genes for autism spectrum disorders**

A total of 193 records were retrieved for the ASD search terms, and a total of six research articles were eligible for review according to our criteria. All studies investigated a single gene and were performed in Caucasian populations. For studies in the healthy population, we obtained 120 records, and 17 were included in the review (**Figure 1**). Twelve of those investigated a single gene in a Caucasian study sample, and five studies used Asian samples (studies for *SLC6A4/5HTT* are included in the ADHD section above). Generally, the ASD case/control samples included mainly childhood and adolescent study samples, whereas the studies in healthy population samples mostly used samples of (young) adults. From the eleven genes selected and listed in **Table VI**, imaging genetics studies could only be retrieved for genetic variants in *CNTNAP2*, *MET*, *OXTR*, and the *SLC6A4/5HTT* gene.

The **contactin-associated protein-like 2** (CASPR2), encoded by the gene *CNTNAP2* (the largest gene in the human genome), is a neural transmembrane protein involved in neuronal-glial interactions and in clustering $K^+$-channels in myelinated axons; as such, it is involved in neuronal cell adhesion, migration, and the formation of neuronal networks (Rodenas-Cuadrado et al., 2014). Several single nucleotide polymorphisms (SNPs) in *CNTNAP2* have been associated with ASDs. During human brain development, *CNTNAP2* expression is broad, with highest levels in frontal and anterior lobes, striatum, and dorsal thalamus. This cortico-striato-thalamic circuitry is important for higher order cognitive functions, including speech and language, reward, and frontal executive function (Rodenas-Cuadrado et al., 2014). This is reflected in the imaging genetics studies having been performed for *CNTNAP2*, which cover studies of brain volume and structural connectivity as well as brain activity and functional connectivity during tasks related to rewarded learning and language (**Table VI**).

Two studies performed DTI in healthy individuals. For the SNP rs2710102 it was found that carriers of the CC risk genotype showed reduced overall path length and increased small-worldness of brain-wide structural connectivity, which appeared to be a general phenomenon rather than being localized to individual tracts (Dennis et al., 2011). A large study in healthy individuals combining sMRI with DTI for the SNP rs7794745 showed that carriers of the ASD risk genotype exhibited reduced gray and white matter volume as well as reduced white matter integrity in the cerebellum, fusiform gyrus, occipital and frontal cortices; distribution of reductions was found to be sex-specific (Tan et al., 2010).

In a case-control study, an association between the SNP rs2710102 and medial prefrontal cortex activation during a rewarded implicit learning task was found, when collapsing patients and controls into one group. The non-risk allele was linked to reduced activation.

Furthermore, the risk carriers had more widespread and bilateral connectivity throughout the frontal cortex and anterior temporal poles. The latter finding was confirmed in an independent healthy sample (Scott-Van Zeeland et al., 2010). An additional fMRI study using a sentence completion paradigm showed that carriers of the risk genotype for one of two SNPs had increased activation of the IFG (Broca's area), the lateral temporal cortex, or right middle temporal gyrus (Whalley et al., 2011).

The **Met proto-oncogene** encoded by the *MET* gene is a cell surface receptor with tyrosine-kinase activity. In the forebrain, *MET* gene and protein expression is regulated in excitatory projection neurons during synaptogenesis (Judson et al., 2011) and is restricted to regions of temporal, occipital, and medial parietal cortex in humans. These regions are known to be of relevance to the processing of socially relevant information (Rudie et al., 2012). The effects of the ASD risk variant rs1858830 have been studied in two imaging genetics studies (**Table VI**).

A case-control study combining fMRI (emotional face task), resting-state fMRI, and DTI modalities showed that the ASD risk genotype predicted wide-spread atypical brain activity patterns to social stimuli, with increased activation in amygdala and striatum, and impaired deactivation patterns in part of the default mode network (DMN) in the posterior cingulate cortex. In addition, reduced functional and structural connectivity was observed in temporo-parietal regions belonging to the DMN suggesting altered white matter integrity. In general, the effects were more pronounced in the ASD group (Rudie et al., 2012). An sMRI study in a large sample of healthy individuals revealed that cortical thickness in temporal, pre- and postcentral gyri, anterior cingulate, and frontopolar cortex was reduced in risk-allele carriers, with reductions increasing with increasing number of risk alleles (Hedrick et al., 2012).

The **oxytocin receptor** (*OXTR*) gene encodes the receptor protein for oxytocin, which has an important role in the regulation of social cognition and behavior (Meyer-Lindenberg et al., 2011). So far, no imaging genetic studies were performed for risk variants in the *OXTR* gene in ASD case-control samples, but twelve studies in healthy samples were found (**Table VI**). Various different SNPs and combinations of those were investigated, not all related to ASD risk.

Two sMRI studies showed that adolescents homozygous for the rs2254298 risk factor for psychopathology displayed an overall increased gray matter volume, but a decreased amygdala volume (Furman et al., 2011); for carriers of the rs53576 SNP, a risk factor for disorders associated with social impairment, a smaller hypothalamus gray matter volume was reported in healthy adults (Tost et al., 2010).

Functional MRI paradigms used to study *OXTR* all covered the cognitive domains of emotion processing and reward (**Table VI**). In a face matching task, adult carriers of the rs53576 risk allele showed increased functional correlation of hypothalamus and amygdala during perceptual processing of facial emotion (Tost et al., 2010). Investigating a large group of 1445 healthy adolescents in a passive face viewing task for effects of 23 SNPs across *OXTR*, the IMAGEN Consortium found significant effects of one SNP on ventral striatal activity in a region of interest analysis. In the presence of stressful life events, this SNP modulated the occurrence of emotional problems in the participants, linking more

emotional problems to reduced striatal activation; no effects of the risk variants for ASD were observed (Loth et al., 2014). A study of brain regions related to processing of social stimuli observed increased functional connectivity between such regions in adult carriers of the risk genotype for rs53576 (Verbeke et al., 2013). Functional MRI of mesolimbic structures during reward processing was modulated by the rs2268493 risk factor for ASD: young adult carriers of the risk genotype showed reduced activation in mesolimbic reward circuitry (nucleus accumbens, amygdala, insula, thalamus, and prefrontal cortical regions) during the anticipation of rewards but not during reward receipt (Damiano et al., 2014). Using a mother-child interaction task, Michalska and coworkers (2014) showed that females carrying the ASD risk genotypes for rs53576 or rs1042778 had lower brain activity in OFC, ACC, and hippocampus in response to child stimuli. When healthy adult females were tested for empathic response and associated brain activation, carriers of the rs2254298 risk factor for psychopathology showed increased responsiveness of the superior temporal sulcus to observed pain (Laursen et al., 2014). In a pharmacologic imaging genetics study in adult males, one of three SNPs modulated the response of the amygdala (only) after oxytocin inhalation, with increased activation to directed gaze and decreased activation to averted gaze under oxytocin in the carriers of the variant allele (Montag et al., 2013). This study did not find any effects of rs2254298 on brain activation.

In summary, genetic variation in the *OXTR* gene has been linked to brain activation during emotional processing. Risk factors for ASD/psychopathology appear to reduce activation during most relevant paradigms, but may increase functional connectivity during those tasks.

Four ASD case-control imaging genetics studies investigated the gene encoding the **serotonin transporter** gene (*SLC6A4*, *5HTT*) in addition to those in healthy individuals (and ADHD case-control samples) described in the section on ADHD candidate genes. Structural MRI, fMRI, and rs-fMRI were used to study the effect of either only the 5HTTLPR or the combination of this variant with rs25531 (**Table VI**).

Whereas a VBM study did not reveal an association between total gray or white matter volume and genotype in adult patients (Raznahan et al., 2009), another sMRI study showed that in 2-4 year old boys with ASD, carriers of the 5HTTLPR S-allele had increased total cortical and frontal lobe gray matter volume (Wassink et al., 2007), suggesting an age-dependent effect of the variant.

The fMRI and rs-fMRI study, performed in overlapping samples of adolescent patients and controls, showed that carriers of alleles that mark low gene expression had increased amygdala activation during an emotional face task, an effect that was observed only in the patients (Wiggins et al., 2014b), and increased posterior-anterior connectivity during a resting-state condition in patients, where the converse was observed in the healthy group (Wiggins et al., 2012).

The findings of those case-control studies are not easily reconciled with those observed in healthy individuals (**Table V** and **VII**), and indeed the latter two studies suggest the existence of differential effects in patients and healthy individuals.

**Imaging genetics of candidate genes in oppositional defiant disorder and conduct disorder**

A total of 158 records were retrieved for the ODD and CD search terms. No case-control studies were identified. For studies in the healthy population, we obtained 38 records, of which 15 were eligible for review (**Figure 1**). This search included imaging genetics studies for *AVPR1A* and *MAOA*, updated up to October 1th, 2017. Studies investigating *SLC6A4/5HTT* in healthy individuals are included in the ADHD section above (**Table V**). Imaging genetics studies for genetic variants in *AVPR1A* and *MAOA* in healthy individuals are summarized in **Table VIII.** Most of these studies investigated Caucasian adolescents or (young) adults.

The **Arginine Vasopressin Receptor 1A** (*AVPR1A*) gene encodes the primary receptor of arginine vasopressin (AVP) in the brain. This is a neuropeptide, which is strongly implicated in emotional and complex social behaviors, including aggressive behaviors (Ebstein et al., 2010). Two repeat polymorphisms (RS1 and RS3) exist in the promoter region of the gene, with shorter alleles of RS3 and the 320 bp allele of RS1 marked as risk alleles (Knafo et al., 2008; Moons et al., 2014). While no imaging genetics studies have been performed so far in case-control samples for ODD or CD, two studies investigated the effect of microsatellite variants RS1 and RS3 on the brain in healthy individuals (**Table VIII**). One study investigated brain activation during a face-matching task in a Caucasian population, and found a significant increase in left amygdala activation in carriers of the 334 bp risk allele of RS3 compared to all other alleles. The 320 bp risk allele of RS1 was associated with decreased left amygdala activity compared to other alleles (Meyer-Lindenberg et al., 2009). The second study investigated effects of RS3 on grey matter volume in a Chinese Han population. Smaller grey matter volume of the right fusiform face area (FFA) was found in male subjects with shorter repeats, representing risk for (decreased) altruistic decision making (Wang et al., 2016). Although more information is necessary, these first studies suggest an association of *AVPR1A* risk factors for psychopathology with the structure and function of brain areas involved in processing socially relevant information (amygdala and FFA).

The **monoamine oxidase A** gene (*MAOA*), located on the X-chromosome, encodes a key-enzyme in the metabolism of monoamine neurotransmitters. The 30 base pair variable number of tandem repeats of the gene (MAOA-uVNTR) is the most commonly studied polymorphism in the context of human antisocial behaviors. Transcription of short repeats (2 or 3 repeated copies) of this VNTR results in reduced MAOA activity (MAOA-L), while long repeats (3.5 or 4 copies) result in increased activity (MAOA-H). Low activity variants, leading to increased serotonin in the synapse, are positively associated with antisocial behaviors in meta-analysis (Ficks and Waldman, 2014).

Only one study investigated the effect of *MAOA* genotype group on brain structure, and found pronounced limbic grey matter volume reductions, including structures like amygdala and ACC, in individuals homo-/hemizygous for MAOA-L compared to MAOA-H alleles (Meyer-Lindenberg et al., 2006). Ten additional studies investigated brain function, and several confirm differential task-related activation of the structurally affected limbic brain regions for subjects with MAOA-L compared to MAOA-H genotypes (**Table VIII**). Studies

using face-matching tasks detected increased activity in the amygdala as well as increased amygdala connectivity with the ventromedial PFC, which was male-specific (Buckholtz et al., 2008; Meyer-Lindenberg et al., 2006). Sex-specificity of brain function differences during face-matching was also highlighted by a later study, where a three-way interaction of *MAOA* genotype group, sex, and stressful life events on brain activity was found: male MAOA-L subjects with a high exposure to life stress showed increased amygdala and hippocampal activity, while a decrease was seen in male MAOA-H subjects. Interestingly, females showed the opposite pattern (Holz et al., 2016). Other fMRI studies using tasks related to emotion processing confirmed increased amygdala activation in male MAOA-L carriers. Adverse emotional memory retrieval (Meyer-Lindenberg et al., 2006), response to the word 'no' in subjects with high anger reactivity (Alia-Klein et al., 2009), and response to anger provocation (Denson et al., 2014) all elicited increased amygdala activation in male MAOA-L carriers. While the same activity increase has been found for female carriers of MAOA-L alleles during passive viewing of sad faces (Lee and Ham, 2008), results are more variable for women (e.g. (Holz et al., 2016)). One report, studying females only, found increased amygdala activation during a rejection-themed emotional Stroop paradigm in MAOA-L adults, but decreased activation in MAOA-L adolescents (Sebastian et al., 2010), suggesting that functional development of the circuitry underlying the processing of social rejection continues throughout adolescence into adulthood. Hence, the effects of MAOA genotype on emotional or social neural responses may vary not only with sex, but also with age.

Functional imaging genetics studies for *MAOA* have also probed cognitive inhibitory control. Studies using a Flanker task show that male MAOA-L carriers have decreased activation in dorsal ACC during response inhibition (Holz et al., 2016; Meyer-Lindenberg et al., 2006). Another study, investigating how MAOA affects brain activity within resting-state networks, confirms reduced activity in brain areas related to inhibitory and executive control for MAOA-L subjects (Clemens et al., 2015).

In summary, available imaging genetics studies in healthy individuals confirm that genetic variation in the *MAOA* gene is linked to brain activation during emotion and inhibitory processing. Increased amygdala activation during emotion processing and reduced activation in regions regulating inhibitory control might be intermediate phenotypes for aggressive behaviour, suggesting a possible mechanism of susceptibility for ODD/CD and other aggression-related phenotypes.



**Imaging genetics in selected intellectual disability disorders**

A total of 579 records were retrieved for the ID syndromes of interest. Eighty research articles were eligible for review according to our criteria, 30 for fragile X syndrome, 24 for neurofibromatosis type 1, 22 for tuberous sclerosis complex, and four for Rett syndrome (**Figure 1**). No imaging studies of Timothy syndrome patients were uncovered by our search term. The reviewed imaging genetics studies in ID syndromes are presented in **Table IX**.

The **fragile X mental retardation 1** gene (*FMR1*) is located on the X chromosome and codes for fragile X mental retardation protein. Large expansions of a CGG repeat (>200 repeats) in the 5'- untranslated (5'UTR) region of the gene, leading to protein deficiency,

are the cause of fragile X syndrome (FXS). FMR1 has a prominent role in synaptic plasticity and maturation (Saldarriaga et al., 2014). In studies including participants with the *FMR1* full mutation, brain structure was most often investigated, followed by task-based brain activation (**Table IX**). A few studies investigated brain structural integrity and resting-state functional connectivity. Several studies compared individuals with FXS with and without ASD or included an idiopathic autism or IQ-matched group (**Table IX**).

The most robust finding in investigations of brain structure in FXS is an increased caudate nucleus volume. This enlargement was observed early in development (Hazlett et al., 2009), throughout adolescence (Bray et al., 2011; Hall et al., 2013; Lee et al., 2007) as well as in adult samples (Hallahan et al., 2011; Molnar and Keri, 2014; Wilson et al., 2009). Studies comparing individuals with FXS and with ASD found increased caudate volumes in children and adults with FXS compared to children/adults with idiopathic autism (Hazlett et al., 2009; Wilson et al., 2009). Consistent volumetric abnormalities have also been found for cerebellar regions in FXS; a reduction in the volume was observed in both children and adults with FXS (Hazlett et al., 2012; Hoeft et al., 2008; Wilson et al., 2009). Several studies found cerebellar volumes to be larger in children and adults with FXS relative to individuals with autism, in whom reduced volume of cerebellar regions compared to control subjects is often seen as well (Hazlett et al., 2012; Wilson et al., 2009). Few studies have investigated white matter integrity in people with the full *FMR1* mutation, and deficits seem most prominent in fronto-striatal connections. Increased density of fibers was found in the left ventral fronto-striatal pathway in boys with FXS compared to typically developing and developmentally delayed controls (Haas et al., 2009), and differences in white matter in frontal-caudate circuits were found in females with FXS compared to controls (Barnea-Goraly et al., 2003). More widespread reductions in white matter integrity have also been observed (Villalon-Reina et al., 2013).

Cognitive and psychiatric characteristics associated with FXS include poor eye contact, repetitive motor behavior, language deficits, inattention, hyperactivity, inhibition, and anxiety (Saldarriaga et al., 2014). Functional neuroimaging studies have focused on these deficits, with a main focus on poor eye contact and behavioral inhibition. Several fMRI studies have investigated the circuitry underlying face/gaze processing in subjects with FXS, as eye-gaze avoidance is common in this population. Abnormal activation was found in several regions, including superior temporal gyrus and fusiform gyrus (Garrett et al., 2004), amygdala and insula (Watson et al., 2008), regions within the ventrolateral prefrontal cortex (vlPFC) (Holsen et al., 2008), and frontal cortex and cingulate and fusiform gyri (Bruno et al., 2014). These regions are associated with visual processing, social cognition, emotion processing, and executive functioning, indicating that eye-gaze avoidance in FXS may be linked to social anxiety. Investigating attention and inhibition, a study using a Go/No-go task found that boys with FXS show reduced activation in the right vlPFC and caudate head. The authors suggested that defective fronto-striatal signaling is a key feature of FXS, leading to impairments in executive functioning (Hoeft et al., 2007), which is in line with the altered white matter connectivity in fronto-striatal connections, described above.

The **neurofibromin 1** gene (*NF1*) located on chromosome 17q11.2 codes for neurofibromin, a protein which is thought to be a regulator of the RAS signal transduction pathway

and necessary for embryonic development. Neurofibromatosis type 1 (NF1) is caused by mutations in the gene, often leading to the synthesis of truncated or otherwise non-functional proteins. We found 14 studies investigating effects of *NF1* on brain structure and four investigating brain function. Additional studies of brain structural and functional connectivity have been conducted. While most studies included children and adolescents, a few studies have included adults as well (Duarte et al., 2014; Karlsgodt et al., 2012; Pride et al., 2014; Violante et al., 2012; Wignall et al., 2010; Zamboni et al., 2007) (**Table IX**).
The structural brain abnormalities most commonly seen in subjects with NF1 are T2 hyperintensities and an increased brain volume. T2 hyperintensities are areas of high signal intensity on T2-weighted MR images also referred to as 'unidentified bright objects' (UBOs). Although their association with cognitive and intellectual deficits remains controversial, thalamic hyperintensities have repeatedly been associated with cognitive impairments (Payne et al., 2010). Multiple studies have investigated the characteristics of UBOs. UBOs are found in almost all children with NF1, but reports on whether their volume and number increases or decreases with age are inconsistent (Gill et al., 2006; Griffiths et al., 1999; Kraut et al., 2004). A few studies have used diffusion tensor imaging (DTI) to characterize white matter microstructure and integrity of UBOs by measuring the degree and directionality of diffusivity. Higher apparent diffusion coefficient (ADC) and (radial) diffusivity values and lower fractional anisotropy (FA) values have been found in UBOs compared to normal appearing white matter (Ertan et al., 2014; van Engelen et al., 2008). These findings can be explained by myelin deficiency and axonal damage. An increase in brain volume is observed in children with NF1, which was found to be due to increases in white matter volume (Said et al., 1996; Steen et al., 2001), gray matter volume (with an increased gray to white matter ratio especially in younger subjects (Moore et al., 2000)), or both gray and white matter volume (Karlsgodt et al., 2012). These volume increases involve temporal, parietal, occipital, and frontal regions (Duarte et al., 2014; Greenwood et al., 2005; Pride et al., 2014). In addition, the corpus callosum seems larger in cases compared to controls, which has been found in children with NF1 as well as adults, marking it as a robust finding for NF1 (Duarte et al., 2014; Moore et al., 2000; Violante et al., 2013; Wignall et al., 2010). In addition to the investigation of UBOs, DTI studies have been used to study microstructural integrity in NF1 more broadly. Increased ADC values (Ertan et al., 2014; Nicita et al., 2014; van Engelen et al., 2008) and decreased FA values (Ertan et al., 2014; Ferraz-Filho et al., 2012) are found widespread across the brain. Karlsgodt et al. also found increased radial diffusion, which may be explained by decreased myelination or axonal packing density (2012). Differences in radial diffusivity have also been observed at the genu and anterior body of the corpus callosum (Wignall et al., 2010). The change in corpus callosum size and connectivity observed in NF1 may have functional importance, as they have been associated with academic achievement and visual-spatial and motor skills (Moore et al., 2000).
Three fMRI studies have investigated visual-spatial processing in subjects with NF1, and one study investigated phonologic processing (**Table IX**). During visual-spatial processing, decreased activation in the primary visual cortex was found for individuals with NF1 compared to controls (Clements-Stephens et al., 2008), although an earlier study reported

contrasting findings of increased posterior (occipital) cortex activation relative to lateral/inferior frontal activation (Billingsley et al., 2004). A later study did confirm that both children and adults with NF1 showed deficient activation of the low-level visual cortex during tasks specifically designed to activate magnocellular and parvocellular pathways (Violante et al., 2012). During such magnocellular-biased stimulation, NF1 patients did not deactivate regions belonging to the brain default-mode network as would be expected during cognitively demanding tasks (Violante et al., 2012).

The tumor growth suppressor genes **tuberous sclerosis 1** (*TSC1*) and **tuberous sclerosis 2** (*TSC2*) code for the hamartin and tuberin proteins, respectively. Mutations in either *TSC1* or *TSC2* disrupt the function of the GTPase-activating protein (GAP) complex formed by these proteins that regulates mTOR signaling. The neurocutaneous syndrome tuberous sclerosis complex (TSC), characterized by benign hamartomas in multiple organ systems, is caused primarily by these mutations. In the brain, the hamartomas manifest as subendymal giant cell astrocytomas, subendymal nodules (SEN), and tubers. Tubers show disrupted cortical architecture and contain a number of atypical cells. For TSC, structural MRI and DTI studies have been conducted investigating both typical neuropathological lesions, especially tubers, and normal-appearing brain matter (**Table IX**). A consistent imaging determinant of the cognitive phenotype in TSC has not been established. Findings of an inverse correlation of tuber number and cognitive functioning have not been consistent (Ridler et al., 2004). Tuber/brain proportion may be a better predictor of IQ than tuber load, although the age of seizure onset in patients seemed to predict cognitive functioning best (Jansen et al., 2008). However, abnormal brain structure and connectivity unrelated to tubers are likely also important factors contributing to the neurobehavioral abnormalities in TSC. Decreased white matter volume of major intrahemispheric tracts has been found in adults with TSC compared to age-matched controls, as has a decrease of gray matter volume in several cortical and subcortical structures (Ridler et al., 2001; Ridler et al., 2007). Reduced volume in the cerebellum has been associated with tuber-associated loss of the underlying parenchyma (Jurkiewicz et al., 2006; Marti-Bonmati et al., 2000). Reduced cerebellar volume was observed in all cerebellar regions in a more recent study, with strongest volume reductions in patients with a mutation in *TSC2* (Weisenfeld et al., 2013). The finding of reduced cerebellar volume is in line with mouse models showing cerebellar involvement in TSC (Reith et al., 2011). White matter abnormalities are another typical finding in TSC. DTI studies generally report increased ADC values and decreased FA values in individuals with TSC compared to controls, in tubers and white matter lesions, but also in other white matter portions (**Table IX**). Compared to contralateral white matter or white matter in control subjects, increased ADC values were found in cortical tubers, and higher ADC and lower FA values were found in white matter lesions (Piao et al., 2009). A recent study also found increased radial diffusivity values and decreased FA values in cortical tubers and white matter lesions (Dogan et al., 2015). Hypomyelination, gliosis, and heterotopic cells may lead to ADC and FA changes observed in such lesions (Alexander et al., 2007). Abnormalities have also been reported in normal-appearing white matter in individuals with TSC compared to control groups. Decreased FA and increased ADC, especially in corpus callosum and internal and external capsules, have been reported

repeatedly (Krishnan et al., 2010; Peters et al., 2012; Simao et al., 2010). A recent whole-brain analysis of white matter connectivity showed that increased radial diffusivity exists throughout the brains of TSC patients and that interhemispheric connectivity is decreased (Im et al., 2015).

The **methyl CpG binding protein 2** gene (*MECP2*) is located on the short arm of chromosome X (Xq28) and codes for the protein MECP2. MECP2 acts as a modifier of gene expression and is highly expressed in the brain. Mutations in *MECP2* are the cause of Rett syndrome, a disorder primarily affecting female patients. Brain weight is reduced in Rett syndrome, particularly that of cerebral hemispheres. Although the anatomical basis for this reduction is not completely clear, it has been suggested that it is caused by defective neuronal maturation for which MECP2 is essential, rather than by atrophy (Armstrong, 2005). Only few imaging studies have been conducted in series of patients with Rett syndrome (**Table IX**). All investigated brain structure in girls. These studies confirmed a wide-spread reduction in cerebral white and gray matter volumes, the latter most pronounced in subcortical nuclei including the caudate nucleus and in prefrontal, posterior-frontal, and anterior-temporal (Reiss et al., 1993; Subramaniam et al., 1997) and parietal regions (Carter et al., 2008). Using DTI, evidence of reduced white matter integrity was found in frontal regions, corpus callosum, and internal capsule. FA was also reduced in the superior longitudinal fasciculus, but only in patients who had little or no ability to speak (Mahmood et al., 2010).



## DISCUSSION

In this review, we set out to summarize the literature on imaging genetics studies in neurodevelopmental disorders. This being a very broad field, we focused on five most frequent and often comorbid disorder spectra, ADHD, ASDs, ODD, CD and selected forms of ID, and we only considered MRI-based imaging genetics studies. Further restriction of the search space was achieved by focusing on genes harboring common genetic variants with the most consistent evidence for association with ADHD, ASDs, and aggression, and by selecting five relatively common ID disorders with frequent ADHD/ASDs/aggression comorbidity implicating single genes. The review was driven by the wish to learn more about the mechanisms by which genetic factors influence disease-related behavior specific to the individual disorders and their clinical overlap.

At the level of the individual genes, the most extensively studied candidate gene is the *SLC6A4* (*5HTT*) gene encoding the serotonin transporter (associated with ADHD and ASDs as well as with ODD and CD). Limitations regarding power of individual studies and hypothesis-driven designs aside, the fMRI-based imaging genetics literature on this gene does show a remarkably coherent picture of functional genetic variation leading to hyperactivation of the amygdala and connected areas in conjunction with functional dysconnectivity amongst those areas. Possibly, this finding is linked to availability of synaptic serotonin, as imaging genetics studies on the *MAOA* gene – coding for an enzyme that metabolizes monoamine neurotransmitters - reveal a similar picture. However, since

much of this research has been performed in healthy individuals only, the link to cognition in patients with neurodevelopmental disorders needs further investigation. Findings for *SLC6A3* (*DAT1*) and *DRD4*, which have also been studied quite often already, still lack the consistency observed for *SLC6A4* (*5HTT*), partly due to the much less restricted focus on a particular cognitive domain, and thus more 'patchy' literature.

The most consistent findings observed in all of the imaging genetics literature reviewed here are for the different genetic variants for ID. This is likely linked to the severity of the variants present in the patients, with those for ID being rare and most damaging. Consistent are finding for increased caudate volume and reduced cerebellum due to *FMR1* mutations, and for T2 hyperintensities and increased brain volume in patients carrying *NF1* mutations. However, in terms of finding overlap between different forms of ID, we find that conclusiveness of studies still is limited, as most concentrated on a limited set of (often non-overlapping) features. Tubers and T2 hyperintensities have received a lot of attention in studies of TSC and NF1, for example, although reports on their contribution to cognitive deficits are inconsistent. In recent years, DTI studies have produced evidence that tissue microstructure and white matter connectivity patterns are affected in all ID disorders, and often in widespread brain areas. Effects on brain volumes are also often widespread, but can go in opposite directions, with reductions in total brain volume in Rett, but increases in NF1. One may conclude that while altered (structural) connectivity is likely to play a role in ID etiology, MRI at its current resolution (1.5 – 4 Tesla), does not allow a sufficiently detailed view on the brain to understand the neuroanatomical overlap between disorders (Williams and Casanova, 2011).

Similar to the situation amongst the ID disorders, there seems to be little overlap between the findings for different genes in ADHD, ASD, and aggression related disorders. This is likely to be heavily influenced by the strong focus on regions and cognitive domains of interest (consistent with the limited power of many of the studies published to date). Some overlap is seen, e.g., for *DAT1* and *DRD2*, both of which have been studied for their effects on striatal phenotypes. (Appropriately powered) brain-wide studies and phenome-wide association study (PheWAS)/RDoc-like approaches (Cuthbert and Insel, 2013; Pendergrass et al., 2011) would help to determine, whether the apparent specificity of brain phenotypes for individual genes is real. An important observation is that gene expression does not predict/limit the location of effects of a genetic factor ( e.g., *SLC6A3/DAT1* shows effects outside of its region of gene expression), most likely through effects on structural and/or functional connectivity.

Did the reported imaging genetics findings help us understand the comorbidity between different neurodevelopmental disorders? This would be expected, since several of the genes implicated in ID, ASD, ADHD, ODD and CD, function in the same or overlapping molecular networks (Poelmans et al., 2011; Rudie et al., 2012; van Bokhoven, 2011). As discussed above, imaging genetics studies of *SLC6A4* and *MAOA* putatively point to a similar mechanism for ADHD and aggression symptomatology based on amygdala hyperactivity/dysconnectivity. However, the limited availability of genes investigated through imaging genetics to date might bias our interpretation of the data. In ID, the genes studied thus far are related to mTOR signaling, RAS signaling, and translation repression/

regulation, thus functioning in very 'basal' cell signaling pathways in comparison to the genes investigated for ADHD and ODD/CD, which regulate the dopamine and serotonin neurotransmitter systems specifically. This could explain the much more widespread cell proliferation/migration defects observed in ID, whereas in for example ADHD defects seem more specific, e.g. limited to individual neurotransmitter systems and or affecting cell-cell communication more acutely. ASD seems to be intermediate between the other disorder spectra, but more studies are necessary to substantiate this view. What is already very clear from the available studies, is that the associations of genetic factors are with behavioral traits, and not with the disorders directly (e.g., (Hoogman et al., 2011). Some level of pleiotropy is highly likely, which may also form the basis of comorbidity between the neurodevelopmental disorders.

In general, we found the existing imaging genetics literature for the neurodevelopmental disorders of our interest lacking in several aspects. Firstly, despite our focus on well-supported candidate genes, several of the selected genes had not been studied at all with MRI in humans. In several additional cases, only single studies were available for different MRI modalities (sMRI, DTI, fMRI), thus limiting the conclusiveness of the reported findings. And in the case of ODD/CD, no case-control studies were retrieved for the selected aggression candidate genes at all. Secondly, most imaging genetics studies, especially the earlier ones, suffer from being underpowered. The small sample sizes are severely hampering the generalization of findings to the population the samples are meant to represent (Button et al., 2013). Although the endophenotype concept postulates that measures, which mediate a genetic effect on behavior (including some of those investigated in the imaging genetics studies), should have stronger effect sizes for gene effects than the behavioral/disease measures (Gottesman and Gould, 2003), the sample size of most studies would still have to be considered too small. The problem of limited number of samples becomes evident from e.g. a recent review by Strike and coworkers. They showed that at the most lenient threshold for significance ($\alpha = 0.05$) studies with at least 1,566 participants would be needed to achieve the canonical 80% power threshold to detect a reasonable effect size (0.5% of the phenotypic variance explained) (Strike et al., 2015). Furthermore, recent work raises doubts about whether larger effect sizes can really be expected for neuroimaging (endo)phenotypes, at least for volumetric MRI measures (Franke et al., 2016; Hibar et al., 2015b). Major challenges are the large inconsistency across genetic variants tested and genotype groups compared, differences in study designs and imaging modalities, and the fact that data acquisition and analysis protocols usually were not standardized across studies. Additionally, we observed large inconsistency across studies in the way how genotypic effects were reported and recommend a standardized way of reporting results, e.g. including at least effect estimates and standard errors. Nevertheless, meta-analyses are strongly needed in order to enable definition of robust findings and realistic effect estimates. Therefore, meta-analytic studies would be beneficial for those brain measures covered by multiple studies, as it was shown for the effect of the serotonin transporter 5HTTLPR on amygdala activation (Murphy et al., 2013). Thirdly, to interpret observed links between genes, brain, and behavior properly, one needs to determine, whether a brain (endo)phenotype is really intermediate between a genetic factor and a behavioral outcome, or if it is only an

epiphenomenon unrelated to the behavior of interest (Kendler and Neale, 2010; Preacher and Hayes, 2008). Only few studies have really studied this, e.g. by mediation analysis including environmental, behavioral, and/or physiological variables (Klumpers et al., 2014; van der Meer et al., 2015), by applying combinations of different imaging modalities (Kobiella et al., 2011; Zhang et al., 2015), or by using causal modeling (Sokolova et al., 2015). The results of those studies show that only part of the brain regions showing genotype effects actually do mediate between genetics and behavior, proving the importance of such multilevel investigations. Fourthly, age effects might also be of importance, but have been neglected in most studies. Our own work has shown, for example, that the risk factor for ADHD in *DAT1* differs between children and adults, which resulted in effects of the 9-6 VNTR haplotype on caudate nucleus volume only in adult patients (Onnink et al., 2016). Age effects have also been observed for the 5-HTTLPR variant (Wiggins et al., 2014a) and for *MAOA* (Sebastian et al., 2010). Fifthly, current brain imaging genetics studies often suffer from additional limitations, such as the low ethnic diversity, as most studies included cohorts of only Caucasian origin, and gender imbalance, especially in studies of childhood ADHD and ASD that showed an over-representation of males.

An important additional aspect is that this review enabled us to look at the overlap between studies in healthy individuals and those in patients (case-control designs). An interaction between genetic variant and diagnosis was indeed observed in some studies (e.g. (Durston et al., 2008; Monuteaux et al., 2008; Wiggins et al., 2012; Wiggins et al., 2014b). With the available limited amount of evidence it is hard to judge though, whether this is a true difference between patients and healthy individuals, or whether it is simply due to power restrictions in the samples investigated. Recent genome-wide studies investigating the genetics of brain structure as part of the ENIGMA Consortium (Thompson et al., 2014) suggest that effects are largely similar for healthy individuals and those with a psychiatric disorder (Hibar et al., 2015b; Stein et al., 2012). This means, that brain imaging genetics studies with healthy participants can be very informative in discovering related brain correlates and in understanding the biological mechanisms leading to diseases of interest.

Did we overlook important literature through the choices made in our review? We did restrict our selection of genes to study. For ASD, we did not include genes harboring rare genetic variants, while those might result in stronger effect sizes, as observed for the ID genes. However, most of the rare variants linked to ASD have only recently been identified, making the availability of imaging genetics studies (with 10 or more cases) unlikely. A similar argument holds true for our selection of ID genes, where the imaging genetics literature is largely focused on the relatively common disorder subtypes we included in our study. We also restricted our search to MRI-based studies, following a first screen of the literature showing that this was the predominant method used for imaging genetics studies of the neurodevelopmental disorders. Nevertheless, for several genes/variants, also other imaging modalities have been employed, which may provide additional insights. EEG and MEG offer a much higher time resolution than MRI, and may allow investigation of genetic influences on neuronal functioning and oscillation patterns. PET can provide information on (acute) protein availability. Especially the integration of modalities in the study of individual participants can provide deeper insights into mechanisms (e.g. (Kobiella

et al., 2011)). Moreover, future studies might want to investigate additional comorbid neurodevelopmental disorders, such as obsessive-compulsive disorder (OCD), once robust association of genetic variants with these disorders has been established and investigated in imaging genetics studies.

To summarize, despite the considerable numbers of imaging genetics studies in neurodevelopmental disorders available for review, this field of research should still be considered in its early stages. More genes need to be studied, and individual genes need to be investigated in larger samples, with more hypothesis-generating brain- and phenome-wide methods. Gene-environment interactions and age effects should be taken into account. While we see consistent findings for single genes and variants, gene-wide and gene-set analyses, with polygenic scores explaining more phenotypic variance and thus improving study power (Bralten et al., 2011), are likely to take the stage in the future. Several early examples reviewed here already show the promise of this work (e.g. (Nikolova et al., 2011; Passamonti et al., 2008; Stice et al., 2012). As the genes in such sets often show different gene expression patterns, (structural and functional) connectivity patterns are likely the best brain phenotypes to be studied with such approaches (see above). In the future, we are also likely to see studies approaching imaging genetics in a different way, by asking the question, whether genes contributing to brain structure/function observed in hypothesis-free, genome-wide approaches also contribute to disease-related phenotypes (Franke et al., 2016). First studies of this kind have been published for schizophrenia (Franke et al., 2016) and obsessive compulsive disorder (OCD) (Hibar et al., 2015a), based on results of findings from the ENIGMA GWAS of brain structure (Hibar et al., 2015b; Stein et al., 2012). To successfully map the biological pathways from gene to disease, imaging genetics studies need to be combined with complementary approaches (Klein et al., in press). Recent examples for this are provided by studies by our own group, in which we investigated effects of ADHD-associated genes for their effects in the fruit fly *Drosophila melanogaster* (Klein et al., 2015; van der Voet et al., 2016), as well as the study by Jia and coworkers, in which the authors identified a genetic variant significantly associated with dysfunctional reward, a cognitive and affective deficit frequently observed in ADHD, then verified gene function in locomotion in the fruit fly model (Jia et al., 2016). In conclusion, although still in its early stages, results from studies available thus far already confirm that the imaging genetics approach is suitable to provide more insight into the link between genes, the brain, and behavior in neurodevelopmental disorders.

## TABLES CHAPTER 5

To improve readability of the article, tables for chapter 5 have been grouped together in this separate section. References for each table can be found directly below it to enable fast lookup of information.

**Table I:** Genes containing common variants most consistently implicated in ADHD, based on (Gizer et al. 2009) and more recent (meta-) analyses.

| Gene | Protein | Associated variant/ polymorphism | Risk allele | Location/chr position | References for reports of association with ADHD |
|---|---|---|---|---|---|
| *DRD2*/ ANNK1 | Dopamine receptor D2/ Ankyrin repeat and kinase domain containing 1 | Taq1A (rs1800497) | T allele = A1-allele | Exon 8/ 3' flanking/11q23 | (Comings et al. 1991)[a]; (Pan et al. 2015)[b] |
| *DRD4** | Dopamine receptor D4 | **48 bp VNTR** | 7 repeat (5 repeat in Asians) | Exon 3/11p15 | (LaHoste et al. 1996)[a]; (Gizer et al. 2009)[b]; (Wu et al. 2012)[b] |
| | | **rs1800955** | T allele | Promoter/11p15 | (Barr et al. 2001)[a]; (Yang et al. 2008)[d]; (Gizer et al. 2009)[b] |
| *DRD5* | Dopamine receptor D5 | **148 bp dinucleotide repeats** | 148 bp allele | 5' flanking/4p16 | (Daly et al. 1999)[a]; (Gizer et al. 2009)[b]; (Wu et al. 2012)[b] |
| *HTR1B* | Serotonin receptor 1B, G protein-coupled | **rs6296** | G allele | Exon 1/6q14 | (Hawi et al. 2002)[a]; (Gizer et al. 2009)[b] |
| *LPHN3* | Latrophilin 3 | rs6551665<br>rs6858066 | G allele<br>G allele | 4q13 | (Arcos-Burgos et al. 2010)[a]; (Hwang et al. 2015)[d]; (Ribases et al. 2011)[d]; (Labbe et al. 2012)[a] |
| *NOS1** | Nitric oxide synthase 1 | 180-210 bp CA repeat | Short allele | Exon 1/12q24 | (Reif et al. 2009)[a]; (Franke et al. 2009)[c]; (Weber et al. 2015)[b] |
| *SLC6A3/ DAT1** | Solute Carrier Family 6 (Neurotransmitter Transporter), Member 3; Dopamine transporter 1 | **40 bp VNTR** | 10 repeat | 3' UTR/5p15 | (Cook et al. 1995)[a]; (Gizer et al. 2009)[b] |
| | | **rs27072** | G allele | 3' UTR/5p15 | (Galili-Weisstub and Segman 2003)[a]; (Gizer et al. 2009)[b] |
| | | **30 bp VNTR** | 6 repeat | Intron 8/5p15 | (Brookes et al. 2006)[a]; (Gizer et al. 2009)[b] |
| *SLC6A4/ 5HTT** | Solute carrier family 6 (neurotransmitter transporter), member 4; serotonin transporter | **5-HTTLPR** | Long allele | Promoter/17q11 | (Manor et al. 2001)[a]; (Gizer et al. 2009)[b]; (Landaas et al. 2010)[b] |
| *SLC9A9/ NHE9* | Solute Carrier Family 9, Subfamily A, Member 9 | rs9810857 | T allele | Region 3p14-q21 | (de Silva et al. 2003)[a]; (Stergiakouli et al. 2012)[c]; (Mick et al. 2010)[c] |
| *SNAP25* | Synaptosomal-associated protein, 25kDa | **rs3746544** | T allele | 3' UTR/20p12 | (Brophy et al. 2002)[a]; (Gizer et al. 2009)[b] |

Bold text indicates significant result at $P < 0.05$ in Gizer et al., 2009. [a]Association first reported by. [b]Meta-analysis article. [c]GWAS finding. [d]Association in large sample or validation using animal model. *Gene with at least one case-control imaging genetics study; ADHD = Attention deficit/hyperactivity disorder, bp = base pair, chr = chromosome, CNV = copy number variation, UTR = untranslated region, VNTR = variable number tandem repeat; no imaging genetics studies found.

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**Table II:** Genes containing common variants most convincingly implicated in ASDs, adapted from Persico and Napolioni (2013). We added CDH9, CDH10, and MSNP1AS, because the locus harbouring these genes has shown genome-wide significant association with ASDs in GWAS (Prandini et al. 2012; Wang et al. 2009). Selection of candidate polymorphisms and risk alleles for ASD was based on recent research articles.

| Gene | Protein | Associated variant/ polymorphism | Risk allele | Location/chr position | References for association with ASD |
|---|---|---|---|---|---|
| CDH9 | Cadherin 9 | rs4307059 | C allele | Intergenic/5p14 | (Wang et al. 2009)a,c; (Prandini et al. 2012)d |
| CDH10 | Cadherin 10 | rs4307059 | C allele | Intergenic/5p14 | (Wang et al. 2009)a,c; (Prandini et al. 2012)d |
| MSNP1AS | Moesin pseudogene 1, antisense | rs4307059 | C allele | Intergenic/5p14 | (Wang et al. 2009)a,c; (Prandini et al. 2012)d |
| CNTNAP2* | Contactin associated protein-like 2 | rs7794745 | T allele | Intron 2/7q35 | (Arking et al. 2008)a; (Li et al. 2010)d |
| | | rs2710102 | C allele | Exon 8/7q35 | (Stein et al. 2011) |
| EN2 | Engrailed homeobox 2 | rs1861972 | G allele | Intron/7q36 | (Gharani et al. 2004)a; (Benayed et al. 2005)d |
| | | rs1861973 | T allele | Intron/7q36 | (Gharani et al. 2004)a; (Benayed et al. 2005)d |
| GABRB3 | Gamma-aminobutyric acid (GABA) A receptor, beta 3 | rs7171512 | G allele | Intron/15q12 | (Warrier et al. 2013)a |
| | | rs7180158 (AS) | G allele | Intron/15q12 | (Warrier et al. 2013)a |
| | | rs7165604 (AS) | T allele | Intron/15q12 | (Warrier et al. 2013)a |
| | | rs12593579 (AS) | C allele | Intron/15q12 | (Warrier et al. 2013)a |
| | | rs9806546 (EQ) | G allele | Intron/15q12 | (Warrier et al. 2013)a |
| | | rs11636966 (EQ) | T allele | Intron/15q12 | (Warrier et al. 2013)a |
| ITGB3 | Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) | rs12603582 | T allele | Intron 11/17q21.32 | (Napolioni et al. 2011)a; (Schuch et al. 2014)d |
| | | rs15908 | A allele | Exon 9/17q21.32 | (Schuch et al. 2014)a |
| MET* | Met proto-oncogene (hepatocyte growth factor receptor) | rs1858830 | C allele | Promoter/7q31 | (Campbell et al. 2006)a; (Sousa et al. 2009)d; (Thanseem et al. 2010) d; (Zhou et al. 2011)d |
| OXTR | Oxytocin receptor | rs7632287 | A allele | 3' flanking/3p25 | (Tansey et al. 2010)a; (LoParo and Waldman 2014)b; (Campbell et al. 2011)d |
| | | rs237887 | A allele | Intron3/3p25 | (Liu et al. 2010)a; (LoParo and Waldman 2014)b |
| | | rs2268491 | T allele | Intron3/3p25 | (Liu et al. 2010)a; (LoParo and Waldman 2014)b |
| | | rs2254298 | A allele | Intron3/3p25 | (Wu et al. 2005)a; (LoParo and Waldman 2014)b; (Liu et al. 2010) d; (Nyffeler et al. 2014)d |
| | | rs2268493 | C allele | Intron3/3p25 | (Yrigollen et al. 2008)a; (Campbell et al. 2011)d; (Di Napoli et al. 2014)d |
| | | rs53576 | A allele | Intron3/3p25 | (Wu et al. 2005)a; (Nyffeler et al. 2014)d |
| | | rs2268494 | T allele | Intron3/3p25 | (Lerer et al. 2008)a |
| RELN | Reelin | rs362691 | Population specific? | Exon 22/7q22 | (Wang et al. 2014)b |
| | | rs362780 | G allele | Intron 41/7q22 | (Holt et al. 2010) a |
| | | rs736707 | Population specific? | Intron 59/7q22 | (Sharma et al. 2013)a |
| | | rs2073559 | T allele | Intron 11/7q22 | (Ashley-Koch et al. 2007) a |
| SLC6A4/ 5HTT* | Serotonin transporter | 5-HTTLPR | Long allele | Promoter/17q11.2 | (Nyffeler et al. 2014)d; (Gadow et al. 2013) |

[a]Association first reported by. [b]Meta-analysis article. [c]GWAS finding. [d]Association in large sample or validation using animal model. *Gene with at least one case-control imaging genetics study; ASD = Autism spectrum disorder, AS = Asperger's syndrome, chr = chromosome, EQ = empathy quotient); no imaging genetics studies found.

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**Table III:** Genes containing common variants most consistently implicated in aggressive behaviors, based on (Ficks and Waldman, 2014) and (Pappa et al., 2015).

| Gene | Protein | Associated variant/poly-morphism | Risk allele | Location/chr position | References for reports of association with ADHD |
|---|---|---|---|---|---|
| *AVPR1A* | Arginine Vasopressin Receptor 1A | Gene-wide | - | 12q14 | (Pappa et al., 2015)[b] |
| | | RS1 and RS3 | 320 allele (RS1); short repeats (RS3) | 5' UTR/12q14 | (Moons et al., 2014); (Knafo et al., 2008) |
| *SLC6A4/ 5HTT* | Solute carrier family 6 (neurotransmitter transporter), member 4; serotonin transporter | 5-HTTLPR | Short allele | Promoter/17q11 | (Ficks and Waldman, 2014)[a] |
| *MAOA* | Monoamine oxidase A | 30bp uVNTR | Short repeats | Promoter/Xp11 | (Ficks and Waldman, 2014)[a] |

[a]Meta-analysis article. [b]GWAS finding.; bp = base pair, chr = chromosome, UTR = untranslated region, VNTR = variable number tandem repeat; no imaging genetics studies found.

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**Table IV:** Genes causing prevalent and well-studied single-gene ID disorders with behavioral and cognitive overlap with ADHD, ASD, and/or aggression.

| Gene | Protein | Chr position | Associated ID disorder | Reported rate of ASD-related phenotype | Reported rate of ADHD-related phenotype | Reported rate of aggression- related phenotype |
|---|---|---|---|---|---|---|
| *FMR1* | Fragile X mental retardation protein | Xq27 | Fragile X syndrome | 30% [Hagerman and others 2009] | 59% [Sullivan and others 2006] | 38% (males)/14%(females) (Bailey et al., 2008) |
| *NF1* | Neurofibromin | 17q11 | Neurofibromatosis type 1 | 40% [Walsh and others 2013] | 38% [Hyman and others 2005] | unknown |
| *TSC1*<br>*TSC2* | Hamartin<br>Tuberin | 9q34<br>16p13 | Tuberous sclerosis complex | 50% [Prather and de Vries 2004] | 30-60% [D'Agati and others 2009] | 50% (Eden et al., 2014) |
| *MECP2* | Methyl-CpG-binding protein 2 | Xq28 | Rett syndrome | 42-58% [Wulffaert and others 2009] | unknown | unknown |
| *CACNA1C* | Voltage-dependent L-type calcium channel subunit alpha-1C | 12p13 | Timothy syndrome | 60% [Splawski and others 2004] | unknown | unknown |

Genes causing prevalent and well-studied single-gene ID disorders with behavioral and cognitive overlap with ADHD and/or ASD.
Phenotypic overlap as adapted from [Vorstman and Ophoff 2013]; ID= intellectual disability; ASD= Autism spectrum disorder; ADHD= Attention deficit/hyperactivity disorder; Chr= chromosome; no imaging genetics studies found.



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**Table V:** Imaging genetics studies in ADHD case-control samples and ADHD candidate genes studies in the healthy population (for selection of candidate genes see Table I).

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| DRD2 | DRD2/ ANKK1-Taq1a (rs1800497, T allele = A1 allele) | sMRI (VBM) | Global GM volume | A1- carriers vs. A2/A2-carriers | 70 HC (30.7) | A1- carriers: ↓ part of midbrain, encompassing substantia nigra bilaterally | (Cerasa et al. 2009) |
| | | sMRI (VBM) | GM and WM volume | A1- carriers vs. A2/A2-carriers | 25 HC (25) | A1-carriers: ↓ Volume in cerebellar cluster | (Wiener et al. 2014) |
| | | fMRI | Temporal or color discrimination task | | | A1-carriers: ↑ Activation in striatum and right dorsolateral PFC | |
| | | | Reward anticipation paradigm | A1- carriers vs. A2/A2-carriers | 24 HC (25.7) | ↑ Nucleus accumbens activation in three-way interaction analysis from placebo to bromocriptine (D2 receptor agonist); ↑ performance under bromocriptine in A1- carriers. | (Kirsch et al. 2006) |
| | | | Striatal activation in response to receiving palatable food (2 fMRI paradigms) | A1- carriers vs. A2/A2-carriers | fMRI 1: 43 HC (20.4) †<br>fMRI 2: 33 HC (15.7) † | ↑ Negative relation between striatal response to food receipt and BMI. A1-non-carriers : striatal activation in response to food intake was positively related to weight gain (negatively related to weight gain for A1- carriers). | (Stice et al. 2008) |
| | | | Emotional face task | A1/A1-carriers vs. A1/A2-carriers vs. A2/A2-carriers | 45 HC (23.2)†§ | TaqIA genotype modifies activations in putamen, ACC, and amygdala in response to negative facial stimuli (higher signal intensity in homozygous groups (A1/A1 + A2/A2) than in heterozygous group (A1/A2)). | (Lee et al. 2011) |
| | | | Flanker task with a motivation manipulation | A1- carriers vs. A2/A2-carriers | 32 HC (22.9) | A1- carriers: ↓ Interference effects to reward alone (as compared to reward + punishment) and ↑ anterior insula activation | (Richter et al. 2013) |
| | | | Task-switching paradigm | A1-non-carriers vs. A1- carriers | 48 HC (22) | A1 non-carriers: ↑ Task-switching costs, ↑ prefrontal switching activity in inferior frontal junction area, and ↑ functional connectivity in dorsal frontostriatal circuits | (Stelzel et al. 2010) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | **Samples size** (mean age in years) | **Primary results** (main effect of genotype) | **Reference** |
|---|---|---|---|---|---|---|---|
| | | | Feedback-based reversal learning task | A1- carriers vs. A2/A2-carriers | 22 HC (age range 20–31) | A1- carriers in placebo condition: ↓ neural responses to positive feedback; cabergoline: ↑neural reward responses in medial OFC, cingulate cortex, and striatum, but ↓task performance and fronto-striatal functional connectivity | (Cohen et al. 2007) |
| | | | Probabilistic reversal learning task | A1- carriers vs. A2/A2-carriers | 28 HC (26.1)‡ | A1- carriers: no graded increase in RCZ activity to preceding negative feedback; ↓ recruitment of right VS and right lateral OFC during reversals. | (Jocham et al. 2009) |
| | | | "Wug" test (knowledge of grammar, opposed to vocabulary) | A2/A2-carriers vs. A1- carriers | 22 HC (22) | A2/A2-carriers: ↑ At concatenative (but not analogical) grammar learning; ↑ striatal responses | (Wong et al. 2013) |
| DRD4 | exon 3 VNTR | sMRI | Superior frontal, middle frontal, anterior cingulate, and cerebellum cortices volumes | ADHD 7R-carriers vs. non 7R-carriers | 24 ADHD (38.1)<br>19 ADHD+BPD (35.8)<br>20 HC (33.2) | 7R- carriers: ↓ volumes of superior frontal cortex and cerebellum cortex compared to non-carriers. No effects in ADHD+BPD or HC. | (Monuteaux et al. 2008) |
| | | | TBV, PFC, cerebellum, CN and pallidum volume | 7R-carriers vs. non-7R-carriers | 41 ADHD (9.7)<br>56 HC (17.6) | No volumetric differences between 7R-carriers and non-7R-carriers. No group x genotype interactions. | (Castellanos et al. 1998) ‡ |
| fMRI<br>fMRI | | DTI | WM integrity | 5R- carriers vs. non 5R-carriers | 765 HC (20.7) § | 5R-carriers : ↑ MD in widespread GM and WM areas of cerebral cortex, and subcortical areas | (Takeuchi et al. 2015) |
| | | Activity related to N-back paradigm | | | 5R-carriers : ↓ Task-induced deactivation in precuneus areas in both attention-demanding working memory task and sensorimotor task; similar patterns were observed in posterior cingulate cortex and areas around midbrain and hippocampus. | | |
| | | MID task | 7R-carriers vs. non 7R-carriers | 78 HC (16.3) | *DRD4* status moderated relation between Behavioral Inhibition (BI) and activation in CN.<br>7R-carriers: ↑ striatal response to incentive cues. DRD4 genotype influenced relations among neural response to incentives, early childhood BI and anxiety. | (Perez-Edgar et al. 2014) | |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | | Emotional rating task | 4R/7R-carriers vs. 4R/4R-carriers | 26 HC (23.3) | 4R/7R-carriers: ↑ activity in response to unpleasant images compared to neutral images in right temporal lobe. | (Gehricke et al. 2015) | |
| | | Go/No-go task | 7R-carriers vs. non 7R-carriers | 62 HC (18) | 7R-carriers "No-Go" trials:, ↓ activation in right anterior PFC/IFG, left premotor cortex, and right occipital/ cerebellar areas (7-repeat status accounted for ca. 5-6% of variance in BOLD response during "No-Go" trials). | (Mulligan et al. 2014) | |
| | | Combined stimulus-response Incompatibility Task (IC) and Time Discrimination Task (TT) | 7R-non-carriers vs. 7R-carriers | 26 HC (11.4) | 7R-non-carriers: ↑activation of left middle and IFG in IC and ↑cerebellar activation in TT; ↑functional connectivity between left IFG and ACC during IC and between cerebellar activation and IFG and ACC during TT. | (Gilsbach et al. 2012) | |
| NOS1 | Exon 1f-VNTR | fMRI | Reward anticipation task/ modified MID task | SS-carriers vs. SL/ LL-carriers | 63 ADHD (38.3)<br>41 HC (38.0) | SS-carriers: ↑ in VS. No group x genotype interactions. | (Hoogman et al. 2011) |
| SLC6A3/ DAT1 | 3'UTR and intron 8 VNTR haplotype | sMRI | Bilateral striatal volumes (nucleus accumbens, CN, and putamen) | Three DAT1 alleles (10/10 genotype, and the haplotypes 10-6 and 9-6) | 118 ADHD (35.9)<br>111 HC (37)<br><br>301 ADHD (17.2)<br>186 HC (16.6)<br><br>1718 HC (26.1) | Adult ADHD 9-6 haplotype carriers ↑ 5.9 % larger striatum volume relative to participants not carrying this haplotype (in adult ADHD patients only).<br>Effect was not replicated in adolescent case-control and adult population-based cohort. | (Onnink et al. 2016) |
| | 3' UTR VNTR | sMRI | CN volume | 9R-carriers vs. 10R/10R-carriers | 33 ADHD (10.5)<br>26 HC (10.6) | 9R-carriers: ↑ volumes of CN. | (Shook et al. 2011) |
| | 3'UTR and intron 8 VNTR haplotype | fMRI | VS and CN activity during reward-predicting cues | *SLC6A3* 10-6 dosage (2 copies vs. <2 copies) | 29 ADHD (combined type; 15.8)‡<br>30 HC (15.6)‡ | ADHD: Activation in CN ↓ as number of copies ↑, but in control group reverse was found. | (Paloyelis et al. 2012) |
| | | | Striatal activity during reward anticipation task | 9-6 haplotype carriers vs. non 9-6 haplotype carriers | 87 ADHD (38.3)<br>77 HC (38) | No differences in striatal activity compared with non 9-6 haplotype carriers nor 9R- and 10R/10R-carriers. | (Hoogman et al. 2013) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | | | | 9-6 haplotype carriers vs. non 9-6 haplotype carriers | 87 ADHD (38.3)<br>77 HC (38); same as above | Bayesian Constraint-based Causal Discovery (BCCD) algorithm confirmed that there is no evidence of a direct link between *DAT1* genetic variability and brain activation, but suggested an indirect link mediated through inattention symptoms and diagnostic status of ADHD | (Sokolova et al. 2015) |
| | 3' UTR VNTR | fMRI | Working memory task | 9R-carriers vs. 10R/10R-carriers | 53 ADHD (35.7)<br>38 HC (31.2) | 9R-carriers: ↓ left medial PFC activation compared to 10R/10R-carriers. Group × genotype interaction showed that 10R/10R-ADHD patients had ↑ activity in pre-SMA/dorsal ACC compared to HC. | (Brown et al. 2011) |
| | | | Go/No-go task | 10R/10R carriers vs. 9R-carriers | 20 ADHD (14.1)<br>38 HC (13.12) | 10R/10R carriers: ↑ activity in frontal, medial, and parietal regions during response inhibition compared to 9R-carriers; ↓error response in the parahippocampal gyrus | (Braet et al. 2011) |
| | | | | 10R/10R carriers vs. 9R-carriers | 33 ADHD (11.1) | 10R/10R carriers: ↑ activity in left striatum, right dorsal premotor cortex, and temporoparietal cortical junction compared to 9R-carriers. | (Bedard et al. 2010) |
| | | | | 9R-carriers vs. 10R/10R carriers | 10 ADHD (14.6)‡<br>10 unaffected siblings (14.8)‡<br>9 HC (15.3)‡ | 9R-carriers: ↑ activity in CN and ↓ in cerebellar vermis compared to 10R/10R-carriers. Group × genotype interaction: effect in CN is observed in ADHD and unaffected siblings, but not HC. | (Durston et al. 2008) |
| | | | Multi-source interference task | 10R/10R carriers vs. 9R-carriers | 42 ADHD (35.4) | 9R-carriers: ↓ activity in dorsal ACC compared to 10R/10R-carriers. | (Brown et al. 2010) |
| | 3' UTR VNTR | rs-fMRI | Striatal FC | 9R/10R-carriers vs. 10R/10R carriers | 50 HC (20.4) | 9R/10R-carriers: stronger connectivity between dorsal CN and insula, dorsal anterior cingulate, and dorsolateral prefrontal regions, as well as between VS and ventrolateral PFC, compared with 10R/10R-carriers. | (Gordon et al. 2015) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | **Samples size** (mean age in years) | **Primary results** (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | | fMRI | Modified version of the MID task | 10R/10R-carriers vs. 9R carriers | 53 HC (29) | 10R/10R-carriers: strong positive correlation between reward sensitivity and reward-related VS activity (relationship is absent in 9R-carriers). | (Hahn et al. 2011) |
| | | | Exposure to threatening faces | 10R/10R-carriers vs. 9R-carriers | 85 HC (45.2) | 9R-carriers: ↑ amygdala reactivity compared with 10R/10R-carriers. | (Bergman et al. 2014) |
| | | | Go/No-Go task under influence of 40 mg MPH or placebo | 9R-carriers vs. 10R/10R-carriers | 50 HC (23.7)‡ | 9R-carriers: MPH induced ↑ activation during successful no-go trials compared with oddball trials in thalamocortical network<br>10R/10R-carriers: ↓ activation in thalamocortical network.<br>Same pattern was observed in CN and IFG (successful no-go trials compared with successful go trials). | (Kasparbauer et al. 2015) |
| | | | Pre-cued task-switching task | 9R-carriers vs. 10R/10R-carriers | 20 HC (21.6) | 9R-carriers: ↑ventromedial striatum activation during reward anticipation compared with 10R/10R-carriers; ↑ influence of anticipated reward on switch costs, and ↑activity in dorsomedial striatum during task switching in anticipation of high reward relative to low reward in 9R-carriers. | (Aarts et al. 2010) |
| | | | Verbal n-back task | 9R/10R-carriers vs. 10R/10R-carriers | 20 HC (10.4) | 9R/10R-carriers: ↑ performance accuracy, ↑ activation in frontal-striatal-parietal regions in high but not low runs compared with 10R/10R-carriers. Genotype x load interaction in right CN.<br>9R/10R-carriers: ↑ activation in striatal and parietal regions under high compared to low load, and genotype differences (9R/10R>10R/10R) were evident only under high load.<br>10R/10R-carriers: ↑ activation of substantial nigra/subthalamic nuclei under low than high load and genotype differences (10R/10R>9R/10R) were evident only under low load. | (Stollstorff et al. 2010) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| SLC6A4/ 5HTT | 5-HTTLPR | sMRI (VBM) | GM volume | S-carriers vs. LL | 291 ADHD<br>78 subthreshold ADHD<br>332 HC;<br>Average age: 17 years | S-carriers: stress exposure is associated with ↓ GM volume in precentral gyrus, middle and superior frontal gyri, frontal pole, and cingulated gyrus. Association of G x E interaction with ADHD symptom count was mediated by GM volume in frontal pole and anterior cingulated gyrus only. | (van der Meer et al. 2015) |
| | 5-HTTLPR | sMRI | Amygdala | SS vs. SL vs. LL | 138 HC (41.2) | SS-carriers x anxiety: ↑ right amygdala volume (only in females) | (Cerasa et al. 2014) |
| | | | Hippocampus | S-carriers vs. LL | 56 HC (71) | ↓ Hippocampal volume in interaction with increased waking cortisol levels | (O'Hara et al. 2007) |
| | | | | SS/SL vs. LL | 357 HC (24.3) | S-carriers: ↓ hippocampal volume (females only); ↓ hippocampal volume correlated with severe CA (males only) | (Everaerd et al. 2012) |
| | | | | S-carriers vs. LL | 51 HC (-21) | ↑ Left hippocampal volumes in woman<br>↓ Left hippocampal volumes in men | (Price et al. 2013) |
| | | | | LL vs. SS/SL | 159 HC (69.5) | LL-carriers x stress: ↓ hippocampal volume | (Zannas et al. 2013) |
| | | | Multiple regions | S-carriers vs. LL | 113 HC (37.6) | ↓ GM volume of right IFG, left anterior cingulate, and superior temporal gyrus | (Selvaraj et al. 2011) |
| | 5-HTTLPR, rs25531 | sMRI | Total GM volume | SS vs. LL, S' vs. L' | 58 HC (18.5) | No significant association with total GM volume | (Walsh et al. 2014) |
| | 5-HTTLPR, rs25531, AluJb methylation of promoter | sMRI (VBM) | Hippocampus, amygdala, insula, anterior cingulated gyrus | S' vs. L' quantitative methylation score | Sample 1: 94 HC (36.9)<br>Sample 2: 95 HC (34.2) | No significant association of genotype. Strong association of methylation and hippocampal GM volume; amygdala, insula, and CN showed similar associations, genotype-independent. | (Dannlowski et al. 2014) |
| | 5-HTTLPR | sMRI (VBM) | GM volume | S-carriers vs. LL | sMRI: 114 HC (32.8)<br>fMRI: 94 HC (31.3)<br>(26 included in both) | S-carriers (VBM): ↓GM volume in limbic regions, particularly perigenual ACC and medial amygdala. | (Pezawas et al. 2005) |
| | | fMRI | perceptual processing of fearful stimuli | | | S-carriers (fMRI): ↓ of amygdala- perigenual ACC connectivity, particularly in rostral ACC; ↓ structural covariance between amygdala and rostral ACC | |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | | | GM volume, attentional interference task | S-carriers vs. LL | 41 HC (adults) | S-carriers (VBM): ↑ volume in left cerebellum<br>LL (VBM): ↑ volume in left superior and medial frontal gyri, left anterior cingulated, and right IFG<br>S-carriers (fMRI): ↑ activation in response to negative, relative to neutral, words in right amygdala (driven by ↓ activation to neutral stimuli, rather than ↑ activation to negative stimuli); for negative-neutral contrast ↑ activation most prominent in insula, putamen, and CN | (Canli et al. 2005) |
| | | sMRI (VBM) | Hippocampus, amygdala | S-carriers vs. LL, interaction with SLEs | 48 HC (24.7); | S-carriers: no correlation of hippocampus and amygdala volume with SLEs.<br>LL-carriers: positive correlation in GM volume with SLEs. | (Canli et al. 2006) |
| | | fMRI | Face-stimuli | | | Negative correlation between SLEs and amygdala and hippocampus activation in response to face stimuli in S-carriers; positive correlation in LL-carriers. | |
| | | rs-fMRI | FC between amygdala and hippocampus; absolute CBF at rest | | 21 HC for perfusion scan | GxE effect altered FC between hippocampus and putamen.<br>Interaction effect of 5-HTTLPR genotype and life stress on resting level activation in amygdala and hippocampus (positive correlation in S-group and negative correlation in L-group). | |
| | | sMRI | GM volume | SS vs. LL | 26 HC (20.3) | SS-carriers: No effect on amygdala and ventromedial PFC volume | (Rao et al. 2007) |
| | | rs-fMRI | resting CBF | | | SS-carriers: ↑ resting CBF in amygdala and ↓ CBF in ventromedial PFC | |
| | | DTI | WM integrity | L-carriers vs. SS | 233 HC (22.7) § | L-carriers: ↓ anatomical connectivity between amygdala and PFC through uncinate fasciculus. | (Long et al. 2013) |
| | | rs-fMRI | TC | | | L-carriers: ↓ FC between right amygdala and right frontal pole. | |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | **Samples size** (mean age in years) | **Primary results** (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | 5-HTTLPR, rs25531 | DTI | Structural connectivity | S'-carriers x SLE vs. L'L' x SLE | 34 HC (25.6) † | ↑ Structural connectivity between hippocampus and putamen (seed-based). | (Favaro et al. 2014) |
| | | rs-fMRI | FC | | | ↑ Positive correlation of co-activation of right parahippocampus and posterior cingulate cortex with SLEs (seed-based). | |
| | 5-HTTLPR | rs-fMRI | Task-free activity | SS vs. LL | 30 HC (20.3) | ↑ Negative correlation of right amygdala activity and depressive symptoms | (Gillihan et al. 2011) |
| | | | FC | SS vs. L-carriers | 200 HC (22.1) ‡§ | SS-carriers: ↑ fractional amplitude of low-frequency fluctuation in amygdala; ↓ rsFC between amygdala and various regions (including insula, Heschl's gyrus, lateral occipital cortex, superior temporal gyrus, hippocampus) and ↑ rsFC between amygdala and various regions (including supramarginal gyrus and middle frontal gyrus) | (Zhang et al. 2015) |
| | 5-HTTLPR, rs25531 | rs-fMRI | FC | S'S' vs. S'L' vs. L'L' | 39 HC (14.8) | ↓ Superior medial frontal cortex connectivity<br>↓ Age-related increase in FC between posterior hub and superior medial frontal cortex | (Wiggins et al. 2012) |
| | 5-HTTLPR | fMRI | Sadness induction - regulation to normal emotion | SS vs. LL | 30 HC (20.3) | ↑ Amygdala activity during mood recovery. | (Gillihan et al. 2010) |
| | | | Emotion regulation task | S-carriers vs. LL | 37 HC (22.6) † | ↑ Right amygdala reactivity to fearful faces.<br>↑ Signal reductions in right amygdala during regulation of fear.<br>↑ Modulatory influence of cognitive regulation on FC between amygdala and bilateral ventrolateral PFC, left medial OFC, subgenual ACC and rostral ACC. | (Schardt et al. 2010) |
| | | | | SS vs. LL | 30 HC (20.3), same sample as above | ↑Anti-correlation between amygdala and posterior cingulate cortex/precuneus during mood recovery. | (Fang et al. 2013) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | **Samples size** (mean age in years) | **Primary results** (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | 5-HTTLPR, rs25531 | fMRI | Emotion regulation task | S'S' vs. L'L' | 30 HC (20.5) | ↓ Posterior insula and prefrontal brain activation during passive perception of negative emotional information. ↑ Prefrontal activation and anterior insula activation during down- and upregulation of negative emotional responses. | (Firk et al. 2013) |
| | 5-HTTLPR | fMRI | Mood induction, sadness (film) | S-carriers vs. LL | 48 HC (8.3) | ↑ Right putamen, right CN, right rostro-ventral ACC, left CN, and left putamen in sad mood. | (Fortier et al. 2010) |
| | | | | S-carriers vs. LL | 49 HC (12) † | ↑ Earlier rise of left amygdala activation as sad mood increases. | (Furman et al. 2011) |
| | 5-HTTLPR | rs-fMRI | FC | LL vs. SS | 38 HC (20.4) § | ↑ Regional homogeneity in right amygdala; no effects on FC of right amygdala. | (Li et al. 2012) |
| | | fMRI | Emotional processing | | | No difference in amygdala activity in response to negative stimuli. | |
| | 5-HTTLPR, rs25531 | fMRI | Emotion processing task | S'S' vs. S'L' vs. L'L' (treatment with escitalopram) | 36 HC (25.1) † | ↑Left amygdala activation with escitalopram treatment linearly related to 5-HTTLPR S' allele load for negative stimuli increased. | (Outhred et al. 2014) |
| | 5-HTTLPR | fMRI | Emotional face task | S-carriers vs. LL | 28 HC | S-carriers: ↑ right amygdala activity | (Hariri et al. 2002) |
| | | | | S-carriers vs. LL | 92 HC (30.5) | S-carriers: ↑ right amygdala activity | (Hariri et al. 2005) |
| | | | | S-carriers vs. LL | 29 HC (40) ‡ | S-carriers: ↑ activation of amygdala and ↑ coupling between amygdala and ventromedial PFC. | (Heinz et al. 2005) |
| | | | | SS vs. SL vs. LL | 29 HC (37.5) | ↑ Activity in right fusiform gyrus to fearful faces. ↑Positive FC between amygdala and fusiform gyrus and between right fusiform gyrus and right ventrolateral PFC. | (Surguladze et al. 2008) |
| | | | | S-carriers vs. LL | 21 HC (15) | ↑ Left amygdala activation in response to anger. | (Battaglia et al. 2012) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | 5-HTTLPR, rs25531 | fMRI | Emotional face task | S'-carriers vs. L'L' | 44 HC (30.3) | ↑ Right amygdala responses to sad faces. | (Dannlowski et al. 2010) |
| | | | | L'L' vs. S'S' | 30 HC (26.6) | No association with amygdala reactivity.<br>↓ Subgenual cingulate cortex activation in response to fearful faces. | (O'Nions et al. 2011) |
| | | sMRI | Amygdala volume | S'-carriers vs. L'L' | 54 HC (41.6) | ↓ Amygdala volume<br>Path analysis suggests effects on left amygdala volume are mediated by right amygdala volume but not through (midbrain) 5-HTT availability. | (Kobiella et al. 2011) |
| | | PET | 5-HTT availability | | | No genotype effect on (midbrain) 5-HTT availability. | |
| | | fMRI | Amygdala activation | | | ↑ Left amygdala activation in response to emotional stimuli. | |
| | | | | S'S' vs. L'L | 67 HC (18.6) | ↑ Left amygdala reactivity in multivariate analysis; additive effects of recent SLEs. | (Walsh et al. 2012) |
| | | | | S'S' vs. L'-carriers, interaction with SLEs | 44 HC (26.8) ‡ | ↑ Bilateral amygdala activation in response to fearful faces.<br>Interaction with SLEs: highest activity in S'S with SLEs for fearful faces in bilateral amygdala. | (Alexander et al. 2012) |
| | | rs-fMRI | | S'S' vs. L'-carriers | 48 HC (14.8) | ↓ Connectivity between right amygdala and ventromedial PFC with age. | (Wiggins et al. 2014) |
| | | fMRI | | | | ↑ Amygdala activation with age (age range 9-19 years) | |
| | | | | S'-carriers vs. L'L' (bright-light intervention) | 30 HC (24.3)‡ | Bright-light dose positively associated with intra-prefrontal (medial PFC coupling with medial PFC seed) functional coupling only in S'-carriers. | (Fisher et al. 2014) |
| | 5-HTTLPR | fMRI | Perceptual task of threatening stimuli | S-carriers vs. LL | 14 HC phobic-prone (32.7)<br>14 HC eating disorders prone (34.3) | S-carriers: ↑ activity in right amygdala | (Bertolino et al. 2005) |
| | | fMRI | Emotional face task with approach-avoidance | S-carriers vs. LL | 48 HC (22.5) ‡ | ↑ Amygdala activity originating from reduced prefrontal inhibitory regulation. | (Volman et al. 2013) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | **Samples size** (mean age in years) | **Primary results** (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | | | Emotional face-emotional word conflict task | S-carriers vs. LL | 26 HC (70.5) | ↓ Connectivity between dorsal ACC and pregenual ACC for incongruent face-word combination. | (Waring et al. 2014) |
| | 5-HTTLPR, rs25531 | fMRI | Emotional face task with self-referential and emotion labeling conditions | S-carriers vs. LL, SLE interaction | 45 HC (23.3) | ↑ Amygdala activation and ↓ FC of amygdala with subgenual ACC in self-referential processing vs. emotion labeling.<br>Negative correlation of bilateral amygdala activation during self-referential with SLEs in S-carriers; positive correlation in LL; pattern opposite during emotion labeling. | (Lemogne et al. 2011) |
| | | | Emotional face- word conflict task (Stroop-like task) | S'-carriers vs. L'L' | 42 HC (-20) | ↓ Recruitment of prefrontal control regions and superior temporal sulcus during conflict when task-irrelevant information was positively-valenced.<br>↑ Recruitment of these regions during conflict when task-irrelevant information was negatively-valenced. | (Stollstorff et al. 2013) |
| | 5-HTTLPR | fMRI | Pain rating task | LL vs. SS | 50 HC (24.9) † | ↑ Positive linear effect of target pain in posterior cerebellum. | (Laursen et al. 2014) |
| | | | (un)predictable electric shocks | SS vs. L-carriers | 51 HC (22) † | ↑ Activity of amygdala, hippocampus, anterior insula, thalamus, pulvinar, CN, precuneus, ACC, and mPFC during threat anticipation.<br>↑ Positive coupling between mPFC activation and anxiety experience; L-carriers show ↑ negative coupling between insula and success of regulating anxiety. | (Drabant et al. 2012) |
| | | | | S- carriers vs. LL | 99 HC (21.9)‡<br>69 HC (33.4) | S-carriers: ↑ dorsomedial PFC, anterior insula, bed nucleus of stria terminalis, thalamus and midbrain activation with increasing threat conditions across both samples. | (Klumpers et al. 2014) |
| | 5-HTTLPR, rs25531 | fMRI | Modified Flanker task | S'-carriers vs. L'L' | 33 HC (23.4) | ↑ Error-related rostral ACC activation.<br>↓ Conflict-related dorsal ACC activation. | (Holmes et al. 2010) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | **Samples size** (mean age in years) | **Primary results** (main effect of genotype) | **Reference** |
|---|---|---|---|---|---|---|---|
| | | | Decision making task | S'S' vs. L'L' | 30 HC (26.6) | ↑ Amygdala activation during decisions made counter to, relative to decisions made in accord with, the frame effect (gain or loss).<br>Anterior cingulate-amygdala coupling during choices to made in counter to, relative to those made in accord with, the frame effect only observed in L'L'. | (Roiser et al. 2009) |
| | | | n-back task | S'S' vs. S'L' vs. L'L' | 33 HC (37) † | ↑ Bilateral prefrontal activation in right and left IFG pars triangularis with increasing S-allele count. | (Jonassen et al. 2012) |
| | 5-HTTLPR | fMRI | Source memory task | S-carriers vs. LL | 23 HC (66.8)<br>[17 (23.3), not analyzed for genotype effects in fMRI] | ↓ Activity in left IFG, middle frontal gyrus and anterior paracingulate cortex. | (Pacheco et al. 2012) |
| | | | Food / non-food pictures | LL vs. S-carriers | 28 HC (25.5) | ↑ Left posterior cingulate cortex activity for food pictures. | (Kaurijoki et al. 2008) |
| | 5-HTTLPR, rs25531 | fMRI | Differential fear conditioning | S'S' vs. L'-carriers | 47 HC (26.8) ‡ | ↑ Activity in fear network: amygdala (right), insula, thalamus (left) and occipital cortex for conditioned stimulus.<br>Interaction with SLEs: ↑ activity in right insula and left occipital cortex in S'S'. | (Klucken et al. 2013) |

ACC = anterior cingulated cortex, ADHD = attention-deficit/hyperactivity disorder, BCCD = Bayesian Constraint-based Causal Discovery, BI = Behavioral Inhibition, BMI = Body mass index, BOLD = blood oxygen level–dependent, BPD = bipolar disorder, CA = childhood adversity, CBF = cerebral blood flow, CN = caudate nucleus, FC = functional connectivity, fMRI = functional magnetic resonance imaging, GM = gray matter, HC = healthy control, IC = Incompatibility Task, IFG = inferior frontal gyrus, MD = mean diffusivity, MID task = monetary incentive delay task, MPH = methylphenidate, OFC = orbitofrontal cortex, PET = positron emission tomography, PFC = prefrontal cortex, RCZ = rostral cingulate zone, rsFC = resting-state functional connectivity, SLE = stressful life events, SMA = supplementary motor area, sMRI = structural magnetic resonance imaging, TBV = total brain volume, TT = Time Discrimination Task, VBM = voxel-based morphometry, VS = ventral striatum, WM = white matter, † only females, ‡ only males; § Asian sample; S'= functional S-allele (S or $L_G$), L'= functional L'-allele ($L_A$); in gray case-control studies.

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**Table VI:** Imaging genetics studies in ASDs case-control samples and ASDs candidate genes studies in the healthy population (for selection of candidate genes see Table II).

| Gene | Polymorphism | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| CNTNAP2 | rs2710102 | fMRI | Reward-guided implicit learning task (fronto-striatal circuits) | C-allele carriers vs. non-risk-carriers | Discovery sample: 16 ASD (12.4) ‡ 16 HC (12.3) ‡ | Non-risk group (collapsed across patients and controls): ↓ Activity in medial PFC during reward feedback processing; Risk group: ↑ long-range anterior-posterior connectivity between medial PFC, medial occipital, and ventral temporal cortices. | (Scott-Van Zeeland et al. 2010) |
| | | | | C-allele carriers vs. non-risk-carriers | Replication sample: 39 HC (13) | Non-risk-group: ↑ long-range anterior-posterior functional connectivity between mPFC, medial occipital, and ventral temporal cortices. | |
| | rs2710102 | DTI | Whole-brain fiber tractography (graph theory analyses) | CC-carriers vs. CT/TT-carriers | 328 HC (23.4); twins from 189 families | CC-carriers: ↓ path length, ↑ small-worldness and global efficiency in whole-brain analyses, and ↓ eccentricity (maximum path length) in 60 of the 70 nodes in regional analyses. | (Dennis et al. 2011) |
| | rs7794745 | sMRI | WM and GM morphology | TT-carriers vs. AT/AA-carriers | 314 HC | TT-carriers: ↓ GM and WM volume in cerebellum, fusiform gyrus, occipital and frontal cortices. Male TT-carriers: ↓ GM in right frontal pole in right rostral fronto-occipital fasciculus. | (Tan et al. 2010) |
| | | DTI | WM integrity | | | TT-carriers: ↓ FA in cerebellum, fusiform gyrus, occipital and frontal cortices. Male TT-carriers: ↓FA in right rostral fronto-occipital fasciculus. Female TT-carriers: ↓ FA of anterior thalamic radiation. | |
| | rs7794745, rs2710102 | fMRI | Language task | Risk group (T- and C-allele) vs. non-risk group | 66 HC (20.54) | Risk group: ↑ activation in right IFG (Broca's area homologue) and right lateral temporal cortex. | (Whalley et al. 2011) |

| Gene | Polymorphism | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| MET | rs1858830 | fMRI | Emotional faces task (n = 144), DMN functional connectivity (n = 71), WM structural connectivity (n = 84). | CC-carriers vs. CG-carriers vs. GG-carriers (non-risk) | 75 ASD (13.1) 87 HC (12.5) | Risk genotype predicted wide-spread atypical fMRI activation (↑ amygdala and striatum) and deactivation patterns (↓ mainly posterior cingulate cortex) to social stimuli. Effects were more pronounced ASD group, especially within heterozygous risk group. | (Rudie et al. 2012) |
| | | rs-fMRI | | | | Risk genotype: ↓ Functional and structural connectivity in temporo-parietal regions (within DMN) | |
| | | DTI | | | | Risk genotype: Altered WM integrity | |
| | rs1858830 | sMRI | Measures of cortical thickness (CT) development | CC-carriers vs. CG-carriers vs. GG-carriers | 222 HC (9-22) | C-carriers: ↓ CT (lowest in CC group) in superior and middle temporal gyri, ventral precentral and postcentral gyri, and anterior cingulate bilaterally, and in right frontopolar cortex. | (Hedrick et al. 2012) |
| OXTR | rs2254298 | sMRI | Amygdala volume, TBV | GG-carriers vs. GA-carriers | 51 HC (13) † | GG-carriers: ↑ GM volume, ↓ amygdala volumes. VBM analysis revealed ↑ volume in region of dorsomedial ACC in GG-carriers and ↑ in posterior brainstem in G/A-carriers | (Furman et al. 2011) |
| | | sMRI (VBM) | Global brain measures (GM, WM, TBV) | AA- carriers vs. AG-carriers vs. GG-carriers | 135 HC (28.8)§ | Male A-allele carrier: ↓ GM volume in right insula (neuroanatomical correlate of ALTs). | (Saito et al. 2014) |
| | rs1042778, rs2254298, rs237887, rs918316, rs2268493, rs53576, rs2268495 | sMRI | Amygdala and hippocampus volume, TBV | rs2254298: AA- carriers vs. AG-carriers vs. GG-carriers | 208 HC (33.9)§ | rs2254298: A-allele carriers: ↑ bilateral amygdala volume. Two 3-SNP haplotypes (including rs2254298 G-allele), showed associations with ↓ bilateral amygdala volume. | (Inoue et al. 2010) |
| | rs53576 | sMRI (VBM) | Global brain measures (GM, WM, TBV) | AA-carriers vs. GG/GA-carriers | 290 HC (23.7)§ | Female AA-carriers: ↓ amygdala volumes bilaterally (especially centromedial subregion, with a trend of allele-load-dependence) | (Wang et al. 2014) |
| | | rs-fMRI | rsFC | | | ↓ Resting-state functional coupling between PFC and amygdala bilaterally (allele-load-dependent trend). | |
| | | rs-fMRI | Functional connectivity density (FCD) using a voxel-wise, data-driven approach | Male AA-carriers vs. male G-allele carriers | 270 HC (24.2)§ | FCD of hypothalamus exhibited main effect of genotype (↓FCD in male AA homozygotes). Gender-by-genotype interaction in resting-state FC (rsFC) between hypothalamic region and left dorsolateral PFC, but no main effect of genotype (↓ rsFC in male AA homozygotes). | (Wang et al. 2013) |

| Gene | Polymorphism | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | | sMRI (VBM) | Regional alterations in GM volume | GG-carriers vs. GA-carriers vs. AA-carriers | VBM: 212 HC (29.9) fMRI: 228 HC (31.9) (98 overlap) | A-allele carriers: ↓ hypothalamus GM volume | (Tost et al. 2010) |
| | | fMRI | Face-matching task | | | A-allele carriers: ↓ amygdala activation, ↑ functional correlation of hypothalamus and amygdala during perceptual processing of facial emotion (specifically in male risk allele carriers lower levels of reward dependence predicted). | |
| | 23-tagging SNPs (including rs7632287, rs237887, rs2268491, rs2254298, rs2268494) | fMRI | Animated angry faces task | rs237915: CC-carriers vs. CT/TT-carriers | 1445 HC (14.4) | CC-carriers: ↓ VS activity (related to more peer problems). | (Loth et al. 2014) |
| | rs53576 | fMRI | Others' suffering task | GG-carriers vs. AA-carriers | 60 HC (20.2)§ | GG-carriers: hierarchical regression analyses revealed ↑ associations between interdependence and empathic neural responses in insula, amygdala, and superior temporal gyrus. | (Luo et al. 2015) |
| | | fMRI | Emotional-valenced stimuli task | GG-carriers vs. AG/AA-carriers | 21 HC (34) | GG-carriers: ↑ functional connectivity between regions of interest. Bilateral amygdala and medial PFC show ↑ influence on other brain regions; bilateral pars opercularis, left amygdala, and left medial PFC are more receptive to activity in other brain regions. | (Verbeke et al. 2013) |
| | rs1042778, rs2268493, rs237887 | fMRI | MID task | rs2268493: TT-carriers vs. CT/CC-carriers | 31 HC (23.6) | rs2268493 TT-carriers: ↓ Activation in mesolimbic reward circuitry (nucleus accumbens, amygdala, insula, thalamus and prefrontal cortical regions) during anticipation of rewards but not during outcome phase. | (Damiano et al. 2014) |
| | rs53576, rs1042778 | fMRI | Mother-child interaction task | 3 genotype groups per SNP | 40 HC † | Both rs53576 and rs1042778 were associated with both positive parenting and hemodynamic responses to child stimuli in OFC, ACC, and hippocampus (rs53576 GG group showed lowest hemodynamic response). | (Michalska et al. 2014) |

| Gene | Polymorphism | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | rs2268498, rs180789, rs401015 | fMRI, double-blind placebo-controlled crossover study | Social–emotional and gaze processing task; amygdala activation after intranasal oxytocin self-administration | rs401015: CT-carriers vs. TT-carriers | 55 HC (24.9) ‡ | rs401015 modulated right amygdala activity under influence of oxytocin (CT-carriers: ↑ amygdala activity). | (Montag et al. 2013) |
| SLC6A4/ 5HTT | 5-HTTLPR | sMRI (VBM) | Total GM and WM volume | LL vs. LS vs. SS | 43 ASD (30) | No associations between total GM or WM volume and genotype. | (Raznahan et al. 2009) |
| | | sMRI (longitudinal) | Cerebral cortical and cerebellar GM and WM volume | SS vs. SL vs. LL | 44 ASD (3.4) ‡ | S-carriers: ↑ cortical and frontal lobe GM | (Wassink et al. 2007) |
| | 5-HTTLPR, rs25531 | rs-fMRI | Functional connectivity | Low vs. high expressing | 54 ASD (13.7) 66 HC (14.5) | Low expressing genotypes (SS, $SL_G$, $L_GL_G$): ↑ posterior-anterior connectivity in ASD group (converse for HC). | (Wiggins et al. 2012) |
| | | fMRI | Emotional faces task | Low vs. high expressing | 44 ASD (13.5) 65 HC (14.7) | Low expressing genotypes (SS, $SL_G$, $L_GL_G$): ↑ amygdala activation in ASD group. | (Wiggins et al. 2014) |

ACC = anterior cingulate cortex, ALT = autistic-like traits, CA = childhood adversity, CN = caudate nucleus, CT = cortical thickness, CV = cortical volume, DMN = default mode network, DTI = diffusion tensor imaging, GM = gray matter, FC = functional connectivity, FCD = functional connectivity density, HC = healthy control, IFG = inferior frontal gyrus, MID = monetary incentive delay task, mPFC = medial prefrontal cortex, OFC = orbitofrontal cortex, PFC = prefrontal cortex, ROI = region of interest, rsFC = resting-state functional connectivity, SA = surface area, SLE = stressful life events, sMRI = structural magnetic resonance imaging, STS = superior temporal sulcus, VBM = voxel-based morphometry, VS = ventral striatum, WM = white matter, TBV = total brain volume, † only females, ‡ only males; § Asian sample; in gray only case-control studies; for *SLC6A4* studies in healthy individuals see Tables IV and VI (ADHD).

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**Table VII:** Imaging genetics studies in ADHD and ASDs case-control samples and candidate genes studies in the healthy population studying more than one single gene.

| ADHD/ASD candidate gene (polymorphism) | Additional gene(s) studied | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared (candidate genes or interaction) | Samples size (mean age in years) | Primary results (main effect of candidate gene genotype or interaction) | Reference |
|---|---|---|---|---|---|---|---|
| SLC6A3 (3' UTR VNTR), DRD4 (exon 3 VNTR) | --- | sMRI | PFC gray matter and CN volume | 9R-carriers vs. 10R/10R-carriers, 4R/4R-carriers vs. rest | 26 ADHD (12.1)<br>26 unaffected siblings (11.6)<br>20 HC (10.7); all ‡ | *SLC6A3* 9R-carriers: ↑ CN volumes<br>*DRD4* 4R/4R-carriers: ↓ prefrontal GM volume.<br>No effects on CN, or TBV. No interactions between ADHD status and genotype. | (Durston et al. 2005) |
| | *DRD1* | sMRI; longitudinal study (mean follow-up, 6 years) | Cortical thickness | 9R-carriers vs. 10R/10R-carriers, 7R-carriers vs. non-7R-carriers | 105 ADHD (10.1; 13.1; 15.9)<br>103 HC (10.0; 12.4; 14.4) | *SLC6A3* 9R-carriers: No effect on cortical development.<br>*DRD4* 7R-carriers: thinner right orbitofrontal/ inferior prefrontal and posterior parietal cortex.<br>ADHD 7R-carriers: distinct trajectory of cortical development; normalization of right parietal cortical region. | (Shaw et al. 2007) |
| | *COMT* | DTI | WM integrity, FA values | 9R-carriers vs. 10R/10R-carriers; 4R/4R-carriers vs. rest | 58 stimulant- and atomoxetine-naïve ADHD (8.7) § | *SLC6A3* 9R-carriers: no effect on WM integrity<br>*DRD4* 4R/4R-carriers: no effect on WM integrity. | (Hong et al. 2015) |
| SLC6A3 (3' UTR VNTR) | *COMT* | fMRI | Episodic memory task | 9R-carriers vs. 10R/10R-carriers | 49 HC (22.7) | 9R-carriers: ↑ midbrain activation (right substantia nigra and the ventral tegmental area) | (Schott et al. 2006) |
| | | | N-back task | 9R/9R-carriers x val/val-carriers | 75 HC (19.6) | No effects on brain activation were found for each genotype independently.<br>Val/val and 9R/9R subjects show highest activation dorsolateral prefrontal region. | (Caldu et al. 2007) |
| | | | Response inhibition (stop-signal) task | 9R-carriers vs. 10R/10R-carriers | 43 HC (22.7) | *SLC6A3* 9-allele carriers: ↑ activation during inhibition in subthalamic nucleus and (pre-)supplementary motor area | (Congdon et al. 2009) |
| | | | Reward anticipation task | 9R-carriers vs. 10R/10R-carriers; val-carriers vs. met/met-carriers | 22 HC (27.9) | *SLC6A3* 9R- carriers: highest activity in CN and VS during reward anticipation and in lateral PFC and midbrain at time of reward delivery.<br>Interaction *SLC6A3* and *COMT*: *DAT1* 9R-allele carriers and *COMT* met/met-allele carriers showing highest activation in VS and lateral PFC during reward anticipation and in lateral prefrontal and orbitofrontal cortices, and in midbrain at time of reward delivery. | (Dreher et al. 2009) |
| | *TREK, COMT* | fMRI | MID task | 9R-carriers vs. 10R/10R-carriers | 32 HC (21.7) | *TREK1* and *SLC6A3 /COMT* genotypes were independently related to basal ganglia responses to gains. | (Dillon et al. 2010) |

| ADHD/ASD candidate gene (polymorphism) | Additional gene(s) studied | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared (candidate genes or interaction) | Samples size (mean age in years) | Primary results (main effect of candidate gene genotype or interaction) | Reference |
|---|---|---|---|---|---|---|---|
| | *COMT* | fMRI | Fear conditioning, extinction and reacquisition task | 9R- carriers vs. 10R/10R-carriers | 69 HC ‡ | 9R- carriers: ↑ learning rates and stronger hemodynamic appetitive prediction error signals in VS. | (Raczka et al. 2011) |
| SLC6A4 (5-HTTLPR) | *BDNF* | sMRI | Global GM volume | S-carriers x val/val | 111 HC (32.60) | ↓ ACC volume | (Pezawas et al. 2008) |
| SLC6A4 (5-HTTLPR, rs25531, STin2) | *OXTR, STMN1* | sMRI | Amygdala volume | SS vs. SL vs. LL. | 139 HC (22)‡ | *SLC6A4* risk alleles are associated with ↓ amygdala volumes. | (Stjepanovic et al. 2013) |
| SLC6A4 (5-HTTLPR) | *COMT* | sMRI (VBM) | Global GM volume | S-carriers vs. LL-carriers x met- carriers vs. val/val-carriers | 91 HC (33) | Interaction: ↓GM volume of bilateral parahippocampal gyrus, amygdala, hippocampus, vermis of cerebellum and right putamen/insula | (Radua et al. 2014) |
| SLC6A4 (5-HTTLPR, rs25531) | *TPH2* | fMRI | MID task | L'L' vs. S'-carriers | 89 HC (27.8) | L'L'-carriers: positive association with amygdala-hippocampus activity and trait anxiety score. | (Hahn et al. 2013) |
| SLC6A4 (5-HTTLPR) | *MAOA* | fMRI | Response inhibition task | S-carriers vs. LL | 35 HC (32.1) ‡ | S-carriers: ↑ activation in ACC<br>Allele–allele interaction: ↑ BOLD activity in ACC. | (Passamonti et al. 2008) |
| SLC6A4 (5-HTTLPR, rs25531) | *COMT* | fMRI | Emotion processing task | S'S'-carriers and L'L'-carriers x val/val –carriers and met/met-carriers | 48 HC (41.2) ‡ | Interaction effects in amygdala, hippocampal and limbic cortical regions elected by unpleasant stimuli. No additive or interaction effects. | (Smolka et al. 2007) |
| | | | Emotional face task | S'-carriers vs. L'L' met/met vs. val-carrier s | 54 HC (24.1) | S'-carriers : ↑right amygdala activity in response to angry stimuli. | (Lonsdorf et al. 2011) |
| SLC6A4 (5-HTTLPR) | *TPH2, HTR1A, HTR2A* | fMRI | Emotional face task | L-carriers vs. SS | 55 HC (23.3) †§ | L-carriers: ↑Bilateral amygdala activation in response to angry faces | (Lee and Ham 2008) |
| SLC6A4 (5-HTTLPR, rs25531) | *COMT* | fMRI | Emotional face task | S'S'-carriers and L'L'-carriers x met/met-carriers and val/val-carriers | 91 HC (32.5) | Interaction: ↓Reciprocal connectivity within bilateral fusiform and inferior occipital regions, right superior temporal gyrus and superior temporal sulcus, bilateral inferior and middle PFC and right amygdala, in fear processing conditions. | (Surguladze et al. 2012) |
| SLC6A4 (5-HTTLPR) | *TPH2* | fMRI | Emotional face task | S-carriers and LL-carriers x *TPH2* | 49 HC (24.0) | Interaction: ↑ activation of putamen and amygdala, most robust for visuospatial and negatively valenced stimuli | (Canli et al. 2008) |

| ADHD/ASD candidate gene (polymorphism) | Additional gene(s) studied | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared (candidate genes or interaction) | Samples size (mean age in years) | Primary results (main effect of candidate gene genotype or interaction) | Reference |
|---|---|---|---|---|---|---|---|
| SLC6A4 (5-HTTLPR, rs25531) | *BDNF* | fMRI | Emotion processing | S-carriers vs. LL; interaction val/met | 28 HC (24.49)† | S-carriers: ↑rostral ACC and amygdala activation during presentation of emotional images. S-carriers and met-carriers: ↑ activation in rostral ACC and amygdala. | (Outhred et al. 2012) |
| DRD2 (A1 allele) | *BDNF* | sMRI | Global GMV | A1-carriers x met-carriers | 161 HC (27.29) | Interaction: ↓GM volume of ACC | (Montag et al. 2010) |
| DRD4 (rs1800955) | *COMT* | fMRI | Gambling paradigm featuring unexpectedly high monetary gains and losses | CC-carriers vs. TT-carriers | 53 HC (21.2) | CC-carriers: ↑ responses in anterior insula and cingulate cortex. | (Camara et al. 2010) |
| DRD2 (rs1800497), DRD4 (exon 3 VNTR) | --- | fMRI | Imagined intake of palatable foods, unpalatable foods, glasses of water (pictures). | A1-carriers and 7R- carriers | 44 HC (15.6) † | ↓ Activation of frontal operculum, lateral OFC, and striatum in response to imagined intake of palatable foods (vs. unpalatable food or water), predicted future ↑ in body mass for those with A1 or 7R-allele. | (Stice et al. 2010) |
| SLC6A3 (3' UTR VNTR), DRD2 (rs1800497) | *COMT* | fMRI | Cue-target reading paradigm | A1-carriers vs. A2/A2, 9R-carriers vs. 10R/10R, met/met vs. val/met vs. val/val | 71 HC (27.6) ‡ | *DRD2* polymorphism did not affect results. 10R-carriers: ↑ dorsal IFG activation. Linear effect of *COMT* val/met and *DAT1* 9R/10R on preparatory activity in left IFG pointed to negative interaction between tonic lateral prefrontal and phasic subcortical DA. | (Arnold et al. 2015) |
| DRD2 (rs1800497), DRD4 (exon 3 VNTR), SLC6A3 (3' UTR VNTR and intron 8 VNTR) | *ADRA1A, ADRA1B, ADRA1D, ADRA2A, ADRA2B, ADRB1, ADRB2, ADRB3, COMT, DBH, DDC, DRD1, DRD3, DRD5, SLC6A2, TH* | fMRI | Stop-signal task | *SLC6A3* rs37020 (T-carriers vs. GG-carriers) | 50 HC (22.1) | Activity in frontal regions (anterior frontal, superior frontal and superior medial gyri) and CN varied additively with T-allele of rs37020. | (Cummins et al. 2012) |

| ADHD/ASD candidate gene (polymorphism) | Additional gene(s) studied | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared (candidate genes or interaction) | Samples size (mean age in years) | Primary results (main effect of candidate gene genotype or interaction) | Reference |
|---|---|---|---|---|---|---|---|
| DRD2 (rs1800497, rs1799732), DRD4 (exon 3 VNTR) SLC6A3 (3' UTR VNTR) | *COMT* | fMRI | Receipt and anticipated receipt of palatable food and monetary reward | Individual risk genotypes and multilocus score | 160 HC (15.3) | Individuals with 5 'risk' genotypes: did not show ↓ activation of DA-based reward regions. *DRD4*-L vs. *DRD4*-S genotype: ↓ middle occipital gyrus activation in response to monetary reward. Multilocus composite score: ↑ number of 'risk' genotypes ↓ activation in putamen, CN, and insula in response to monetary reward. | (Stice et al. 2012) |
| | | | Card guessing game task | Multilocus DA profile | 69 HC (44.5) | ↑ Reactivity correlated with ↑ number of risk factors. Multilocus DA profile scores accounted for 10.9% of inter-individual variability in reward-related VS reactivity. None of individual polymorphisms accounted for significant variability. | (Nikolova et al. 2011) |
| SLC6A4 (5-HTTLPR, rs25531), OXTR (rs2268498 and rs53576) | --- | fMRI | Empathic performance task (facial responses of target person to electric stimulation) | SS-carriers vs. LL-carriers; rs2268498: CC- vs. CT- vs. TT-carriers; rs53576: AA-vs. AG-vs. GG-carriers | 50 HC (24.9) † | rs2268498 CC-carriers: high empathic accuracy was associated with ↑ responsiveness of right STS to observed pain. | (Laursen et al. 2014) |

ACC = anterior cingulate cortex, ADHD = Attention deficit/hyperactivity disorder, BOLD = blood oxygen level–dependent, CN = caudate nucleus, DA = dopamine, DTI = diffusion tensor imaging, FA = fractional anisotropy, fMRI = functional magnetic resonance imaging, GMV = gray matter volume, HC = healthy control, MID task = monetary incentive delay task, OFC = orbitofrontal cortex, PFC = prefrontal cortex, sMRI = structural magnetic resonance imaging, UTR = untranslated region, TBV = total brain volume, VAC task = variable attentional control task, VNTR = variable number tandem repeat, VS = ventral striatum, VSWM = visuospatial working memory, WM = white matter, † only females, ‡ only males, § Asian sample; in gray only case-control studies.

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**Table VIII:** Imaging genetics studies of aggression candidate genes studied in the healthy population (for selection of candidate genes see **Table III**).

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| AVPR1A | RS3 | sMRI (VBM) | Global GM volume | SS/SL/LL | 278 HC (22.3) § | SS and SL vs. LL: ↓ GM volume right fusiform face area. | (Wang et al., 2016) |
| | | fMRI | Face-matching | 334bp allele | 258 HC (31.5) | RS3 334bp allele: ↑ Activation left amygdala. | (Meyer-Lindenberg et al., 2009) |
| | RS1 | | | 320bp/312bp alleles | | RS1 320bp allele: ↓ Activation left amygdala. | |
| MAOA | 30bp uVNTR | sMRI (VBM) | total GM | MAOA-L/ MAOA-H | 97 HC (32.1) | MAOA-L: ↓volume of cigulate gyrus, amygdala and ACC area, insula and hypothalamus.<br>MAOA x sex; MAOA-L ↑ volume of OFC in males only. | (Meyer-Lindenberg et al., 2006) |
| | | fMRI | Face-matching | MAOA-L/ MAOA-H | 142 HC (29.6)<br>90 HC (30.5) | MAOA-L: ↑ activity in left amygdala, ↓ activity ventral cingulate cortex, left lateral OFC, left insular cortex.<br>MAOA x sex x task; MAOA-L men ↑ reactivity in left amygdala and hippocampal formation for retrieval of emotionally aversive stimuli. | |
| | | | Neutral and aversive encoding and retrieval<br>Flanker task | | 82 HC (29.4) | MAOA x sex; MAOA-L men ↓ activation in dorsal anterior cingulate during response inhibition. | |
| | | fMRI | Face-matching and Flanker NoGo | MAOA-L/ MAOA-H | 125 HC (24.6) | MAOA x SLE x sex interaction during face-matching; MAOA- L ↑ (and MAOA-H ↓) activity in amygdala and hippocampus in males with high SLE, reverse in females. Effect in opposite direction in ACC during NoGo task. | (Holz et al., 2016) |
| | | fMRI | Dorsal ACC response to anger provocation | MAOA-L/ MAOA-H | 38 HC (22.0) ‡^ | MAOA-L: ↑ activation dorsal ACC and amygdala in response to provocation | (Denson et al., 2014) |
| | | fMRI | Rejection-themed emotional Stroop | MAOA-L/ MAOA-H | 19 HC (15.4; adolescents)<br>16 HC (28.7; adults) † | MAOA x valence x age interaction for rejection>neutral contrast; MAOA-L adults ↑ activation in left amygdala for rejection>neutral contrast compared to MAOA-H adults, opposite in adolescents. | (Sebastian et al., 2010) |
| | | fMRI | 'No' task | MAOA-L/ MAOA-H | 27 HC (30.4) ‡ | MAOA-L: ↓activity in left middle frontal gyrus in response to 'No'.<br>MAOA-L x high anger reactivity: ↑ activity in left amygdala and thalamus. | (Alia-Klein et al., 2009) |
| | | fMRI | Emotional faces (passive viewing) | MAOA-L/ MAOA-H | 47 HC (unknown) †§ | MAOA-L: ↑ activity in left amygdala in response to sad faces.<br>MAOA-L: ↑ activity in right ACC and hippocampus. | (Lee and Ham, 2008) |
| | | fMRI | Face-matching, FC | MAOA-L/ MAOA-H | 123 HC (29.2) | MAOA-L x sex: ↑ connectivity between amygdala and ventromedial PFC in males, mediated by vmPFC effect on perigenual cingulate.<br>MAOA-L: negative correlation between amygdala-vmPFC connectivity and amygdala activation in males. | (Buckholtz et al., 2008) |

| Gene | Variant | Imaging modality | Imaging/cognitive phenotype | Genotype groups compared | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|---|
| | | rs-fMRI | Amygdala connectivity ROIs affected by acute tryptophan depletion | MAOA-L/ MAOA-H | 64 HC (25.0) ‡ | MAOA-L: ↑ Reduction of amygdala connectivity with right PFC. MAOA-L: ↑ Increase of amygdala connectivity with insula and dorsal PCC. | (Eisner et al., 2017) |
| | | rs-fMRI | FC | MAOA-L/ MAOA-H | 56 HC (15.7)‡ § | MAOA-L: ↓ ALFF pons. | (Lei et al., 2014) |
| | | rs-fMRI | Activity in resting-state networks | MAOA-L/ MAOA-H | 54 HC (27.1) | MAOA-L: ↑ activity in frontoparietal and temporal parts of the DMN and in the cerebellum; ↓ activity in right middle frontal gyrus of the executive control and salience networks | (Clemens et al., 2015) |

ACC = anterior cingulated cortex, ALFF = amplitude of low-frequency fluctuation, BOLD = blood oxygen level–dependent, bp = base pair, DMN = default mode network, FC = functional connectivity, fMRI = functional magnetic resonance imaging, GM = gray matter, HC = healthy control, MAOA-L = MAOA low-expression variants, MAOA-H = MAOA high-expression variants, OFC = orbitofrontal cortex, PFC = prefrontal cortex, PPC = posterior cingulated cortex, ROI = region of interest, SLE = stressful life events, sMRI = structural magnetic resonance imaging, VBM = voxel-based morphometry, VNTR = variable number tandem repeat, † only females, ‡ only males; § Asian sample; ^ mixed sample; in gray case-control studies; none identified.

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**Table IX:** Imaging genetics studies in intellectual disability syndromes (fragile X syndrome (FMR1), tuberous sclerosis (TSC1 and TSC2), neurofibromatosis type 1 (NF1), and Rett syndrome (MECP2)). No studies for Timothy syndrome (CACNA1C) were retrieved in our search of the literature.

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| Fragile X syndrome | *FMR1* full mutation | sMRI | Quantitative morphometry | Subgroups of 51 FXS; 120 HC | ↑ CN volume, and lateral ventricle (in males). | (Reiss et al. 1995) |
| | | | Hippocampus and amygdala volume | 10 FXS (5 ‡ (6.3); 5 † (9.0)); 10 HC (5 ‡ (6.4); 5 † (8.7)) | ↑ Right hippocampal volume. | (Kates et al. 1997) |
| | | | Regional brain volumes | 10 FXS (9.0); 10 HC (8.5) | ↑ CN and ventricular volumes. | (Kaplan et al. 1997) |
| | | | Tissue volumes | 10 FXS (9.1); 10 HC (8.5) | ↑ CN GM volume. | (Reiss et al. 1998) |
| | | | TBM | 36 FXS (14.66); 33 HC (14.67) | ↑ CN and lateral ventricle volumes, and trend-level parietal and temporal WM ↑. | (Lee et al. 2007) |
| | | | GM VBM and manual tracing; multivariate pattern classification | 51 FXS (35 months); 32 HC (29.7 months); 18 DD (34.8 months) ‡ | ↓ GM volumes in regions including hypothalamus, insula, medial and lateral PFC.<br>Spatial patterns that discriminated FXS from other groups included a medial to lateral gradient of increased and decreased regional brain volumes in posterior vermis, amygdala, and hippocampus. | (Hoeft et al. 2008) |
| | | | CN, hippocampus, putamen, amygdala volume | 52 FXS (2.9); 63 ASD (2.8); 19 DD (3,0); 31 HC (2.6) ‡ | ↑ CN volume compared to all control groups.<br>FXS: ↓ amygdala volume.<br>ASD:↑ amygdala volume. | (Hazlett et al. 2009) |
| | | | VBM of regional GM | 10 FXS (28.9); 10 ASD (30.1); 10 HC (29.4) | FXS: ↑ GM volumes within frontal, parietal, temporal, and cingulate gyri, and in CN and cerebellum compared to ASD.<br>FXS: ↑ GM volumes in frontal gyri and CN and ↓ GM volumes in cerebellar, parietal and temporal regions compared to HC.<br>ASD: ↑ GM volumes in frontal and temporal gyri compared to FXS and ↓ GM cerebellar volumes compared to HC. | (Wilson et al. 2009) |
| | | | Total and regional insular volumes | 11 FXS (5 † (15.3); 6 ‡ (16.3)); 8 HC ((5 † (16.5); 3 ‡ (13.3)); 11 DD (6 † (16.4); 5 ‡ (16.0)) | ↓ Total, anterior and posterior insular volumes compared to HC and DD. | (Cohen et al. 2011) |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| | | | Univariate VBM; multivariate pattern classification and clustering. | 52 FXS (2.90); 63 ASD (2.77); 31 HC (2.55); 19 DD (2.96) ‡ | ↓ (for FXS) and ↑ (for ASD) volumes of frontal and temporal GM and WM regions (including medial PFC, OFC, superior temporal region, temporal pole, amygdala, insula, and dorsal cingulum) compared to HC.<br>Overall pattern of brain structure in ASD resembles that of HC more than FXS. | (Hoeft et al. 2011) |
| | | | Regional brain bulk volumes (stereology) and GM and WM volume (VBM) | 17 FXS (30); 18 HC (35) ‡ | ↑ CN, parietal lobes and right brainstem bulk volume.<br>↓ Left frontal lobe volume.<br>↑ GM volumes of fronto-striatal regions including CN.<br>↑ WM in regions extending from brainstem to para-hippocampal gyrus, and from left cingulate cortex to CC. | (Hallahan et al. 2011) |
| | | | Age-related change in regional brain volumes | 59 FXS (36 † (16.0); 23 ‡ (15.2)) (19 with longitudinal data); 83 HC (47 † (15.8); 36 ‡ (15.5)) (17 with longitudinal data) | Consistent FXS related volume differences in CN compared to HC across adolescence. Aberrant maturation of PFC gyri. | (Bray et al. 2011) |
| | | | Cortical volume, thickness, complexity, surface area and gyrification index | 11 FXS (9.16) (6 FXS; 5 FXS+ASD); 10 HC (8.25) ‡ | FXS: ↑Cortical volume, thickness and complexity compared to HC.<br>FXS+ASD: ↑ Left parietal lobe volume, ↓ gyrification specifically in the left temporal and a trend for ↓ right frontal surface area compared to FXS. | (Meguid et al. 2012) |
| | | | Total brain, regional (lobar) and subcortical volumes; brain growth | 53 FXS (2.9); 68 ASD (2.8); 19 DD (3.0); 31 HC (2.6) ‡ | FXS: ↑ Global brain volumes compared to HC but not ASD.<br>↑ Temporal lobe WM, cerebellar GM, and CN volume compared to ASD.<br>↓ Amygdala volume compared to ASD.<br>Rate of brain growth from 2 to 5 years similar to HC. | (Hazlett et al. 2012) |
| | | | Relationship repetitive behaviors and CN volume | 41 FXS (4.6) (16 FXS+ASD (4.8)); 30 ASD (4.7) ‡ | FXS: Positive correlation of self-injury with CN volume.<br>ASD: Positive correlation of compulsive behaviors with CN volume. | (Wolff et al. 2013) |
| | | | CN volume and topography | 48 FXS (21.3); 28 IQ-matched controls (19.5); 36 HC (19.7) | ↑ CN compared to both control groups, with ↑ bilateral CN radial distance, ↑ dorsolateral CN head and ventromedial CN body radial distances. | (Peng et al. 2014) |
| | | | CN and hippocampal volume | 14 FXS+ASD (22.6); 17 HI (22.0); 25 HC (21.6) ‡ | FXS: ↑ Hippocampus and CN volume compared to HC.<br>HI: ↓ Hippocampal volumes. | (Molnar and Keri 2014) |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| | | DTI | Whole-brain, frontal-caudate, and sensory-motor tract FA | 10 FXS (16.7); 10 HC (17.1) † | ↓ FA in WM in fronto-striatal pathways and parietal sensory-motor tracts. | (Barnea-Goraly et al. 2003) |
| | | | Ventral frontostriatal WM | 17 FXS (2.8); 13 HC (2.3); 8 DD (3.0) ‡ | ↑ Density of fibers localized in left ventral frontostriatal pathway. | (Haas et al. 2009) |
| | | | Voxel-based comparison of anisotropy and diffusivity | 18 FXS (11.01); 25 22q11.2DS (10.75); 17 TS (10.56); 41 HC (10.6) † | FXS: ↓ FA in posterior limbs of internal capsule, posterior thalami, and precentral gyrus. | (Villalon-Reina et al. 2013) |
| | | sMRI | GM density (VBM) | 17 FXS (17.5); 16 HC (16.3) | ↑ GM density in bilateral caudate head, left hippocampus, left planum temporale, left angular gyrus, and left superior parietal lobule.<br>↓ GM density in bilateral insular cortex, precuneus cortex, thalamus, and subgenual cingulate cortex. | (Hall et al. 2013) |
| | | rs-fMRI | Fractional Amplitude (fALFF); functional connectivity (group ICA and dual regression) | | ↓ Functional connectivity in salience, precuneus, left executive control, language, and visuospatial networks.<br>↓fALFF in bilateral insular, precuneus, and ACC. | |
| | | fMRI | ROI activation during 1-back and 2-back visuospatial working memory tasks | 10 FXS; 15 HC † | No change in activation between 1-back and 2-back tasks in IFG, middle frontal gyrus, superior parietal lobule, and supramarginal gyrus, while HCs showed ↑ activation. | (Kwon et al. 2001) |
| | | | Activation during a counting Stroop task | 14 FXS; 14 HC † (15.4) † | ↓ Activation in orbitofrontal gyrus, insular cortex, superior temporal gyrus. No activation in inferior/superior parietal lobe as seen in HC. | (Tamm et al. 2002) |
| | | | FG and STS activation in response to face and gaze stimuli | 11 FXS (16.4); 11 HC (15.5) † | ↓ Left STS activation to all stimuli.<br>No greater FG activation to forward faces compared to angled faces as seen in HC. | (Garrett et al. 2004) |
| | | | Go/nogo task | 10 FXS (15.4); 10 DD (14.6); 10 HC (16.7) ‡ | ↓ Activation in right ventrolateral PFC and right caudate head, and ↑ left ventrolateral PFC activation compared with both control groups.<br>Positive correlation between task performance and activation in left ventrolateral PFC. | (Hoeft et al. 2007) |
| | | | Emotional attribution task | 10 FXS (16.4); 10 HC (15.6) † | ↓ ACC activation for neutral compared to scrambled faces.<br>↓ CN activation for sad compared to scrambled faces.<br>FXS: ↑ Negative correlation between IQ and insula activation for neutral compared to scrambled faces.<br>HC: ↑ Positive correlation between IQ and ACC activation for neutral compared to scrambled faces. | (Hagan et al. 2008) |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| | | | Activation during face encoding | 11 FXS (18.5); 11 HC (18.7) | ↓ Activation of prefrontal regions including medial and superior frontal cortex during successful face encoding.<br>Negative correlation social anxiety and brain activity during face encoding. | (Holsen et al. 2008) |
| | | | Whole-brain and ROI activation during directed or averted eye gaze stimuli | 13 FXS (15.5); 10 DD (16.1); 13 HC (15.0) ‡ | ↓ PFC activation and ↑ left insula activation to direct eye gaze stimuli.<br>↑ Sensitization in left amygdala with successive exposure to direct gaze compared to controls. | (Watson et al. 2008) |
| | | | Auditory temporal discrimination task | 10 FXS (18.7); 10 HC (14.7) † | ↑ Activation in a left-lateralized network including left medial frontal gyrus, left superior and middle temporal gyrus, left cerebellum, and left brainstem (pons). | (Hall et al. 2009) |
| | | | Brain activity during a gaze habituation task | 30 FXS (20.9); 25 HC (19.0) | ↓ Neural habituation and significant sensitization in cingulated gyrus, fusiform gyrus and frontal cortex in response to gaze stimuli. | (Bruno et al. 2014) |
| Neurofibromatosis type 1 | *NF1* | sMRI | Cerebral GM and WM | 22 NF1; 20 HC | ↑ Brain volume, especially WM. | (Said et al. 1996) |
| | | | Number, volume, distribution and change in time of UBOs | 46 NF1 (7.8) (28‡; 18†) | UBOs found in 93% of subjects, localized most commonly in GP (30.4%), cerebellum (23.5%), and midbrain (16.2%).<br>↑ UBO number and volume between 4 to 10 years with a reduction in subjects aged 10+ years. | (Griffiths et al. 1999) |
| | | | 24 ventricular and parenchymal dimensions and area calculations | 27 NF1 (8.8) (20‡; 7†); 43 HC (5.9) (22‡;21†) | ↑ Bicaudate width, biatrial width, and biparietal diameter, but not hemispheric length.<br>↑ Iter measures, descending sigmoid sinus, and ↑ brainstem height (age-specific). | (DiMario et al. 1999) |
| | | | TBV, GM, WM, CSF, CC regions and hyperintensities | 52 NF1 (10.9); 19 HC (9.8) | ↑ TBV due to ↑ GM volume.<br>↑ CC size.<br>↑ Group differences in GM to WM ratio in younger compared to older subjects. | (Moore et al. 2000) |
| | | | Morphometric and volumetric measures of (midline) structures; GM and WM volume | 18 NF1 (range 6.2-14.7); 60 HC (range 4.5-16.1) | ↑ Bilateral hyperintensities and ↑ midline structure size in macrocephalic compared to normocephalic NF1.<br>↑ Brain volume and WM volume but not GM or ventricular volume in macrocephalic subjects compared to HC. | (Steen et al. 2001) |
| | | | Surface area, GM volume, and asymmetry of the PT and PP | 24 NF1 (11.1); 24HC (11.8) | ↓ Left PT surface area and GM volume and ↑ symmetry between left and right PT in NF1 boys compared to NF1 girls and HC. | (Billingsley et al. 2002) |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| | | | Number of affected regions, UBO volume and number | 12 NF1 (13.0) | UBO prevalent in GP/internal capsule.<br>↓UBO locations, number and/or volume for all regions except cerebellar hemispheres between ages 7 to 12 years and ↑ during adolescence. | (Kraut et al. 2004) |
| | | | GM and WM volumes | 36 NF1 (9.3); 39 HC (9.5) | ↑ GM volumes predominantly in temporal, parietal and occipital regions and WM volumes predominantly in frontal lobe. | (Greenwood et al. 2005) |
| | | | Frequency, signal characteristics and localization of T2 hyperintensities at different ages | 103 NF1 (13.9) | ↓ Frequency, size, and intensity of T2 hyperintensities in BG and cerebellum/brainstem with age.<br>No differences in hemispheric lesions with age. | (Gill et al. 2006) |
| | | | Regional subcortical volumes; cortical volume, thickness, surface area and gyrification | 14 NF1 (11.3); 14 HC (11.9) | ↑ Volume of thalami, right CN and middle CC.<br>↓ Gyrification indices in frontal and temporal lobes, insula, cingulate cortex, parietal and occipital regions.<br>No differences in cortical volume, thickness and surface area. | (Violante et al. 2013) |
| | | | SVM; VBM | 21 NF1 (11.1); 29 HC; 18 NF1 (33.1); 31 HC (35.0) | SVM classifiers correctly classified 94% of cases (sensitivity 92%; specificity 96%). | (Duarte et al. 2014) |
| | | | GM and WM volume | 16 NF1 (29.8); 16 HC (33.1) | ↓ GM volume of superior frontal gyrus, orbital gyrus and right STG<br>↑ GM volume in frontal, temporal, ,parietal and limbic lobes | (Pride et al. 2014) |
| | | sMRI | TBV; CC morphology | 10 NF1 (range 20-68): 10 HC (range 21-64) | No differences in TBV.<br>↑ CC length (10%), CC area (20%). | (Wignall et al. 2010) |
| | | DTI | CC diffusion characteristics | | ↑ Minor eigenvalues at genu of CC. | |
| | | | GM and WM volume | 14 NF1 (24); 12 HC (22.7) | ↑ GM and WM volume. | (Karlsgodt et al. 2012) |
| | | | TBSS | | ↓ FA and radial diffusion and ↑ ADC with greatest magnitude in frontal lobe. | |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| | | DTI | FA and ADC brainstem, basal ganglia, thalamus, CC, and frontal and parietooccipital WM regions | 10 NF1 (25.8); 10 HC (26.3) | ↑ ADC and ↓ FA in all regions of interest. | (Zamboni et al. 2007) |
| | | | Diffusion characteristics (ADC, FA, A(m), eigenvalues) healthy and disordered brain matter | 50 NF1 ((21 female (12.2); 29 male (12.3)); 8 HC | ↑ ADC and eigenvalues in UBO compared to normal-appearing sites<br>↑ ADC in normal-appearing sites compared to HC.<br>No differences in FA or A(m) in most regions. | (van Engelen et al. 2008) |
| | | | FA BG, cerebellum, pons, thalamus | 44 NF1(12.8); 20 HC (14.1) | ↓ Bilateral cerebellar and thalamic FA. | (Ferraz-Filho et al. 2012) |
| | | | FA, ADC CN, putamen, GP, thalamus | 14 NF1 (16.3) (8 with UBOs; 6 without UBOs and 9 <18 years;5 > 18 years); 8 HC (16.1) | ↑ ADC in CN, putamen, GP, thalamus. | (Nicita et al. 2014) |
| | | | ADC,FA, RD, eigenvalues for 7 GM and 8WM ROIs; WM trajectories for adjacent WM tracts of NBOs | 14 NF1 (7.2); HC | ↑ ADC and eigenvalues in GM and WM UBOs compared to contralateral normal-appearing sites and HC and ↓ FA compared to HC.<br>Three out of 18 UBOs disrupt WM tracts.<br>↑ADC, lambda(2) and radial diffusivity of WM UBOs in patients with neurological symptoms compared to patients without. | (Ertan et al. 2014) |
| | | fMRI | Activity in ten ROIs during phonologic processing | 15 NF1 (14.4); 15 HC (15.3) | Inferior and dorsolateral PFC activation relative to posterior activation ↑ during auditory phonologic processing and ↓ during orthographic processing. | (Billingsley et al. 2003) |
| | | | Occipital and parietal cortex activity during visual-spatial processing | 15 NF1 (14.4); 15 HC (15.3) | ↑ Posterior cortex activation relative to lateral and inferior frontal activation. | (Billingsley et al. 2004) |
| | | | Activation in frontal, temporal, parietal, and occipital regions during visuospatial processing | 13 NF1 (9.8); 13 HC (9.8) | ↑ Left instead of right hemisphere activation.<br>↓ Activation in primary visual cortex. | (Clements-Stephens et al. 2008) |
| | | | Early cortical visual pathway and DN activation during visual stimuli activating magnocellular and parvocellular pathways | 15 NF1 (11.7); 24 HC (12.0); 13 NF1 (33.1)†; 15 HC (32.7)† | ↓ Activation of low-level visual cortex.<br>↓ Deactivation or ↑ activation of midline regions of DN during magnocellular-biased stimulation. | (Violante et al. 2012) |
| | | rs-fMRI | Ventral ACC, amygdala, OFC, PCC RSFC | 14 NF1 (12.5); 30 HC (12.3) | ↑ Connectivity between: left ventral ACC and frontal cortex, insula, and subcortical areas (CN, putamen); left amygdala and frontal cortex, insula, supramarginal gyrus, and PCC/precuneus; left OFC and frontal and subcortical areas (CN, pallidum). | (Loitfelder et al. 2015) |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| Tuberous sclerosis complex | *TSC1*/ *TSC2* | sMRI | Number and location of cerebellar tubers and volumes of underlying parenchyma | 34 TSC (8.9) | Mean tuber number was 14.3 and 44.1% of subjects showed both cerebral and cerebellar tubers and had more global cortical lesions than subjects with cerebral tubers only.<br>↓ Focal volume associated with tubers in cerebellum. | (Marti-Bonmati et al. 2000) |
| | | | GM, WM and CSF volume | 10 TSC (41.5); 8 HC (40.0) | ↓ GM volume in medial temporal lobes, posterior cingulate gyrus, thalamus, BG and right fronto-parietal cortex.<br>↓ Of limbic and subcortical GM volume negatively correlated with tuber count.<br>↓ WM of longitudinal fasciculi and other major intrahemispheric tracts.<br>↑ Cerebellar WM. | (Ridler et al. 2001) |
| | | | Tuber distribution and lesion load | 25 TSC (39.0) | Highest tuber frequency in frontal lobes and highest tuber density in parietal regions with variation in tuber density but no lateralization of tubers. Nodules were located predominantly in CN. Tuber and nodule volumes positively correlated.<br>↑ Tuber volume in subjects with a history of epilepsy. | (Ridler et al. 2004) |
| | | | Characteristics of cerebellar lesions | 73 TSC (range 0-28 years) | 16.4% of TSC subjects showed cerebellar lesions. Six subjects showed atrophy of cerebellar parenchyma around tubers. | (Jurkiewicz et al. 2006) |
| | | | GM, WM and CSF volume and lesion load | 25 TSC (39.3); 25 HC (34.3) | ↓ Subcortical GM volume in regions including thalamus, BG, insula, and cerebellum.<br>↓ WM in intrahemispheric tracts. | (Ridler et al. 2007) |
| | | | Tuber number and tuber/ brain proportion | 58 TSC (20.6) (19 *TSC1* (25.0); 34 *TSC2* (19.0)) | ↑ Tubers and tuber/brain proportion in *TSC2* compared to *TSC1* subjects and in subjects with a mutation deleting or directly inactivating tuberin GAP domain compared to subjects with an intact GAP domain. | (Jansen et al. 2008a) |
| | | | Tuber number and tuber/ brain proportion as determinants of seizures and cognitive function | 61 TSC (17.9) (14 *TSC1*; 30 *TSC2*) | Tuber/brain proportion was inversely related to age at seizure onset and intelligence. | (Jansen et al. 2008b) |
| | | | Presence of SENs and SGCTs | 81 TSC (28) | 15% of TSC subjects showed SGCTs. 62% showed SENs, 24% of which also showed SGCTs.<br>↑ SGCT volume at follow-up. | (Michelozzi et al. 2013) |
| | | | Cerebellar volume | 36 TSC (9.7)(19 *TSC2*; 7 *TSC1*); HC (9.7) | ↓ Cerebellar volume, with strongest effect in subjects with *TSC2* mutations. | (Weisenfeld et al. 2013) |
| | | | (Cyst-like) tuber/ brain proportion and tuber number in relation to age at seizure onset | 23 TSC (12.4) | Tuber/brain proportion and number of tubers, but not cyst-like tuber/brain proportion and number of cyst-like tubers, were negatively correlated with age at seizure onset. | (Nakata et al. 2013) |
| | | DTI | ADC, FA of epileptogenic tubers | 15 TSC | ↑ ADC values in subtuber WM in epileptogenic tubers compared to nonepileptogenic tubers. | (Chandra et al. 2006) |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| | | | ADC of NAWM in frontal, parietal and occipital lobes, and pons | 23 TSC (12); 18 HC | ↑ ADC values in frontal WM and pons for age group between 96 and 144 months and in right parietal and occipital WM for subjects older than 144 months. | (Arulrajah et al. 2009) |
| | | | ADC, FA in tubers and WM lesions | 14 TSC (15.1) | ↑ ADC values in cortical tubers.<br>↑ ADC values and ↓ FA values in WM lesions compared with contralateral regions. | (Piao et al. 2009) |
| | | | FA, diffusion characteristics in ROIs in or adjacent to cortical tubers in epileptogenic and non-epileptogenic zones | 12 TSC (8.2) | ↓ FA of cortical tubers in epileptogenic compared to non-epileptogenic zones.<br>↑ Radial diffusivity and ↓ FA in NAWM in epileptogenic zones compared to non-epileptogenic zones. | (Widjaja et al. 2010) |
| | | | FA, trace, eigenvalues CC and internal capsules, in relation to tuber load | 12 TSC (9.2); 23 HC (11.1) | Tubers were found in frontal lobes (144), parietal lobes (64), temporal lobes (42), occipital lobes (57) and insular cortex (7). ↓ FA, ↑ trace and average lambda(3) in CC and ↑ trace in internal capsules.<br>Tuber volume correlated with multiple DTI characteristics in CC and internal capsules. | (Simao et al. 2010) |
| | | | Diffusion characteristics geniculocalcarine tract, internal capsule, temporal gyri and splenium of the CC | 10 TSC (range 1.5-25 years); 6 HC (range 1.1-25 years) | ↓ FA in geniculocalcarine tracts and splenium of CC.<br>↓ Axial diffusivity in internal capsule, STG, and geniculocalcarine tracts.<br>↑ Mean and radial diffusivity in splenium of CC. | (Krishnan et al. 2010) |
| | | | FA, mean radial and axial diffusivities of CC | 40 TSC (7.2) (12 with ASD); 29 HC (7.7) | ↓ Average FA and ↑ diffusivity values in CC.<br>↓ Average FA in TSC +ASD subjects compared to HC and TSC -ASD subjects (who showed no differences). | (Peters et al. 2012) |
| | | | Diffusion characteristics in major tracts | 16 TSC (13.0); 12 HC (15.3) | ↓ FA and axial diffusivity in wide-spread WM regions.<br>↓ Number of fibers and number of tract points of commissural fibers, projection fibers and major WM tracts. | (Wong et al. 2013) |
| | | | Diffusion characteristics of RMLs, tubers, SENs, cerebellar lesions and SGCT and NAWM | 30 TSC (15.5); 16 HC (7 children (9); 9 adults (36)) | Mean of 47 RMLs, 27 tubers, and 10 SENs per TSC subject. Inverse correlation of RML FA and MD. No differences NAWM FA and MD. | (van Eeghen et al. 2013) |
| | | | FA dorsal language circuit tract | 38 TSC (10 TSC + ASD; 17 TSC - ASD); 24 HC | ↓ FA values in dorsal language circuit tract.<br>↓ FA in WM close to Geschwind's territory and WM close to Broca's area in TSC +ASD compared to TSC -ASD subjects. | (Taquet et al. 2014) |
| | | | FA, ADC, axial and radial diffusivity of tubers and WM lesions | 18 TSC (9.3) | ↓ FA and ↑ ADC and axial and radial diffusivity values in tubers compared to contralateral normal regions.<br>↑ Radial diffusivity and ↓ FA in WM lesions. | (Dogan et al. 2015) |

| Disorder | Gene | Imaging modality | Imaging phenotype | Samples size (mean age in years) | Primary results (main effect of genotype) | Reference |
|---|---|---|---|---|---|---|
| | | | Global and regional WM connectivity | 20 TSC (range 3-24 years)(11 TSC+ DD; 9 TSC - DD; 20 HC (range 2-23 years) | ↓ Interhemispheric connectivity.<br>↑ MD, positively correlated with tuber load severity.<br>↑ MD in TSC + DD subjects compared to TSC – DD subjects. | (Im et al. 2015) |
| Rett syndrome | *MECP* | sMRI | TBV, cortical GM and WM, subcortical gray nuclei, CSF volumes | 11 RTT (10.1); 15 HC (11.2) † | ↓ Cerebral volume<br>↑ Loss of GM in comparison to WM, with largest decrease in frontal regions and CN and midbrain volume. | (Reiss et al. 1993) |
| | | | TBV, cortical GM and WM, subcortical GM, CSF and posterior fossa volumes | 20 RTT (9.8); 20 HC (9.0) † | ↓ GM volume most pronounced in prefrontal, posterior-frontal, and anterior-temporal regions.<br>↓ WM volume uniformly throughout brain.<br>↓ CN volume. No differences in midbrain volumes. | (Subramaniam et al. 1997) |
| | | | Absolute and relative changes in GM and WM volumes | 23 RTT (8.6) (12 more severe (8.8); 10 less severe (8.3)); 25 HC (8.9)† | ↓ Absolute volume throughout the brain<br>↓ Relative parietal lobe GM volume, particularly dorsal.<br>↓ Cortical WM volume.<br>↓ Anterior frontal lobe volumes in more severely affected subjects. | (Carter et al. 2008) |
| | | DTI | Regional FA | 32 RTT (5.5); 37 HC (6.1)† | ↓ FA in genu and splenium of CC and external capsule, and regions of cingulate, internal capsule, posterior thalamic radiation, and frontal WM. No differences in visual pathways.<br>↓ FA in superior longitudinal fasciculus in patients who were nonverbal or speaking only single words. | (Mahmood et al. 2010) |

22q11.2DS= 22.q11.2 deletion syndrome, ACC= anterior cingulate cortex, ADC= apparent diffusion coefficient, A(m)= axial anisotropy, ASD= autism spectrum disorder, BG=basal gangli, CC= corpus callosum, CN= caudate nucleus, CSF= cerebrospinal fluid, DD= developmental delay, DN= default network, DTI = diffusion tensor imaging, FA= fractional anisotropy, fALFF = fractional amplitude of low-frequency fluctuations, FG= fusiform gyrus, fMRI= functional MRI, FXS= fragile X syndrome, GAP= GTPase activating protein, GM= grey matter, GP= globus pallidum, HI= hypoxic injury, IFG= inferior frontal gyrus, IPL= inferior parietal lobule, IPS= intraparietal sulcus, MCP= middle cerebellar peduncle, MD= mean diffusivity, MTI=magnetization transfer imaging, NAWM= normal-appearing white matter, NF1= neurofibromatosis 1, OFC= orbitofrontal cortex, PCC= posterior cingulate cortex, PFC= prefrontal cortex, PP= planum parietale, PT= planum temporale, RML= radial migration lines, ROI= region of interest, RSFC= resting state functional connectivity; RTT= Rett syndrome, SCP= superior cerebellar peduncle, SEN= subependymal nodule, SGCT= subependymal giant cell tumour, sMRI= structural MRI, STG= superior temporal gyrus, STS= superior temporal sulcus, SVM= support vector machines, SWM= spatial working memory, T1= timepoint 1, T2= timepoint 2, TBM = tensor-based morphometry, TBV= total brain volume, TPJ= temporoparietal junction, TS= turner syndrome, TSC= tuberous sclerosis, TWM = temporal working memory, UBO = unidentified bright objects, VBM= voxel based morphometry, WM= white matter, † female, ‡ male

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# Chapter 6

## Pleiotropic contribution of genes to aggression and subcortical brain volumes

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## ABSTRACT

Reactive and proactive subtypes of aggression have been recognized to help parse etiological heterogeneity of this complex phenotype. With a heritability of about 50%, genetic factors play a role in the development of aggressive behavior. Imaging studies implicate brain structures related to social behavior in aggression etiology, most notably the amygdala and striatum. This study aimed to gain more insight into the pathways from genetic risk factors for aggression to aggression phenotypes.

To this end, we conducted genome-wide gene-based cross-trait meta-analyses of aggression with the volumes of amygdala, nucleus accumbens, and caudate nucleus to identify genes influencing both aggression and aggression-related brain volumes. We used data of large-scale genome-wide association studies of a) aggressive behavior in children and adolescents (EAGLE, N=18,988) and b) Magnetic Resonance Imaging (MRI)-based volume measures of aggression-relevant subcortical brain regions (ENIGMA2, N=13,171). Second, the identified genes were further investigated in a sample of healthy adults (mean age (SD)=25.28 (4.62) years; 43% male) who had genome-wide genotyping data and questionnaire data on aggression subtypes available (BIG, N=501) to study their effect on reactive and proactive subtypes of aggression.

Our meta-analysis identified two genes, *MECOM* and *AVPR1A,* significantly associated with both aggression risk and nucleus accumbens *(MECOM)* and amygdala *(AVPR1A)* brain volume. Subsequent in-depth analysis of these genes in healthy adults (BIG), including sex as an interaction term in the model, revealed significant subtype-specific gene-wide association of *AVPR1A* with reactive aggression due to external provocation or threat ($p$=0.016), an association that was driven by males in the sample.

Using cross-trait meta-analysis of brain measures and psychiatric phenotypes, this study generated new hypotheses about specific links between genes, the brain, and behavior. Results indicate that *MECOM* and *AVPR1A* may exert an effect on aggression through mechanisms involving nucleus accumbens and amygdala volumes, respectively. Additionally, we replicate association of *AVPR1A* with aggression in an independent sample. We show that this association is specific to a threat-/provocation-based reactive subtype of aggression in males, which may help explain contradictory findings for this gene. Our subtype- and sex-dependent association results highlight the importance of taking into account issues of heterogeneity in aggression research.

## INTRODUCTION

Aggression is a common but heterogeneous phenotype often associated with psychiatric disorders that may be harmful to others (Baron and Richardson 1994; Miczek et al. 2002). The term covers a wide range of human behaviors, varying from verbal aggression and bullying to physical violence. Together, these behaviors have been associated with a large emotional and financial burden on society, while interventions typically still have small effects (McGuire 2008; Bakker et al. 2017). To address aggression-related negative outcomes more successfully, a better understanding of the genes and neural mechanisms that control this behavior is essential.

Twin studies show that about 50% of the variance in aggression can be explained by genetic influences, implicating a role for genetics in the development of aggressive behavior (Tuvblad and Baker 2011; Veroude et al. 2016). The main focus of candidate gene studies of aggression has been on genes related to brain neurotransmitter function, in particular to serotonergic and dopaminergic genes, and on genes related to neuroendocrine signaling, like sex-steroid receptors and stress-related circuitry (Waltes, Chiocchetti, and Freitag 2016). Besides these candidate gene studies, several genome-wide association studies (GWAS) of aggressive phenotypes have been conducted. Recently, a large-scale GWAS meta-analysis was conducted within the framework of the early genetics and lifecourse epidemiology (EAGLE) consortium, including nearly 19,000 subjects. The researchers combined GWAS data on childhood and adolescent aggression from nine population-based cohorts, and found suggestive evidence of association for a region on chromosome 2, near a gene involved in the regulation of excitatory synapse development (Pappa et al. 2015). While most other GWASs of aggression were relatively small-scaled, these studies together with bioinformatics approaches have highlighted the importance of neurodevelopmental and synaptic plasticity genes for aggression risk (Fernandez-Castillo and Cormand 2016).

Investigation of the neural correlates of aggression has highlighted the involvement of several brain phenotypes in aggression. Imaging studies point towards an important role for subcortical brain regions in the neurobiology of aggressive phenotypes (Siever 2008). Of specific interest in the context of aggression are the amygdala and the striatal subregions nucleus accumbens and caudate nucleus (Blair, Veroude, and Buitelaar 2016). The amygdala has been strongly linked to aggression through its role in emotion processing and threat reactivity (Fusar-Poli et al. 2009; Mobbs et al. 2010). A large number of studies have reported differences in the size of the amygdala between aggressive and comparison subjects, predominantly volume reductions (Noordermeer, Luman, and Oosterlaan 2016; Caldwell et al. 2015; Fairchild et al. 2013; Pardini et al. 2014; Sterzer et al. 2007; Thijssen et al. 2015; Wallace et al. 2014; Zhang et al. 2013). The striatum has been associated with aggression through its central role in reward sensitivity, processing of punishment, and regulation of avoidance behaviors (Crowley et al. 2010; Finger et al. 2008; Finger et al. 2011; White et al. 2013). Impairments in these functions are thought to be the basis of poor decision-making in individuals with aggressive behavior (Fairchild et al. 2009; Blair,

Veroude, and Buitelaar 2016). Both volume reductions and volume increases of the striatum have been related to aggressive phenotypes, especially for the caudate nucleus and nucleus accumbens (Cha et al. 2015; Fairchild et al. 2013; McAlonan et al. 2007; Ducharme et al. 2011; Schiffer et al. 2011; Nosarti et al. 2005). Brain volume has been shown to be heritable, and the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium recently conducted a GWAS meta-analysis on volumes of seven subcortical brain structures and intracranial volume, to identify genetic variants that influence brain structure (Hibar et al. 2015)). Identification of such genetic variants may help to uncover mechanisms underlying neuropsychiatric disorders.

Since both aggression risk and brain volumes are heritable, one may hypothesize that part of the genes contributing to aggression neurobiology do so by influencing aggression-related brain volumes. Identification of these genes may highlight specific pathways from gene to aggressive behavior via the brain. However, research into the underlying genetic and neurobiological mechanisms of aggression is complicated by the fact that aggression is a behaviorally and etiologically complex phenomenon. Efforts have been made to recognize different subtypes of aggressive behavior, presumed to differ in their underlying neurobiology. A frequently used system divides aggression into three subtypes; proactive aggression, reactive aggression due to external provocation or threat, and reactive aggression due to internal frustration (Dodge and Coie 1987; Raine et al. 2006; Smeets et al. 2016; Brugman et al. 2016). Proactive aggression has been related to psychopathic traits and delinquent behavior (Cima et al. 2013). In this subtype, dysfunction in neural circuitry involving (venteromedial) prefrontal and striatal areas is thought to underlie observed difficulties with decision making and reinforcement learning, while a decreased responsiveness of the amygdala to distress cues is thought to reflect deficits in emotional empathy (Blair 2013). Reactive subtypes of aggression, on the other hand, have been associated with impulsivity, anxiety, and hostile interpretation bias (Brugman et al. 2015; Bubier and Drabick 2009). It has been suggested that reactive forms of aggression are mediated by an overly responsive amygdala-related threat response circuitry, which is dependent on regulation by cortical brain regions (Blair 2013). Hence, different pathways to the maladaptive behavior are thought to exist. An added complication for the identification of genetic and neurobiological mechanisms underlying aggression are the marked sex-differences in aggressive behaviors. Sex-differences in aggression are pronounced with respect to prevalence and with respect to type of aggression displayed (Stephenson, Woodhams, and Cooke 2014; Hill 2002; Collett, Ohan, and Myers 2003). For example, males are overrepresented among patients with aggression-related disorders such as conduct disorder (Hill 2002), and are more prone to display physical aggression compared to females (Baillargeon et al. 2007). Identification of subtype- and sex-dependent genetic association may help in elucidating specific links from gene to brain to behavior.

Based on the above, the aim of the current study was two-fold. First, we sought to identify genes influencing both aggression and aggression-related brain volumes. To this end, we conducted genome-wide gene-based cross-trait meta-analyses of aggression and amygdala,

nucleus accumbens, and caudate nucleus volume, using GWAS meta-analysis data of two large-scale consortia (EAGLE, N=18,988; ENIGMA2, N=13,171). Second, we aimed to assess subtype- and sex-specificity of association for identified genes. For this, we conducted gene-wide association analyses with aggression subtypes for these genes in a population sample of healthy adults with available genome-wide genotyping and questionnaire data on aggression subtypes (BIG, N=501).

## MATERIALS AND METHODS

### Samples

*EAGLE*

GWAS-MA data on aggression were obtained from the EAGLE consortium which investigated childhood aggressive behavior using nine population-based studies with a total of 18,988 subjects (mean age = 8.44 years, SD = 4.16) (Pappa et al. 2015). Different well-validated parent-report questionnaires were used to assess aggressive behavior. Depending on study sample, aggressive behavior was assessed with the aggression scale of the Childhood Behavioral Checklist (CBCL), the conduct problem scale of the Strengths and Difficulties Questionnaire (SDQ), or comparable items in general questionnaires. Scores derived from SDQ and CBCL questionnaires were shown to be highly correlated and interchangeable for the assessment of children's behavior problems (Goodman and Scott 1999). Genomic data were imputed to the HapMap reference panel (release 22) and comprised only samples of European ancestry. GWAS was performed for each cohort, followed by filtering of SNPs with low minor allele frequency (<0.05) and imputation quality (RSQ <0.3 or INFO >0.4). Results were combined using the sample-size weighted z-score method as implemented in METAL (Willer, Li, and Abecasis 2010), controlling for genomic inflation. Access to the summary statistics was requested through http://www.tweelingenregister.org/EAGLE. All sites involved in this study obtained approval from local research ethics committees, and written parental consent was obtained for all participants.

*ENIGMA2*

GWAS Meta-Analysis (GWAS-MA) data on the aggression-related subcortical volumes of nucleus accumbens, amygdala, and caudate nucleus were obtained from the ENIGMA consortium. The ENIGMA consortium conducted GWAS-MA on intracranial volume (ICV) and seven subcortical brain volumes, to identify common genetic variants contributing to volume differences. They used MRI brain scans and genome-wide genotype data of 13,171 subjects of European ancestry from 28 cohorts (discovery sample). Brain scans were examined and processed at each site following a standardized protocol. Subcortical volumes had been adjusted for ICV to identify specific genetic contributions to individual volumes. Genomic data comprised only European samples and were imputed to the 1000 Genomes, v3 phase1 reference panel using MaCH for phasing and minimac for imputation (Fuchsberger, Abecasis, and Hinds 2015). GWAS was performed at each site, and SNPs with an imputation score of RSQ <0.5 and minor allele count <10 were removed. Results

were combined using an inverse-variance-weighted model as implemented in the software package METAL (Willer, Li, and Abecasis 2010), controlling for genomic inflation. Further details of the original analysis can be found in Hibar et al. (2015). Access to the summary statistics of ENIGMA was requested through the ENIGMA website (http://enigma.ini.usc.edu/download-enigma-gwas-results/). All sites involved in this study obtained approval from local research ethics committees or Institutional Review Boards, and all participants gave written informed consent.

*BIG*

To assess subtype-specific association of identified genes and for mediation analysis, data from the Brain Imaging Genetics (BIG) study was used. This study was conducted at the Donders Institute for Brain, Cognition and Behavior (Franke et al. 2010), and consists of self-reported healthy adults who participated in smaller-scale imaging studies at the institute. Participants gave consent to use their acquired brain data, donated saliva and performed online testing. In the current study, a sub-sample of 501 subjects with available Reactive Proactive Questionnaire (RPQ) data (Raine et al. 2006), genome-wide genotype data, and structural MRI data was used (age range 18-45 years).

All participants were of Caucasian descent and were screened using a self-report questionnaire for the following exclusion criteria before study participation: a history of somatic disease potentially affecting the brain, current or past psychiatric or neurological disorder, medication (except hormonal contraceptives) or illicit drug use during the past 6 months, history of substance abuse, current or past alcohol dependence, pregnancy, lactation, menopause, and magnetic resonance imaging contraindications (Gerritsen et al. 2012). All participants gave written informed consent, and the study was approved by the regional ethics committee.

**Behavioral and genetic measures in BIG**

*Aggression Questionnaire*

The Reactive Proactive Questionnaire (RPQ) was used to assess subtypes of aggression in the BIG study (Raine et al. 2006). The RPQ is a self-report questionnaire consisting of 23 items. For each item, subjects are asked to indicate, how often they have engaged in a given type of behavior, like 'had temper tantrums'**.** Items are rated on a three-point Likert scale ('never' =0, 'sometimes' =1, 'often' =2). Responses were summed to yield the three factors that best described the RPQ in earlier exploratory factor analysis (Smeets et al. 2016; Brugman et al. 2016) as well as in the current sample (van Donkelaar et al., in press): 'proactive aggression' (range 0-12), 'reactive aggression due to internal frustration' (range 0-9), and 'reactive aggression due to external provocation or threat' (range 0-10). RPQ proactive aggression scores were dichotomized into high- and low-scoring (score ≥ 2 and score ≤ 1, respectively), because of a highly positively skewed distribution in both males and females (**Supplementary Figure 1**). An overview of RPQ items can be found in **Supplementary Table 1**.

*Genotyping and imputation*

Genetic analyses for the BIG study were carried out at the Department of Human Genetics of the Radboud university medical center. Saliva samples were collected using Oragene kits (DNA Genotek, Kanata, Canada), and genomic DNA was extracted as specified by the manufacturer. Genome-wide genotyping was performed on three different genotyping platforms; Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix Inc., Santa Clara, CA, USA), Infinium PsychArray-24 v1.1 BeadChip (www.illumina.com), and Infinium OmniExpress-24 array (www.illumina.com). Quality control steps and imputation were performed using the Ricopili Rapid Imputation Consortium Pipeline (https://sites.google.com/a/broadinstitute.org/ricopili/home). Pre-imputation quality control included pre-filtering of single nucleotide polymorphisms (SNPs) with call rate <0.95, filtering of individuals with a genotyping rate <.98 or inbreeding coefficient >.02, filtering of SNPs with a call rate <0.98 or Hardy-Weinberg p-value <1e-06, removal of invariant SNPs, and removal of population ancestry outliers. SHAPEIT (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) and IMPUTE2 (Howie, Donnelly, and Marchini 2009) software were used for haplotype phasing and imputation with 1000 Genomes Phase 3.v5a reference data. For the current study, best-guess genotypes were inferred with a minimum probability threshold of 0.8. Post-imputation quality control included a strict SNP imputation quality threshold ≥0.8, removal of duplicated and related individuals (pi hat >0.25), removal of individuals with a call rate below 95%, and removal of SNPs with a call rate below 95%, a minor allele frequency of less than 1%, or failing the Hardy-Weinberg equilibrium test at a threshold of p≤10−6.

**Analyses**

*Genome-wide gene-based cross-trait meta-analyses*

We first conducted genome-wide cross-trait meta-analyses of aggression and three different aggression-related brain volume measures (amygdala, nucleus accumbens, caudate nucleus). We used summary statistic data of two large-scale genome-wide association studies of 1) aggressive behavior in children and adolescents (EAGLE, N=18,988) and 2) MRI-based volume measures of the aggression-relevant brain regions (ENIGMA2, N=13,171). First, four separate genome-wide gene-based analyses with a 50 kb flanking region around genes were performed for the summary statistic data of aggression and the volumes of amygdala, nucleus accumbens, and caudate nucleus using MAGMA v1.06 (de Leeuw et al. 2015). SNPs were mapped onto genes using 1000 Genomes Phase 3 reference data followed by computation of gene p-values by aggregating the effect of common variants within the genes. Next, fixed-effects meta-analyses where performed of aggression with amygdala volume, aggression with caudate nucleus volume, and aggression with nucleus accumbens volume, using the weighted Stouffer's Z method as implemented in MAGMA software. Results were considered significant if they reached the Bonferroni-corrected *P*-value-threshold for testing 18310 genes (p < 2.731e-6). Significant genes with stronger association p-values in meta-analysis, compared to the separate analyses of aggression and brain volume, were reported and selected for further investigation.

*Gene-wide association analyses for aggression subtypes*

Gene-wide association of selected genes with three subtypes of aggression, reactive aggression due to internal frustration, reactive aggression due to external provocation or threat, and proactive aggression, was assessed. One phenotypic outlier (>4 SD) was removed for all analyses. Gene-wide analyses again included a 50 kb flanking region. Three base gene analysis models are available in MAGMA, each of them sensitive to different genetic architectures; Principal Component Analysis, mean-SNP, and top-SNP models. For the current analysis, a multi-model approach was used, combining the results from the base analysis models into an aggregate p-value. Separate analyses were run for subjects genotyped on the three different genotyping arrays. Age and four population components derived from multidimensional scaling analysis were included as covariates. Sex was included as an interaction term in the model, yielding a gene p-value for main and interaction effects combined (P_full). This was followed by meta-analysis of the full model output of the three genotyping arrays, using the weighted Stouffer's Z method as implemented in MAGMA software (de Leeuw et al. 2015). To protect against type I error, the conventional significance threshold was corrected for multiple comparisons (three aggression subtype outcomes) using the effective number of independent tests (*Meff*, see Li and Ji (2005)), calculated to be 2.5, taking into account the correlation matrix of the three aggression measures. Hence, results were considered significant if they reached a significance threshold of 0.02.

## RESULTS

### Genome-wide gene-based cross-trait (aggression-brain) meta-analyses

The MDS1 and EVI1 complex locus gene (*MECOM*) was significantly associated with the cross-trait construct of aggression and nucleus accumbens volume (p=4.94E-07), and the Vasopressin Receptor 1A gene (*AVPR1A*) showed significant association with the cross-trait construct of aggression and amygdala volume (p=1.64E-06). Both associations were more significant compared to the separate analyses of aggression and the respective brain volume (**Table 1**).

**Table 1:** Significant results of the genome-wide cross-trait meta-analyses of aggression and aggression-related brain volumes using gene-wide association statistics.

| Gene | N SNPs | Brain volume | P EAGLE aggression | P ENIGMA2 volume | P cross-trait meta-analysis* |
|---|---|---|---|---|---|
| *MECOM* | 219 | Nucleus accumbens | 1.67E-06 | 2.10E-02 | 4.94E-07 |
| *AVPR1A* | 1132 | Amygdala | 3.40E-05 | 6.77E-03 | 1.64E-06 |

Displayed are genes showing genome-wide significant association in the cross-trait meta-analysis of aggression with an aggression-related brain volume. These genes show more significant association in meta-analysis compared to gene-wide association with aggression and brain volume phenotypes separately. *Bonferroni-corrected P-value-threshold for testing 18310 genes: p<2.73e-6.

### Gene-wide association analyses for aggression subtypes

The general characteristics of the 501 participants from the BIG sample included in the aggression subtype analysis are shown in **Supplementary Table 2**. Gene-wide association analyses with three aggression subtypes were conducted for *AVPR1A* and *MECOM* to identify gene-behavior relationships, including sex as an interaction term in the model. The *AVPR1A* gene was significantly associated with the score for reactive aggression due to external provocation or threat (P_full =0.016) (**Table 2**). The interaction was driven by a significant association in males (P_males =0.037; P_females =0.517). The *MECOM* gene was not associated with any of the aggression subtypes in the population sample.

**Table 2:** Gene-wide association results for aggression subtypes in healthy adults from the BIG sample (N=501).

| Gene | Chr. | N SNPs | Start | Stop | P reactive Internal* | P reactive External* | P proactive* |
|---|---|---|---|---|---|---|---|
| *MECOM* | 3 | 372 | 168751287 | 169431563 | 0.959 | 0.896 | 0.739 |
| *AVPR1A* | 12 | 1583 | 63486539 | 63597971 | 0.709 | **0.016** | 0.734 |

*P-value for main and sex interaction effect combined. **Bold**: Association significance corrected for multiple comparisons.
Chr. = Chromosome.

## DISCUSSION

Using cross-trait meta-analyses of gene-wide association statistics, this study identified two genes as potentially pleiotropic loci for aggression and aggression-related subcortical brain volumes. We identified *MECOM* as a gene potentially contributing to both aggression risk and nucleus accumbens volume, and we identified *AVPR1A* as a gene potentially contributing to both aggression risk and amygdala volume. We replicate the association of *AVPR1A* with aggression in an independent sample of healthy adults, showing gene-wide sex-dependent, subtype-specific association of *AVPR1A* to reactive aggression due to external provocation/threat.

The MDS1 and EVI1 complex locus gene (*MECOM)* codes for a protein known as transcriptional regulator and oncoprotein (Yoshimi and Kurokawa 2011). *MECOM* plays an important role in early development, with Evi1 homozygous mutant mouse embryos dying approximately 10.5 days *post coitum* showing disrupted cell proliferation and disrupted development of cardiovascular and neural systems (Hoyt et al. 1997). The association p-value for *MECOM* in the study of aggression improved in the cross-trait meta-analysis of this behavioral trait with nucleus accumbens volume. According to our hypothesis, this might indicate that it exerts its effect on aggression through mechanisms involving the nucleus accumbens. However, we did not observe the association of *MECOM* with aggression when investigating specific subtypes of aggression in our own, smaller sample of adults. To our knowledge, little is known about *MECOM* in relation to psychiatric behavioral phenotypes so far, and future work needs to investigate this association in more detail.

Our study provides further evidence for a role of candidate gene *AVPR1A* in aggression. The Arginine Vasopressin Receptor 1A gene (*AVPR1A*) codes for the primary receptor of arginine vasopressin (AVP) in the brain. AVP is a neuropeptide strongly implicated in complex social and emotional behaviors, including aggression, through a host of animal studies (Ebstein et al. 2010). Also in humans, AVP was shown to play a role in enhancing aggressive behavior. For example, evidence exists for a positive correlation between aggression and cerebro-spinal fluid AVP in humans (Coccaro et al. 1998). Additional evidence comes from genetic association studies. The original aggression GWAS-MA that we used for cross-trait meta-analysis reported gene-wide association of the *AVPR1A* gene with childhood aggression (P=1.61E-03), using VEGAS gene-based analysis and correcting for 21 candidate genes tested (Pappa et al. 2015). Using the MAGMA multi-model approach, which has the advantage of yielding a more even distribution of statistical power and sensitivity for a wider range of different supposed underlying genetic architectures compared to other methods (de Leeuw et al. 2015), an even lower p-value was reported in the current study. The cross-trait meta-analysis of aggression and amygdala volume resulted in gene-wide genome-wide significance. Other human genetic association studies of variants in the *AVPR1A* gene reported association with anger (Moons, Way, and Taylor 2014), gender-specific nominally significant association with pervasive aggression (Malik et al. 2014), but no association in an early study of antisocial traits (Prichard et al. 2007). The current study further extends such findings by showing evidence for a subtype-specific gene-wide association of the *AVPR1A* gene with reactive aggression due to external provocation or threat in a sample of healthy adults, an association driven by male subjects. This subtype of aggression specifically measures social responses to threat and provocation by others, or actions of self-defense in response to others. Our observed association of *AVPR1A* with this subtype is in line with existing data highlighting the importance of AVP in social context and social communication. Vasopressin signaling is thought to be an important determinant of the intensity and range of social responses displayed in different social situations (Albers 2012). For example, AVP can alter the extent to which social stimuli are threatening, by modulating sensory information (Thompson et al. 2006). Sex-dependent association of *AVPR1A* to aggression is also in line with animal research, finding opposite effects of both vasopressin and V1a receptor blockade on aggressive behavior. For example, AVP injection increases aggression in male hamsters but decreases it in females, while injection of V1a receptor antagonists has the opposite results (Gutzler et al. 2010). This data suggests that there may be a difference between males and females in the effects of vasopressin signaling on aggression. Less is known about sex differences in V1a receptor expression. Nevertheless, research in a number of species indicates that receptor distribution might vary in a sex-dependent manner as well (reviewed in (Albers 2015)). Moreover, gonadal hormones can modulate the expression of vasopressin and vasopressin receptors (e.g. (Young et al. 2000; Dubois-Dauphin et al. 1994)), thus partly explaining sex differences in the vasopressin system. Our finding illustrates that reducing phenotypic heterogeneity and taking into account sex-related heterogeneity may facilitate the search for genes involved in the etiology of aggression.

Our cross-trait meta-analysis results indicate that that amygdala volume might serve as a (proxy for related) mechanisms through which the vasopressin receptor could influence aggressive behavior, and that *MECOM* may exert its effect on aggression through mechanisms involving the nucleus accumbens. Thus, we provide specific hypotheses about shared genetic risk and generated specific hypotheses about links from gene to brain to behavior for future studies to focus on. It is often assumed in imaging genetics research that genetic risk for a neurodevelopmental disorder passes through the brain phenotype to behavior. However, another possibility is that genetic factors influencing behavior also influence the brain in a way that is independent of the behavioral phenotype of interest (Kendler and Neale 2010). Only a few studies have investigated this issue of causality earlier. Those studies showed that only part of the brain regions showing genotype effects actually do mediate between genetics and the behavior under study (Sokolova et al. 2015; van der Meer et al. 2015), proving the importance of such multilevel investigations to elucidate the biological mechanisms, by which brain alterations may be involved in aggression etiology. Currently, available methods for making causal inferences focus on SNP-level investigations, and future studies would benefit from the development of approaches for aggregating common genetic variant data to gene- or gene-set-level in mediation frameworks.
This study has several strengths and limitations. The current study used the largest data-sets available to investigate pleiotropic genetic factors for aggression and brain volumes at gene-level. Cross-trait meta-analysis of brain measures and psychiatric phenotypes is a useful way of detecting shared genetic risk and generating new hypotheses about specific links between genes, the brain, and behavior (Franke et al. 2016). We were also able to use a well-phenotyped population cohort to clarify the specific subtypes of aggression involved. The added value of using data from such smaller cohorts over large consortium based data lies in the possibility of in-depth phenotyping and reducing sources of heterogeneity that come with the use of pooled data-sets. Nevertheless, some limitations apply to the current study. This study only investigated selected subcortical MRI measures, and future work should be extended to include cortical regions as well as connectivity measures that have been shown to play a role in aggression (Meyer-Lindenberg et al. 2006).

In summary, we identified MECOM and AVPR1A as genes contributing to aggression risk in conjunction with nucleus accumbens and amygdala brain volume, respectively. We find a subtype-specific gene-wide association of AVPR1A to reactive aggression due to external provocation/threat, when taking sex-related heterogeneity into account. In this, our findings may help in explaining previous contradictory association findings for this gene. Future studies may elucidate causality of gene-brain-behavior relationships. Comprehension of sex-specific physiological pathways associated with aggression subtypes is needed to enhance our understanding of the determinants of aggression. Only by understanding the mechanisms underlying different forms of aggression will we be able to develop effective treatment approaches and minimize the social costs of aggression.

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## SUPPLEMENTARY MATERIAL

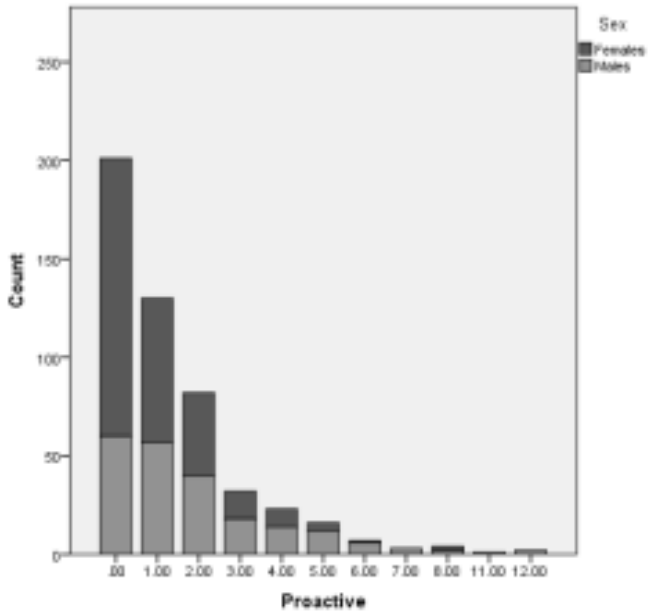
**Supplementary Table 1:** RPQ items relating to each of the aggression subtypes.

| Item | Proactive aggression |
|---|---|
| 2 | Had fights with others to show who was on top |
| 4 | Taken things from other students |
| 6 | Vandalized something for fun |
| 9 | Had a gang fight to be cool |
| 10 | Hurt others to win a game |
| 12 | Used physical force to get others to do what you want |
| 15 | Used force to obtain money or things from others |
| 17 | Threatened and bullied someone |
| 18 | Made obscene phone calls for fun |
| 20 | Gotten others to gang up on someone else |
| 21 | Carried a weapon to use in a fight |
| 23 | Yelled at others so they would do things for you |
| **Item** | **Reactive internal frustration** |
| 1 | Yelled at others when they have annoyed you |
| 5 | Gotten angry when frustrated |
| 8 | Damaged things because you felt mad |
| 11 | Become angry or mad when you do not get your way |
| 13 | Gotten angry or mad when you lost a game |
| **Item** | **Reactive external provocation** |
| 3 | Reacted angrily when provoked by others |
| 7 | Had temper tantrums |
| 14 | Gotten angry when others threatened you |
| 16 | Felt better after hitting or yelling at someone |
| 19 | Hit others to defend yourself |
| 22 | Gotten angry or mad or hit others when teased |

**Supplementary table 2:** Sample characteristics Brain Imaging Genetics sample.

| | Total sample | Males | Females |
|---|---|---|---|
| N | 501 | 215 | 286 |
| Mean age (SD) | 25.28 (4.62) | 24.83 (3.83) | 25.63 (5.12) |
| Mean reactive internal frustration score (SD) | 3.00 (1.81) | 3.14 (1.80) | 2.9 (1.81) |
| Mean reactive external provocation score (SD) | 2.36 (1.90) | 2.53 (1.95) | 2.23 (1.86) |
| Mean proactive score (SD) | 1.38 (1.80) | 1.93* (2.16) | 0.97* (1.33) |

*RPQ proactive aggression scores were dichotomized into high- and low-scoring (score ≥ 2 and score ≤ 1, respectively), because of a highly positively skewed distribution in both males and females. Males scored significantly higher on proactive aggression than females (X2(1)=22.22, p <0.001; discussed in: (van Donkelaar et al., under review)).




**Supplementary Figure 1:** Distribution of RPQ proactive aggression score in males and females from our healthy adult population.

# Chapter 7

## Summary and general discussion

## OVERVIEW

The work described in this thesis is focused on improving our understanding of the genetic and neurobiological architecture of aggression. In general terms, aggression may be defined as hostile behavior with the intention of inflicting damage or harm (Miczek et al., 2002), but it is a behaviorally and etiologically complex phenomenon. It has been shown to have moderate but significant heritability (Veroude et al., 2016) and has been associated with several neuro-cognitive deficits (Blair et al., 2016). However, the research field of aggression has covered an exceptionally wide range of behaviors, contexts, and populations, presenting a constant challenge for researchers to integrate inconsistent research findings. This thesis explores the potential of reducing phenotypic heterogeneity to identify genetic mechanisms involved in aggression. It also investigates pathways to disease, integrating genetics and brain imaging disciplines to help shed light on differential etiological pathways to aggression. In this final chapter, the main findings of the thesis will be summarized and discussed in the context of the current literature. Opportunities for improvement of future aggression etiology research will be discussed, as well as clinical implications of the main findings from this thesis.

## SUMMARY OF MAIN FINDINGS AND METHODOLOGICAL APPROACHES

Part 1 of this thesis (*chapters 2-4*) focuses on the identification of genetic mechanisms involved in aggression, and the potential of reducing phenotypic heterogeneity for uncovering genetic association. In part 2 of this thesis (*chapters 5-6*), we move the focus to imaging genetics approaches, to study associations between genes and brain phenotypes implicated in externalizing behaviors as well as potential links between genetic risk factors, brain correlates, and subtypes of aggression.

In *chapter 2*, a combination of hypothesis-driven and hypothesis-generating genetic association approaches was used with the aim to increase knowledge on the molecular genetics of oppositional behavior in children and adolescents with attention-deficit hyperactivity disorder (ADHD). Research on genetic factors underlying this behavior had been sparse, merely some candidate gene polymorphism associations had been described (*DRD4* exon3 Variable Number Tandem Repeat polymorphism (VNTR), *5-HTTLPR,* and seven *OXTR* Single Nucleotide Polymorphisms (SNPs)) in studies limited by small sample sizes (Beitchman et al., 2012; Campbell et al., 2011; DiLalla et al., 2009; Ficks and Waldman, 2014; Holmes et al., 2002; Johansson et al., 2012a; Johansson et al., 2012b; Malik et al., 2012; Park et al., 2010). To reduce the known heterogeneity of the phenotype, we defined conceptually meaningful dimensions and subtypes of oppositionality based on literature (a defiant/vindictive and an irritable dimension) and Latent Class Analysis (LCA, 2 severity-based subtypes). We found no significant associations with oppositionality subtypes for previously postulated candidate SNPs, genes, or pathways related to serotonin, dopamine, and oxytocin. In contrast, parenting behavior (an environmental risk factor) was

moderately to strongly associated with all defined subtypes of oppositionality. Subsequent hypothesis-generating multivariate genome-wide association analysis (GWA) did not yield genome-wide significant associations, as expected based on sample size. However, by conducting bioinformatics analysis on top-ranked genes, we could provide new insights into the molecular basis of oppositionality, identifying neurite outgrowth as an important biological process involved. The strongest GWA signal resided in a region on chromosome 20 including the *BCL2L1* gene. This gene encodes an anti-apoptotic regulator expressed at high levels in both the developing and the adult brain (Krajewska et al., 2002). The protein regulates neurotransmitter release and retrieval of vesicles in neurons, thereby influencing presynaptic plasticity (Li et al., 2013).
This study contributed to the literature in several ways. First, we confirmed the existence of various subgroups of youths with different oppositional symptom profiles. This improved power of our genetic analyses by reducing the known heterogeneity of oppositional behavior in ADHD. Second, with a sample size of 750 subjects, and by aggregating effects of multiple common variants in our candidate based approach, power for candidate (gene-set ) association analysis was higher than in many previous, smaller candidate studies. Third, by covering a wide range of genetic association approaches - covering hypothesis-based analyses at candidate SNP level, gene-wide level, and pathway-wide level, as well as hypothesis-generating multivariate genome-wide association and genetic bioinformatics approaches – we conducted a large-scale overview of the genetics of oppositional behavior within ADHD. Fourth, using a multivariate association framework, we were able to incorporate genetic overlap as well as differences between subtypes of oppositionality and to maximize power of genome-wide analysis. To summarize, our findings suggest that oppositional behavior in childhood ADHD is not associated with genetic variation in serotonin, dopamine, and oxytocin systems specifically, but rather an extended landscape of molecular signaling cascades involved in neurite outgrowth might be of importance.

*Chapter 3* continues on the topic of aggressive behavior in ADHD, more specifically, it explores the genetic architecture of childhood aggression in adult ADHD. We performed genome- wide association tests to shed light on the genetic susceptibility loci and biological processes involved in the etiology of childhood aggression in ADHD. The measure of childhood aggression in adult ADHD was derived retrospectively from the Wender Utah Rating Scale (WURS) in several studies across Europe. Meta-analysis of GWAS in these separate samples yielded several nominally significant variants in biologically interesting genes. The strongest signal resided in the transcript of a lncRNA with uncertain coding potential. Non-protein-coding RNAs play a critical role in the regulation of gene expression and have been previously associated with neuropsychiatric disorders (Ning et al., 2014). The second most significant locus resided in the neurotrimin (*NTM*) gene, which codes for a cell adhesion molecule predominantly expressed in the central nervous system (CNS) (Struyk et al., 1995). In line with the findings of *chapter 2*, neurotrimin has important functions in neurite outgrowth, regulating the development of neuronal projections via attractive and repulsive mechanisms (Gil et al., 1998). Building on results from *chapter 2*, we also found significant enrichment of top-associated SNPs for adult ADHD in GWA

signals from both the defiant/vindictive and the irritable dimension in childhood ADHD. This study contributed significantly to ADHD literature, as childhood aggression is a feature of the disorder that is associated with worse outcomes later in life (Klassen et al., 2010), but that nevertheless has received little attention so far. In summary, our GWAS meta-analysis results implicate mechanisms of cell adhesion as well as regulation of gene expression in the etiology of childhood aggression in adult ADHD. Moreover, the results tentatively support overlap of association signals with those of oppositionality in childhood ADHD, paving the way for more elaborate statistical methods to test this overlap.

In *chapter 4*, the focus was on reducing phenotypic heterogeneity to elucidate different genetic mechanisms involved in distinct aggression subtypes. The aim was to assess, whether common genetic variation in the main candidate genetic systems involved in aggression (the serotonin, dopamine, neuroendocrine system) show subtype- and sex-specific patterns of association. This was investigated in a sample of healthy adults, where we could confirm the existence of three subtypes of aggression based on the Reactive Proactive Questionnaire- (RPQ) by use of Confirmatory Factor Analysis (CFA); the three aggression subtypes were proactive aggression, reactive aggression due to internal frustration, and reactive aggression due to external provocation or threat. Using gene-set tests, we found a significant female-specific association of reactive aggression due to internal frustration with genetic variation in a gene-set combining genes involved in serotonergic, dopaminergic, and neuroendocrine signaling. This association was mainly driven by variation in serotonergic and neuroendocrine signaling.
This study was the first to investigate the combined effect of common genetic variants related to monoaminergic and neuroendocrine signaling (rather than single candidate variants) on aggression subtypes, thereby increasing power to find association. Moreover, the study highlighted the value of reducing sources of heterogeneity in aggression research. The results identify variation in genes involved in neuroendocrine and serotonergic signaling as biological risk factors for frustration-based reactive aggression in females. Subtype specificity of the finding underscored a biological meaningfulness of reactive-proactive distinctions in aggression research.

In *chapter 5*, imaging genetics studies of the effect of disease-linked genetic variants on brain structure and function were systematically reviewed for a group of highly heritable, often comorbid neurodevelopmental disorders. The aim of this work was to extract core brain mechanisms affected by disease-linked genetic factors related to the individual disorders as well as mechanisms relating to their clinical overlap. Common neurodevelpmental disorders were selected for investigation based on their frequent comorbidity, and included ADHD, autism spectrum disorders (ASD), selected intellectual disability disorders (ID), oppositional defiant disorder (ODD), and conduct disorder (CD) (Vorstman and Ophoff, 2013). We report a remarkably coherent picture of functional genetic variation in *SLC6A4* (*5HTT*; the serotonin transporter gene) leading to hyperactivation of the amygdala and connected areas in conjunction with functional dysconnectivity amongst those areas. Other alterations in brain volume, activity, and connecitivity were also observed, but no conclusions could

be drawn about shared mechanisms across disorders. In this chapter we provide specific recommendations for future imaging genetics research, highlighting the need for larger sample sizes, for hypothesis-free, brain-wide studies, and for extension of imaging genetics studies to a wider pool of genes. Additionally, there is a need to investigate, whether brain phenotypes are intermediate between genetic factors and behavioral outcomes.
This study contributed to the literature by providing a comprehensive overview of imaging genetics studies for five neurodevelopmental disorders with high comorbidity. In summary, results thus far confirm that imaging genetics approaches are suitable to provide more insight into the link between genes, the brain, and behavior in neurodevelopmental disorders. Nevertheless, the field should still be considered to be in its early stages and cannot yet provide definite conclusions for our understanding the comorbidity between different neurodevelopmental disorders.

In *chapter 6,* the aim was to gain more insight into the mechanisms leading from genetic risk factors for aggression to aggression phenotypes. This was investigated using a 2-step approach. Genome-wide gene-based cross-trait meta-analyses of aggression with the volumes of amygdala, nucleus accumbens, and caudate nucleus were conducted to identify genes influencing both aggression and aggression-related brain volumes. Then, the subtyping approach of *chapter 4* was revisited, to study subtype-specific effects of identified genes. The meta-analysis identified significant associations of the *MECOM* and *AVPR1A* genes with both aggression and nucleus accumbens/amygdala volume, respectively. For *AVPR1A,* gene-wide association with aggression was found to be subtype- and sex-specific. *AVPR1A* was significantly associated with reactive aggression due to external provocation or threat, an association that was driven by males in the sample.
This study contributed to the existing literature by pointing out possible gene-brain-behavior relationships for specific genes. This was done by combining use of large-scale consortium-based association statistics with analyses in a well-phenotyped local cohort. *MECOM* was identified as a gene for aggression that potentially exerts its effect through a mechanism involving nucleus accumbens volume. Additionally, this study provided replication of the association of candidate gene *AVPR1A* with aggression in a cohort of healthy adults and showed that association is subtype- and sex-dependent. Potentially it exerts its effect on aggression through a mechanism involving amygdala volume.



## GENERAL INTERPRETATION IN THE CONTEXT OF EXISTING LITERATURE

### An integrated view on identified genetic systems

One of the main aims of this thesis was to identify genetic mechanisms involved in aggression etiology. The genetic association studies in part 1 of this thesis highlight a role for several biological systems. Hypothesis-based analyses stressed the importance of neuroendocrine and serotonergic systems in aggression (*chapter 4*), and hypothesis-generating investigations pointed us towards a role for neurite outgrowth and cell adhesion in the development of aggressive behavior (*chapters 2 and 3*).

The relevance of alterations in the hypothalamic–pituitary–adrenocortical (HPA), sex steroid hormone, and serotonin systems for the regulation of aggression has long been studied and confirmed in rodent, primate, and other animal models (Veenema, 2009). Also in humans, multiple studies have been conducted to investigate, how key molecules in these systems relate to aggressive behavior. Those studies found associations for testosterone, cortisol, serotonin, and vasopressin (Rosell and Siever, 2015). This thesis further increases our insight into the role of neuroendocrine and serotonergic systems in human aggression, by showing that common genetic variation in related genes is associated specifically with reactive aggression (*chapter 4*). In previous literature, a large number of candidate gene studies for aggression and violence had been conducted, some investigating specific genetic markers relating to the above mentioned systems. However, meta-analysis has not yet picked up association significance for any polymorphism analyzed (Vassos et al., 2014), with the exception of two polymorphisms in *MAOA* and *SLC6A4* (Ficks and Waldman, 2014). By aggregating genetic effects across multiple autosomal genes in biological systems of interest, we could now show association of these genetic systems to aggression. It should be noted that in this thesis, we restricted analyses to the most established candidate systems for aggression. Future studies could extend this approach to other promising candidate systems, like GABAergic signaling (de Almeida et al., 2015). In *chapter 6* the *AVPR1A* gene, coding for a vasopressin receptor, was found to be of importance in reactive aggression. While this gene was not included in the neuroendocrine system studied in *chapter 4* (we based our gene-selection on an independent database that had not yet incorporated information on vasopressin), recent literature has shown that it is nonetheless tightly linked to the neuroendocrine stress response (Ramos et al., 2016). Vasopressin has now been recognized as a principal nervous system regulator influencing HPA axis signaling in response to stress (Ramos et al., 2016), providing additional evidence for the association of common variance in the neuroendocrine system with aggression.

In *chapter 2 and 3,* hypothesis-generating approaches where used with the aim to find out more about the genetic architecture of aggression in ADHD. Our results pointed towards brain-related genetic mechanisms. More specifically, the results indicate that neurite outgrowth and cell adhesion mechanisms in the brain are important in aggression etiology. This is in line with brain imaging studies reporting many alterations in brain structure and brain function (for and overview see the introduction of *this thesis*). However, neurite outgrowth in general is a broad concept, and this begs the question, whether it is a brain-wide phenomenon that contributes to aggression or rather a localized one. A next step might be to map neurite outgrowth related genes on brain structure and function to identify genotype effects on the brain.

The connection of brain phenotypes with genes, neurotransmitters, and hormones in neuroendocrine and serotonergic systems has been investigated in previous literature, pointing to several brain regions of interest, where alterations in neurite outgrowth may specifically lead to changes associated with the development of aggressive behaviors. Effects of testosterone, cortisol, and serotonin on the brain mostly implicate amygdala-prefrontal circuitry (Montoya, 2012). For example, gonadal (sex steroid) hormones are thought to have

an important influence on the connection of the amygdala and the orbitofrontal cortex (OFC), influencing regulation of amygdala activity by the OFC (van Wingen et al., 2011). Testosterone has been observed to increase amygdala activity (Derntl et al., 2009; Manuck et al., 2010; van Wingen et al., 2009) and to reduce OFC coupling with the amygdala (Hermans et al., 2008; van Wingen et al., 2010), which can increase aggression (Mehta and Beer, 2010). Cortisol, on the other hand, has been shown to decrease amygdala activity (Henckens et al., 2010). Hence, it has been hypothesized that the testosterone/cortisol ratio can influence amygdala reactivity and inhibitory control by prefrontal regions, thereby influencing aggressive behaviors (Honk et al., 2003). Interestingly, a high serotonin receptor density has been found in these same regions as well. For example, 5-HT1A receptors, which inhibit the activity of target neurons, are abundant in both PFC and amygdala (Albert et al., 2014). Meta-analysis has revealed an inverse but small correlation between central serotonin functioning and aggression, so it has been suggested that the widely accepted view that decreased serotonin lead to increased aggression should be revised to account for serotonin's functional complexity (Duke et al., 2013). From those and similar findings, the influential triple imbalance theory of aggression has been derived, which hypothesizes that a high testosterone/cortisol ratio combined with low serotonin levels increases aggression, by enhancing amygdala reactivity and reducing inhibitory control by the PFC (de Almeida et al., 2015; Montoya et al., 2012b). Although it is unknown what the underlying mediating mechanisms of neurite outgrowth are, it may be hypothesized that alterations in the efficiency or direction of neurite outgrowth specifically in these regions underlies aggression-related dysfunction.

In general, a useful next step in the research of aggression etiology would be to conduct more extensive research on how brain phenotypes in aggression relate to genetic variation and molecular and cellular alterations associated with the phenotype. Our genetic association findings for neuroendocrine and serotonergic signaling are in line with the triple imbalance theory of aggression development. However, the theory will be further discussed later in this chapter in the context of subtype- and sex-specificity of the findings described in this thesis.

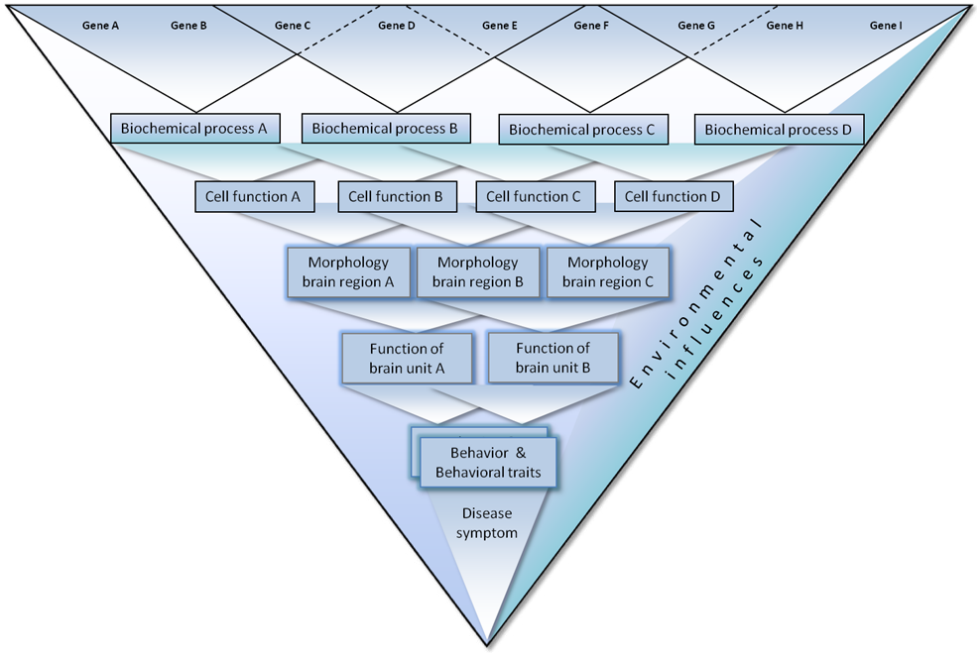



**Figure 1, published in Klein et al., 2017:** This schematic convergence model shows potential pathways leading from gene to disease and suggest endophenotypes relevant to psychiatry at different levels of complexity.
Polygenicity (schematically depicted by gene A to I) is involved in causing disease symptoms. A reduced number of genes is involved in disease-related endophenotypes. These can be studied at various biological levels, e.g. biochemical processes and cell function can be assessed by biological assays in cell or animal models by measuring e.g. neuron morphology or synaptic functioning. Neuroimaging methods (structural and functional) can be applied to assess relevant endophenotypes at the level of brain morphology ('Morphology brain region A-C'). Endophenotypes, related to the 'function of brain units', can be e.g. investigated by functional MRI or through performance measurements on neuropsychological tests. Aberrations at this level can result in altered behavior and disease-related behavioral traits, that subsequently lead to disease symptoms. Environmental influences can impact on all levels and need more attention in future studies. Bioinformatic pathway and network analyses can help to integrate data from various sources and to identify molecular networks or cellular processes in which ADHD-related genes are enriched.

## Brain phenotypes of aggression: the endophenotype model

The main aim of part 2 of this thesis was to gain more insight into how genes exert their effect on aggression and aggression-related phenotypes. In this section of the discussion, the findings are discussed in the context of the endophenotype model. While the specific biological mechanisms contributing to neurodevelopmental disorders are still largely unknown, it is thought that genetic variation will alter molecular and cellular processes, which in turn will lead to altered brain development, and subsequently to altered behavior (**Figure 1**; (Franke et al., 2009; Klein et al., 2017). Phenotypes that mediate the effects of genetic variation on behavioral phenotypes have been termed endophenotypes or intermediate phenotypes (for an overview of endophenotype criteria see Gottesman and Gould (2003); Kendler and Neale (2010)). This thesis focuses on Magnetic Resonance Imaging (MRI)-based endophenotypes.

While the model of endophenotypes has proven useful in understanding the mechanisms of genes (Franke et al., 2009), one should take caution not to oversimplify it. For example, genes can affect multiple (endo)phenotypes (Kendler and Neale, 2010; Matsumoto et al., 2003), because they are expressed in many parts of the brain, and gene expression does not even predict or limit the location of effects of a genetic factor, most likely because of effects on brain connectivity (Braet et al., 2011). In addition, single endophenotypes can be linked to multiple disorders. We made use of this prior knowledge in *chapter 5*, and aimed to extract core brain mechanisms affected by disease-linked genetic factors, related both to individual neurodevelopmental disorders and to their clinical overlap. Reviewing available imaging-genetics literature, we concluded that imaging-genetics studies of aggression (specifically ODD and CD) are still scarce, despite the need for the integration of genetic and brain imaging findings. Definite conclusions helping our understanding of the comorbidity with different neurodevelopmental disorders also could not be drawn yet (*chapter 5*). Partly, this was due to the limited number of genes investigated in imaging genetics studies to date. One exception is the *SLC6A4* gene encoding the serotonin transporter, which has been investigated relatively frequently. Based on imaging genetics literature available, we concluded that aggression-relevant variation in this gene is associated with the above-described pattern of hyperactivation of the amygdala and connected areas. This may be significant not only to cognition in ODD and CD, but also in ADHD and ASD. In general, knowing about the existence of phenotypic (and genetic) continuous distributions of psychiatric traits in the population, with clinical disorders being the extreme of such continua, the associations of genetic risk factors are likely to exist with behavioral traits, and not with disorders directly (e.g., (Hoogman et al., 2011). Likely the identified gene-brain relationship is associated with a trans-diagnostic phenotype, relating to symptoms expressed across all these disorders (Nolen-Hoeksema and Watkins, 2011). As this would have highly important implications for the way we conceptualize psychiatric disorders and for genetic and neurobiological research of neuropsychiatric disease, a separate subsection of this discussion is devoted to a more detailed discussion of diagnostic boundaries in aggression research (see: *Crossing diagnostic boundaries)*. The conclusions we could draw based on existing imaging genetics literature were also limited, because studies to date had limited power. Although the endophenotype concept postulates that imaging genetics measures should have stronger effect sizes for gene effects than the behavioral measures (Gottesman and Gould, 2003), recent work has raised doubt about this assumption. Larger effect sizes have not been found for neuroimaging (endo)phenotypes, at least not for volumetric MRI measures (Franke et al., 2016), underscoring the need for larger sample sizes in imaging genetics literature, or better ways to measure (neuroimaging) phenotypes. Efforts to accomplish this are already underway, for example, large sample sizes with consistently preprossessed neuroimaging measures are continuously collected by the ENIGMA consortium (Thompson et al., 2014). Another factor hampering interpretation is the fact that there has been a strong focus on regions and cognitive domains of interest in most studies published to date. Hence, caution is warranted in claiming specificity of brain phenotypes for individual genes based on existing studies. This underscores the need for appropriately powered hypothesis-generating brain- and phenome-wide methods in the imaging genetics field.

Since we found that imaging genetics literature, especially for aggression-related phenotypes, is still in its early stages (*chapter 5*), in *chapter 6* we aimed to connect genes, brain phenotypes, and aggressive behavior in an hypothesis-generating way, using the largest available GWAS datasets on aggression and subcortical brain volumes available. Cross-trait meta-analysis identified two genes with pleiotropic effects affecting both aggression and subcortical brain volumes (*MECOM, AVPR1A*). We hypothesized that these genes may thus exert their effect on aggression by affecting subcortical brain volumes. However, we should be careful about causality assumptions. It is often assumed that genetic risk passes through the endophenotype to behavior (**Figure 1)**, but another possibility is that genetic factors influencing aggression also influence the brain in a way that is independent of the behavioural phenotype of interest. Only a few studies have investigated the issue of causality earlier. Those studies showed that only part of the brain regions showing genotype effects actually do mediate between genetics and the behavior under study, proving the importance of such multilevel investigations (Sokolova et al., 2015; van der Meer et al., 2015).

On the one hand, this thesis aimed to identify common and unique genetic effects on the brain for genes implicated in different comorbid neurodevelopmental disorders. Specific gene-brain-behavior relationships could not be established based on existing imaging-genetics literature, stressing the need for larger studies using standardized measures that investigate more genes and brain-wide phenotypes (*chapter 5*). On the other hand, this thesis aimed to identify subtype-specific pathways to aggression, that is, to draw lines from specific genes to specific brain regions to specific aggression subtypes. While specific genes with pleiotropic effects on aggression and subcortical brain volumes were detected, it is not certain that these brain volumes mediate the gene-behavior relations of interest (*chapter 6*).

**Reducing heterogeneity in aggression research**

*Aggression subtypes*

Across this thesis, it has been hypothesized that genetic overlap as well as differences exist among aggression subtypes, and broader, across different neurodevelopmental disorders. On the one hand, we tackled this by capturing both broad and specific effects across phenotypic subtypes, for example in *chapter 2,* by using a multivariate association model. On the other hand, we aimed to improve power for finding genetic effects by looking at subtype-specific gene association, for example in *chapters 4* and *6. Chapter 4* showed that neuroendocrine and serotonergic genetic variation might be important specifically to a reactive type of aggression, and *chapter 6* showed that the *AVPR1A* gene might also be of importance in reactive aggression. These subtype-specific genetic association results are in line with the triple imbalance theory of aggression, which is thought to be specific to reactive impulsive types of aggression (Montoya et al., 2012a; van Honk et al., 2010). As shortly mentioned above, it hypothesizes that levels of testosterone, cortisol, and serotonin in the brain work together, facilitating the fight/flight response through acting on amygdala-brainstem networks, and at the same time affecting prefrontal cortex regions responsible for impulse control. This is thought to lead to reduced top-down inhibitory control, thus giving rise to

reactive aggression (Montoya, 2012). While the association results are largely in line with the model of gene-to-behavior relationships depicted in *Figure 1 of the thesis introduction*, not all gene-subtype relationships could be confirmed. Previous literature speculated that serotonergic and dopaminergic neurotransmission regulate both reactive and proactive aggression, whereas endocrine signaling seems to be more involved in the regulation of reactive aggression (Waltes et al., 2016). While our association results affirm the role of neuroendocrine and serotonergic variation in reactive aggression, no link of serotonergic signaling with proactive aggression was found. Association was specific for the frustration-based reactive subtype of aggression, which is thought to be related to poor decision-making (Blair et al., 2016). As the prefrontal cortex is important in decision-making (Fairchild et al., 2009), we may speculate that serotonergic effects are strongest on our cognitive control system. Results from *chapter 6* suggest that for the *AVPR1A* gene, strongest association is with threat-/provocation-based reactive aggression. Hence, vasopressin signaling may be of specific importance in facilitating the acute threat response, which is regulated by the amygdala network (Coker-Appiah et al., 2013; Mobbs et al., 2010). This is in line with our finding that *AVPR1A* association significance increases, when meta-analyzing genetic association results for aggression and amygdala volume (*chapter 6*).
While contributing to knowledge on the specificity of genes and neurobiological defects for aggression subtypes, we provide no specific evidence of genetic systems that might be related to proactive symptoms of aggression. The neurite outgrowth-related genes identified in *chapter 2* may still play a role, but subtype specificity for this system has yet to be investigated. All in all, there is a need for increased knowledge on proactive aggression, also reflected by the limited treatment options available to date for this subtype.

*Gender*
Gender differences in aggressive behavior are pronounced (Card et al., 2008; Collett et al., 2003; Georgiev et al., 2013; Hill, 2002; Stephenson et al., 2014), and might be an important factor accounting for another part of the inconsistent findings in aggression research. Therefore, work in this thesis included sex-specific gene identification in aggression.
Association of serotonergic and neuroendocrine gene-sets with reactive aggression was female-specific. Moreover, we showed that association of *AVPR1A* candidate gene with reactive aggression was male-specific, possibly explaining previous contradictory association results (Malik et al., 2014; Moons et al., 2014; Prichard et al., 2007). Our findings are in line with numerous reports on the sex-specificity of neuroendocrine signaling. Multiple studies have sought to explain sex differences in aggression based on hormonal signaling. For example, a pattern of increased testosterone levels in competitive situations compared to defeat has been found in men but not women, and mediated effects of winning on aggressive behavior (Carre et al., 2013). Progesterone has been shown to interact with HPA axis function, influencing aggressive behavior of females (Kirschbaum et al., 1999; Ossewaarde et al., 2010). Opposite effects of vasopressin on eliciting aggressive behavior have been found in animal studies, as well as sex differences in receptor distribution (Albers, 2015; Gutzler et al., 2010). Less is known about the role of serotonin in sex differences of aggressive behavior (Duke et al., 2013), but variations in the levels of sex hormones have

been shown to lead to changes in serotonin receptor distribution (Witte et al., 2009). Of note, gonadal hormones, glucocorticoids, and neuropeptides interact closely, and further research should be directed at elucidating the effect of these interactions on sex differences in aggression (de Almeida et al., 2015).

Our genetic findings are in line with current theories of reactive aggression, but suggest that these theories should be further specified to incorporate and allow for sexual dimorphism. Results confirm the idea that subtypes and sexes are (at least partly) different with regard to underlying pathophysiology. Heterogeneous genetic susceptibility patterns and the complex nature of the phenotype may explain the lack of robust association signals in aggression genetics to date. It is also likely that earlier, smaller association studies might have been underpowered to detect gender effects. Our work, combined with prior literature, points towards the crucial role of sex neuro-steroids and certain neurotransmitters for sex-specific aggression, and the investigation of those intricately connected systems might be the future of the aggression research field.

**Crossing diagnostic boundaries**

Progress in understanding the genetic etiology of aggression and other psychiatric disorders has been hampered by limited sample sizes available for genome-wide association approaches. Enormous sample sizes are necessary to detect genome-wide significant findings for neurodevelopmental disorders. Reasons for this are the phenotypic heterogeneity discussed above, as well as small expected effect sizes of common genetic variants and polygenicity. Additionally, diagnostic categories for neurodevelopmental disorders do not necessarily follow underlying biological mechanisms (Cross-Disorder Group of the Psychiatric Genomics et al., 2013), further limiting power of genetic association studies.

*Population-based studies*

Part of the research conducted in this thesis was performed in general population samples. Pleiotropic genetic effects for brain and behavior were detected based on one of the largest aggression GWAS meta-analyses to date, which investigated childhood aggressive behavior in healthy subjects (Pappa et al., 2015). In-depth analysis of detected pleiotropic genes (*chapter 6*) as well as genetic association studies of candidate gene-sets with aggression (*chapter 4*) were preformed in a large local sample of healthy volunteers (Brain Imaging Genetics). These latter subjects had detailed phenotyping available on reactive and proactive subtypes of aggression. Interestingly, the measures of reactive aggression followed a normal distribution in this general population sample. Moreover, we were able to find association for genetic variants, selected based on their implication in aggression disorders, with aggressive traits in the general population. This indicates that common genetic variants underlying aggression phenotypes are potentially similar in general and psychiatric populations. Our findings are in line with studies providing evidence that psychiatric disorders represent the extreme ends of continuous distributions of disorder-like traits within the general population. Several lines of research have shown that this model is relevant in psychiatric traits such as ADHD and ASD (Martin et al., 2014; Middeldorp et al., 2016; Riglin et al.,

2016; Robinson et al., 2016; Bralten et al., 2017). Shared genetic etiology between clinical disorders and population traits has also been shown to exist for these disorders. For example, genetic risk factors for clinical ASD predicted ASD-traits in adult and childhood general population samples (Bralten et al.,2017; Robinson et al., 2016). The use of association studies of disorder-like traits in the general population can greatly increase sample sizes at relatively low costs, providing tremendous opportunities for gene finding in neurodevelopmental disorders. For aggression, this point is highlighted by the recent publication of another large-scale population-based GWAS meta-analysis of antisocial behavior, providing new genome-wide suggestive (and sex-specific) signals of interest (Tielbeek et al., 2017). In the context of aggression, the existence of phenotypic (and genetic) continuous distributions of psychiatric traits in the population is especially relevant, particularly since aggressive traits in the general population at sub-diagnostic level give rise to increased crime rates, socially disruptive behaviors and inceased costs for society.

*Cross-disorder studies*

Another part of the research in this thesis focused on aggression phenotypes in comorbid psychiatric disorders (mainly ADHD). Currently, psychiatric disorders are classified based on clinical presentation rather than underlying etiology. It has been shown though, that substantial genetic correlations between psychiatric disorders exist (e.g. for five major disorders: Cross-Disorder Group of the Psychiatric Genomics et al., 2013), providing evidence for shared genetic etiology among different psychiatric disorders as currently classified in clinical practice. In *chapter 2* of this thesis, genetic mechanisms underlying oppositional behavior in childhood ADHD were investigated. In line with evidence for shared genetic etiology, the association findings of this chapter may in part reflect shared genetic risk factors for psychiatric disorders. This notion is supported by the fact that 15 out of the 53 top-associated genes in the study had previously been associated with other neuropsychiatric and neurodevelopmental disorders. As there is a substantial degree of overlap in aggressive behaviour among neuropsychiatric disorders, it could be beneficial to analyze conditions in which aggression is present together, in order to pin-point biological processes in dysfunctional forms of aggression. However, possibilities for this are limited to date, because, although aggression is a cross-disorder trait, aggression is often measured differently across disorders. More generally speaking, consistent investigations of traits occurring across different (co-morbid) neurodevelopmental disorders is needed to provide crucial insights into genetic and biological susceptibility factors that are common to multiple disorders. The importance of heterogeneity and specificity issues relating to categorical diagnostic groups in research of mental disorders is also emphasized by the strategic plans of the U.S. National Institute of Mental Health (NIMH). They underscore the Research Domain Criteria (RDoC) Project, which provides a research framework for a better understanding and classification of mental disorders based on behavioral and neurobiological dimensions (https://www.nimh.nih.gov/research-priorities/rdoc/index.shtml). Consistent assessment of genetic, molecular, cellular, neural, physiological, and behavioral variables might be able to contribute to this approach. By promoting the use of dimensional data for research purposes in psychiatry, the project aims to improve understanding of current diagnoses as

well as normal human behavior, and to improve the search for treatment targets in multiple domains. Reviewing imaging genetics literature across comorbid disorders in *chapter 5*, we found genetic variation in the *SLC6A4* risk gene to be associated with hyperactivity of the amygdala and related regions, and impaired connecitivity amongst those areas. This could be an example of a possible trans-diagnostic phenotype at brain level. Given that this phenotype may be relevant in multiple of the investigated comorbid disorders, this finding provides more insight into the shared neurobiological features among diagnostic categories. Recent neuroimaging studies confirm the existence of shared as well as disorder-specific brain abnormalities in neurodevelopmental disorders (Goodkind et al., 2015). These findings support once more the potential of going beyond diagnostic boundaries to improve power for detecting certain neurobiological signals. Stepping away from clinical diagnosis will be a crucial starting point for defining alternative ways to classify (heterogeneous) psychiatric disorders.
In summary, we highlight the opportunities for sample size maximization offered by population-based studies of aggression, as well as the significance of going beyond diagnostic boundaries in aggression research and using dimensional approaches to the phenotype to shed light on comorbidity (Docherty et al., 2016).

**Strengths and limitations**
A great strength of the analyses in this thesis is that they try to integrate findings of many different samples and data-sets. We combined information derived from large-scale, consortium-based association statistics (EAGLE, ENIGMA) with analyses in well-phenotyped and appropriately powered local (BIG) and European (IMpACT, IMAGE) cohorts. Throughout this thesis, we stress that maximization of sample size is not the only important factor in increasing power of neurobiological studies. Rather, minimization of phenotypic heterogeneity is just as essential for finding genetic and neural associations related to neurodevelopmental disorders like aggression. By using different types of information from different datasets, optimized use was made of sample size on the one hand, and in-depth phenotyping on the other hand.

Combination of information from the above-mentioned datasets was partly made possible by funding by the European Commission (EC). All work described in this thesis was performed within the context of the Aggressotype consortium, which focuses on aggression subtyping for improved insight and treatment innovation in paediatric psychiatric disorders (www.aggressotype.eu). Such large, multidisciplinary consortia are useful in bringing together not only data-sets, but also researchers from all over the world, stimulating discussion and dissemination of research results and methodology as well as the conception of new research ideas. Aggressotype is also successful in combining expertise on multiple levels of aggression research, from genetic, cellular, neuroimaging, and behavioral levels to treatment-focused approaches, thus contributing many pieces to the puzzle of the the complex multi-level etiology of aggression and comorbid neurodevelopmental phenotypes.

Well-phenotyped samples enabled us to study aggression at multiple levels of the

endophenotype model (Figure 1 of the *thesis introduction*). For our candidate genetic studies in BIG and IMAGE, data of hundreds of individuals was available for analyses, thus using sample sizes much larger than those used in much of the published literature to date (*chapters 2, 4,* and *6*). We were able to study factors such as gender and comorbidity, that many smaller studies in literature have lacked power for. However, our hypothesis-generating genome-wide approaches on childhood aggression in ADHD based on IMAGE and IMpACT data (*chapters 2* and *3*) suffered from being underpowered, and the suggestive threshold we used could contain false-positive findings. Nevertheless, we maximized power by using a multivariate statistical approach in *chapter 2*. Also, bioinformatics approaches confirmed relevance of top-findings for neurodevelopmental phenotypes, providing support for true association results. The genome-wide study in *chapter 6*, on the other hand, was based on the summary statistic of two of the largest available datasets on brain imaging and aggression. Thus we maximized power for this cross-trait genome-wide meta-analysis.

The availability of large sample sizes in this thesis allowed for applying a wide range of genetic association approaches. These covered hypothesis-based analyses at candidate SNP level, gene-wide and pathway-wide levels, as well as hypothesis-generating (multivariate) genome-wide association and meta-analysis. The multivariate framework used in *chapter 2* enabled us to incorporate genetic overlap as well as differences between subtypes to maximize power of genome-wide analysis. In *chapter 4*, we were the first to investigate the combined effect of common genetic variants related to monoaminergic and neuroendocrine signaling (rather than single candidate variants/genes) on aggression subtypes. This methodological approach aggregates genetic variants to test their joint effect, combining information to increase statistical power. A downside of this approach is that it relies on prior hypotheses. This eliminates the possibility to find new, unexpected mechanisms. Hence, hypothesis-based approaches and hypothesis-generating approaches were combined in this thesis to uncover different aspects of aggression etiology.

This thesis also contributes to the literature by providing a comprehensive overview of imaging genetics studies for five neurodevelopmental disorders with high comorbidity (CD, ODD, ADHD, ASD, selected IDs). Although we could not yet provide definite conclusions for understanding the comorbidity among these disorders, that observation is valuable in itself, and we were able to formulate important directions for future imaging genetics studies based on our extensive review.

Our results show that not only genetic, but also environmental influences play an important role in aggression. In *chapter 2*, parental ability to cope with disruptive behavior was found to be associated with our defined subtypes of oppositional behavior in children with ADHD. However, we did not address environmental influences in the rest of the chapters. This is a main limitation of the thesis, because environmental influences are another important source of heterogeneity in aggression. This is perhaps best illustrated by the association pattern of the most well-known aggression-related gene, *MAOA*. This X-linked gene contains a variable number tandem repeat polymorphism in its promoter region,

which influences activity of the encoded enzyme. Meta-analysis of 27 studies found an interaction effect of *MAOA* genotype and maltreatment in males, such that maltreatment presaged antisocial outcomes more strongly in persons with low-activity *MAOA* genotype compared to those with high-activity *MAOA* genotype (Byrd and Manuck, 2014). Future studies should further investigate interactions of the environment with genetic factors in contributing to development of aggressive behaviors, as gene-environment interactions may partly explain the lack of candidate genetic association findings in aggression literature to date (Vassos et al., 2014).

**Clinical implications**

The results of this thesis provide new information on the underlying biology of aggression. Although the main perspective was not a clinical one, there are some implications of this work that may be of interest to clinicians. The underlying mechanisms of aggressive behavior are still poorly understood, and treatment options are limited. By increasing knowledge of aggression etiology, this thesis contributes to the search for diagnostic, preventive, and treatment options based on underlying mechanisms. First, our genetic association findings highlight the potential of pharmacological interventions targeting neuroendocrine molecules. Second, this thesis also shows that subtypes of aggression may be fundamentally different with regard to their underlying pathophysiology. Third, this thesis shows that sex differences exist with regard to the etiology of aggression. This is relevant for therapeutic interventions, in the sense that optimal treatment will have to be tailored to the individual patient. Hence, while genetic and neurological measures identified in this thesis cannot be used to diagnose aggression directly, they do emphasize the large neurobiological heterogeneity and behavioral dimensionality of aggression, indicating that detailed subtyping is highly useful in intervention frameworks. Current pharmacological interventions are especially effective in treating reactive forms aggression, stressing the need to develop new pharmacological and/or behavioral interventions for people with proactive aggression symptoms. At the same time, treating reactive components of disease symptomatology in patients with combined reactive and proactive aggression might alleviate part of the symptoms or create openings for behavioral interventions. Additionally, male and female needs of intervention might be quite different. Males and females might react differently to medication acting on neurotransmitter or neuroendocrine systems, stressing again that treatment should be tailored to the individual.

**Recommendations for future research**

Based on the findings described in this thesis, several suggestions for future research can be formulated. With regard to imaging genetics studies, results from this thesis lead to several specific recommendations necessary to successfully map the biological pathways from gene to disease. *More genes* need to be studied, and individual genes need to be investigated in *larger samples*. Imaging genetics studies also need to be combined with *complementary approaches*, such as the verification of gene functions and effects in animal models (van der Voet et al., 2016). It will also be important to determine, whether a brain (endo)phenotype is *intermediate* between a genetic factor and a behavioral outcome, or whether it is only an

epiphenomenon unrelated to the behavior of interest (Kendler and Neale, 2010; Preacher and Hayes, 2008). Furthermore, the future of imaging genetics studies might change significantly, as genome-wide approaches to imaging genetics are being developed. These methods will significantly contribute to knowledge on whether genes contributing to brain measures as observed in hypothesis-generating, genome-wide approaches also contribute to disease-related phenotypes (Franke et al., 2016; Klein et al., 2017). These statistical methods are highly dependent on sufficient polygenic signal of brain and behavior GWAS results (Bulik-Sullivan et al., 2015), again stressing the need for GWAS studies with sufficient power.

Both imaging studies and genetic studies will benefit from increasing sample sizes. This will increase reliability of neuroimaging findings as well as provide genome-wide significant associations of brain phenotypes with common genetic variants. Increasing sample size can be achieved by (international) collaborations, such as ENIGMA (http://enigma.ini.usc.edu/; (Thompson et al., 2014)) or the Psychiatric Genomics Consortium (http://www.med.unc.edu/pgc; (Cross-Disorder Group of the Psychiatric Genomics et al., 2013)). However, this thesis shows that one should not only focus on sample size to achieve maximal study power. Working on smart ways to maximize information within data-sets, and reducing heterogeneity, can be strong determinants of study power. Combining data from different studies often comes with a necessary increase in heterogeneity, as study methodology and measurements scales will likely differ between studies. New data collection within the context of large-scale collaborations, with standardized collection protocols, are needed to overcome this problem. For example, at the moment we are collecting a large imaging genetics sample, uniformly collected across different locations in Europe, within the context of Aggressotype. At the same time, already collected, deeply phenotyped data from single sites, which are more homogeneous than combined data-sets, will remain highly useful in elucidating the underlying mechanisms involved in different aspects of aggressive behavior.

Lastly, future studies should consider ways to take into account heterogeneity in the aggression phenotype. Subtype- and sex-specific findings in this thesis highlight a crucial role for sex neuro-steroids and certain neurotransmitters for sex-specific reactive aggression. Investigation of those intricately connected systems should become a main line of research in the aggression field. Other sources of heterogeneity, like environmental influences, should also be taken into account in future studies. Environmental exposures are also able to cause changes in epigenetic modifications, such as DNA methylation, and the study of epigenetic marks in aggression may identify genes that are differentially regulated in individuals with high and low levels of aggression (van Dongen et al., 2015).

Research frameworks looking across diagnostic boundaries, such as the RDoC approach, will be highly useful in improving our understanding of dimensions of functioning underlying the full range of human behavior from normal to abnormal. In a sense, the aggression research field can provide an example for other psychiatric phenotypes, as research has covered an exceptionally wide range of aspects, conditions, contexts, and populations related

to the behavior. Moreover, many different research fields have contributed information on aggression etiology. While lack of standardization and consensus about which measures to study has created a lot of information, integration of interdisciplinary research findings, and complementary approaches, whether looking at global or specific gene-brain-behavior relationships, will be necessary to paint a complete picture of aggression etiology.

**Key findings of this thesis**

- Conceptually meaningful subtypes and dimensions of oppositionality exist in childhood ADHD (*Chapter 2*).
- Environmental influences like parenting style are promising determinants of the development of oppositionality in ADHD (*Chapter 2*).
- Neurite outgrowth plays a role in etiological mechanisms of aggressive behavior in ADHD (*Chapters 2* and *3*).
- Tentative support is provided for overlap of common genetic variants associated with childhood aggression measured in adult ADHD and oppositionality measured in childhood ADHD, paving the way for more elaborate statistical methods to test this hypothesis (*Chapter 3*).
- Common variation in genes involved in neuroendocrine and serotonergic signaling is a biological risk factor for frustration-based reactive aggression in females (*Chapter 4*).
- Subtype- and sex-specific genetic association stresses the value of efforts to reduce heterogeneity in research of aggression etiology (*Chapter 4* and *6*).
- Population-based studies and studies crossing diagnostic boundaries of aggression offer opportunities for sample size maximization (*Chapters 4* and *5*).
- Large-scale and comprehensive overview of the imaging genetics literature showed that imaging genetics studies provide insight into the links between genes, disease-related behavior, and the brain (*Chapter 5*).
- The imaging genetics research field is still in its early stages, and conclusions about shared mechanisms of neurodevelopmental disorders cannot yet be drawn (*Chapter 5*).
- *MECOM* is a potential new candidate gene for aggression that may exert its effect through a mechanism involving nucleus accumbens (volume) (*Chapter 6*).
- Association of candidate gene *AVPR1A* with aggression is subtype- and sex-dependent (*Chapter 6*).
- *AVPR1A* potentially exerts its effect on aggression through a mechanism involving amygdala (volume) (*Chapter 6*).

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# Appendix

# Nederlandse samenvatting

'Agressie' is een brede term waarmee veel verschillende soorten gedrag kunnen worden bedoeld, van verbale uitingen tot fysiek geweld. Deze complexiteit maakt het moeilijk om de onderliggende oorzaken van agressief gedrag te kunnen vinden, omdat ze wellicht verschillen voor de verschillende vormen van agressie. Wat we weten uit eerder onderzoek is dat genetische factoren een rol spelen. Daarnaast zijn er op groepsniveau verschillen gevonden in de bouw en het functioneren van de hersenen tussen mensen met en zonder agressieproblematiek. Het doel van dit proefschrift is om meer inzicht te krijgen in welke genetische en brein- gerelateerde factoren een rol spelen bij verschillende vormen van agressie.

Een deel van het onderzoek uit dit proefschrift, richt zich op vormen van agressief of oppositioneel gedrag die kunnen voorkomen bij ADHD (attention-deficit/hyperactivity disorder), een veelvoorkomende aandoening die gekenmerkt wordt door aandachtsproblemen en/of hyperactief en impulsief gedrag. Een ander deel van dit proefschrift maakt onderscheid tussen reactieve en proactieve agressie. Reactieve agressie wordt ook wel impulsieve agressie genoemd, en wordt gekenmerkt door een emotionele reactie op een bedreiging of frustratie. Proactieve agressie wordt ook wel instrumentele agressie genoemd, en wordt gekenmerkt door georganiseerd agressief gedrag om een doel te bereiken, waarbij meestal juist weinig emotie of empathie gevoeld wordt. Er wordt gedacht dat deze soorten agressie deels verschillende onderliggende oorzaken hebben.

Deel 1 van dit proefschrift (hoofdstuk 2 t/m 4) richt zich op de identificatie van genetische mechanismen die betrokken zijn bij agressie, en op hoe het verminderen van heterogeniteit op gedragsniveau kan helpen bij het vinden van genetische associaties. In deel 2 van dit proefschrift (hoofdstuk 5 en 6), ligt de nadruk op het combineren van hersenmaten met genetica, om verbanden te leggen tussen genetische risicofactoren, veranderingen in de hersenen, en vormen van agressief gedrag.

Hieronder wordt kort beschreven wat er in de individuele hoofdstukken werd onderzocht en gevonden.

In **hoofdstuk 2** werd een combinatie van statistische methoden gebruikt om genetische associaties met oppositioneel gedrag bij kinderen met ADHD te vinden. Eerder werden hier al kleinschalige studies aan gewijd, waarbij slechts enkele genetische varianten werden onderzocht. In deze studie definieerden we conceptueel betekenisvolle subtypes van oppositioneel gedrag op basis van literatuur en Latent Class Analysis (LCA) om de heterogeniteit te verminderen op gedragsniveau. We vonden geen significante associaties met deze subtypes voor eerder onderzochte kandidaat genen, of sets van genen die te maken hebben met serotonine, dopamine en oxytocine. We vonden wel dat opvoedingsgedrag van ouders matig tot sterk geassocieerd was met oppositioneel gedrag van de kinderen. Daarna deden we een genoomwijde associatiestudie (GWAS) om nieuwe links met de genetica te

kunnen vinden. Door het toepassen van een krachtige multivariate associatie methode, konden we in deze analyse zowel de overlap als de verschillen tussen de subtypes van oppositioneel gedrag meenemen. Uitgaande van de sterkst geassocieerde genen, gebruikten we een bioinformatica methode om een moleculair landschap te bouwen voor oppositioneel gedrag. Hierdoor konden we identificeren dat de uitgroei van hersencellen een belangrijk biologisch proces is dat betrokken is bij oppositioneel gedrag.

In **hoofdstuk 3** keken we ook naar agressief gedrag bij ADHD. We onderzochten deze keer volwassenen met ADHD, om inzicht te krijgen in de genetische factoren die een rol spelen bij de hoeveelheid agressie die zij ervaarden in de kindertijd. We deden genoomwijde associatiestudies in verschillende groepen deelnemers uit verschillende Europese landen, en meta-analyseerden de resultaten. Hoewel de gecombineerde groep nog niet groot genoeg was om significante genetische associaties te vinden, wees het onderzoek op verschillende genen die interessant zouden kunnen zijn voor aggressieproblematiek bij ADHD. Een van de gevonden genen, het neurotrimine (NTM) gen, speelt een rol bij celadhesie, en komt voornamelijk tot expressie in het centrale zenuwstelsel. Omdat het ook een belangrijke rol speelt in de uitgroei van hersencellen, sluit deze bevinding goed aan bij de resultaten van hoofdstuk 2, waarin kinderen met ADHD werden onderzocht. Het is belangrijk om meer te weten te komen over de oorzaken van agressie in de kindertijd, omdat dit bij volwassenen met ADHD geassocieerd is met een slechtere kwaliteit van leven.

In **hoofdstuk 4** keken we naar de biologische systemen die in de literatuur het meest in verband worden gebracht met agressie (serotonine, dopamine, en het neuro-endocriene systeem). We keken of genetische variatie in deze systemen subtype- en geslacht- specifiek geassocieerd is met agressie. Dit werd onderzocht in een groep van gezonde volwassen mannen en vrouwen, die een vragenlijst over reactieve en proactieve symptomen van agressie hadden ingevuld. Door het toepassen van Confirmatory Factor Analysis (CFA) vonden we drie verschillende subtypes: proactieve agressie, reactieve agressie als gevolg van interne frustratie, en reactieve agressie als gevolg van externe provocatie of bedreiging. We vonden dat de dopaminerge, serotonerge en neuroendocriene systemen associatie laten zien met reactieve agressie als gevolg van interne frustratie in de vrouwelijke deelnemers. De specifieke associatie met één van de subtypes, laat zien dat het onderscheid tussen subtypes van agressie ook op genetisch niveau relevant is.

In **hoofdstuk 5** werd het effect van genetische risicofactoren op de structuur en de functie van de hersenen onderzocht. We gingen uit van bekende genetische risicofactoren voor een groep van aandoeningen die regelmatig samen voorkomen: ADHD, Autisme Spectrum Stoornis, oppositioneel- opstandige gedragsstoornis, antisociale gedragsstoornis en bepaalde vormen van verstandelijke beperkingen. We onderzochten per aandoening welke mechanismen in het brein worden beïnvloed door de genetische risicofactoren en probeerden mechanismen te vinden die geassocieerd zijn met de klinische overlap tussen de aandoeningen. We vonden dat genetische variatie in het serotonine transporter gen (*SLC6A4/5HTT*) consistent geassocieerd is met verhoogde activiteit van de amygdala en

gerelateerde hersengebieden, in combinatie met een verminderde connectiviteit tussen deze gebieden. Op basis van de onderzochte literatuur, konden we specifieke aanbevelingen voor toekomstig onderzoek geven. Er is een grote noodzaak voor grotere studies, die hypothesevrij naar alle hersengebieden kijken, en die meer genen onderzoeken. Daarnaast is het nodig om te onderzoeken of veranderingen in de hersenen de effecten van genetische risicofactoren op het klinische fenotype kunnen verklaren. Naast het geven van een uitgebreid overzicht van de literatuur toonde dit onderzoek aan dat het combineren van genetica en hersenmaten nuttig is om meer te weten te komen over verbanden tussen genen, hersenen en gedrag. Toch is het werkveld nog in een vroeg stadium en kunnen er nog geen definitieve conclusies getrokken worden over de overlap tussen de verschillende onderzochte aandoeningen.

In **hoofdstuk 6** wilden we meer inzicht krijgen in de rol van genetische risicofactoren bij agressief gedrag. Dit werd onderzocht door een genoomwijde associatiestudie van agressie met genoomwijde associatiestudies van het volume van verschillende hersengebieden die betrokken zijn bij agressie te meta-analyseren op gen- niveau. Op deze manier konden genen geïdentificeerd worden die zowel agressie, als hersenvolume beïnvloeden. Het *MECOM* gen toonde associatie in de meta-analyse van agressie en de nucleus accumbens en het *AVPR1A* gen in de meta-analyse van agressie en de amygdala. Mogelijk beïnvloed genetische variatie in *MECOM* agressief gedrag via een mechanisme dat geassocieerd is met het volume van de nucleus accumbens, en mogelijk beïnvloed genetische variatie in *AVPR1A* agressief gedrag via een mechanisme dat geassocieerd is met het volume van de amygdala. We onderzochten deze genen in een groep gezonde volwassen mannen en vrouwen, en toonden aan dat *AVPR1A* geassocieerd is met reactieve agressie als gevolg van externe provocatie of bedreiging in mannen. Vervolgonderzoek moet uitwijzen of er een oorzakelijk verband is tussen genetische variatie in deze genen, veranderingen in de gevonden hersengebieden en agressie.

Door gebruik te maken van uiteenlopende analysemethoden, geven de studies beschreven in dit proefschrift nieuwe inzichten in de genetische risicofactoren en hersenmechanismen betrokken bij verschillende vormen van agressie.

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## About the author

Marjolein van Donkelaar obtained bachelor degrees in both Medicine and Philosophy (cum laude), followed by a cum laude masters degree in Cognitive Neuroscience in 2011 at Radboud University Nijmegen. After this, she worked as a research assistant investigating the neural, cognitive, and motivational basis of developmental behavioral disorders such as Attention Deficit Hyperactivity Disorder (ADHD) at the Behavioural Science Institute, Radboud University Nijmegen. From end 2013 onwards she was a PhD student at the department of Human Genetics of the Radboudumc and the Donders Institute for Brain, Cognition and Behaviour. Her work focuses on the complex genetics of aggression and aggression imaging genetics conducted within the 'Aggressotype' consortium, which aims to gain new insights into the mechanisms underlying pathological aggression and to develop therapeutic strategies for the benefit of patients with aggression disorders.

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