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Facts of aggression

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FACTS OF AGGRESSION

PETER JOSSE ROETMAN

Facts of Aggression

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Facts of Aggression

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CHAPTER 1

GENERAL INTRODUCTION

Aggression in its different manifestations comprises an integral part of history and everyday life; ranging from cataclysmic armed conflicts to acts as mundane as an employer giving its employee "the silent treatment". The famous 18th century philosopher Jean-Jacques Rousseau proposed that this has not always been the case, painting an image of a prehistoric world populated by peaceful hunter-gatherers where aggression and violence were almost non-existent (Rousseau, 1754). He proclaimed that modern civilization, primarily through private ownership, caused man to stray from their natural disposition of peacefulness, luring it into a deviant, unnatural, and aggressive state. Logically, when the modern 18th century man was allowed to approximate its original natural state, serious conflict would be a thing of the past. Especially children were considered a prime target for his endeavor, because they would not have been exposed to the degenerative influences of modern society.

Although Rousseau's point of view appeals to many, archaeological findings suggest his vision of aggression and antisocial behavior to be more nuanced. Very clear indications of inter-group violence have been found in Kenya dating back to 10.000 BCE (Lahr et al., 2016). At the edge of a lagoon, at least 10 hunter-gatherers met their violent ends, showing signs of lethal sharp-force (probably by arrows) and blunt-force trauma. Even so, less conclusive evidence of human aggression dates back 24.000 years ago (Trinkaus & Buzhilova, 2012). Descending even further into the abyss of time, the 780.000-year-old remains of Homo Antecessor, which is considered a common ancestor of Homo Sapiens, showed signs of non-ritual cannibalism (Fernández-Jalvo, Díez, Bermúdez de Castro, Carbonell, & Arsuaga, 1996).

Of course, these indications that aggression has been around for the entirety of mankind's existence and their common ancestors' does not automatically render it a good thing or negates the evil and suffering which some aggressive acts entail. This is also a reason why Rousseau's ideas resonate with a lot of people; it provides a feeling that man, especially children, is essentially good and that its surroundings predispose it to aggressive and antisocial acts. Importantly, these archeological findings do indicate that an inclination to resort to aggression under certain circumstances is a very integral part of the human condition. In this thesis I aim to gain a deeper understanding of childhood aggression and its consequences.

Human aggression consists of a very diverse set of behaviors, ranging from explicitly overt expressions, like physical aggression and name calling, to covert

expressions like gossiping, and social exclusion (Vitaro, Brendgen, & Barker, 2006). Because of this heterogeneity a wide variety of aggressive typologies have been proposed, for example: based on the intentions of the perpetrator (Raine et al., 2006), its visibility to bystanders (Crick, Casas, & Mosher, 1997), whether the aggressive act is direct or indirect (Card, Stucky, Sawalani, & Little, 2008), and more. Each of these subtypes try to answer different questions that arise when studying aggression and antisocial behavior, think of sex differences (i.e., direct or indirect aggression) or relating aggression to biology (i.e., reactive or proactive). Although these different definitions answer different questions with varying success, they do emphasize that aggression is a very multifaceted and complex construct to study.

Beside this heterogeneity, it is also crucial to realize that the occurrence of these different types of aggression fluctuate drastically depending on developmental stage (Vitaro et al., 2006). For example, physical aggression has its onset 12 months after birth and peaks at ages 2-4 years after which it decreases substantially (Tremblay, 2014). This desistance from physical aggression can reflect either improved inhibition of aggressive tendencies or the ability to engage in types of aggression which require more cognitively demanding strategies like manipulation or deception. Therefore, more "refined" types of aggression arise later in childhood and adolescence, while other less sophisticated types typically subside. Because children also differ greatly in their environments, experiences, and propensities, there is large individual variety to what extent and in which forms aggressive behaviors are expressed.

Interestingly, it seems beneficial to be aggressive from time to time; with aggression being correlated to positive outcomes, like the assertion of social dominance or social desirability (Little, Rodkin, & Hawley, 2007). This would also explain why human aggression has been around for a long time; under certain circumstances it pays to be aggressive, therefore ensuring its continuity as a prevalent human behaviour. However, despite of this, aggression often seems to come at a considerable cost: an overwhelming majority of the literature indicates that aggression is associated with lower functioning. A wide variety of problems are related to aggression, proximal child characteristics like ADHD symptoms, internalizing problems, (Bartels, Hendriks, Mauri, Krapohl, Whipp, Bolhuis, Conde, Luningham, Fung Ip, et al., 2018), accident proneness (King & Parker, 2008), lower cognitive abilities (Barker et al., 2007), and poor school performance (Vuoksimaa et al., 2020), as well as distal child characteristics, like

low socioeconomic status, exposure to harsh and insensitive parenting (Campbell, Spieker, Vandergrift, Belsky, & Burchinal, 2010; Wakschlag & Keenan, 2001), peer victimization (Barker et al., 2008), exposure to violence, and substance abuse (Ondersma, Delaney-Black, Covington, Nordstrom, & Sokol, 2006). In sum, although aggression is associated with some positive characteristics it seems to be predominantly a risk indicator for various problems.

In addition to the strong relationship between aggression and concurrent worse functioning, there is a general consensus that especially childhood-onset aggression is associated with a considerable risk of future adverse outcomes spanning into adulthood (Fergusson, John Horwood, & Ridder, 2005; Rivenbark et al., 2018). The seminal work of Moffit (1993) first described that childhood-onset antisocial behavior, which includes aggression, confers an important risk marker for life-course-persistent antisocial behavior as compared to adolescent-onset antisocial behavior. Children on this life-course-persistent path are very likely to belong to a tiny fraction of the population which commits a vast majority of crimes, for example, 1% of Sweden's population is responsible for 63% of all violent crime convictions (Falk et al., 2014). Besides severe antisociality, the life-course-persistent antisocial lifestyle is associated with all sorts of other negative long-term outcomes, like physical health problems (e.g., chronic bronchitis, decreased oral health), homelessness, unemployment, having no qualifications, and overall financial problems (Odgers et al., 2008). Notably, twenty-five to sixty percent of adults with a psychiatric disorder had a disruptive behavior disorder in childhood (Kim-Cohen et al., 2003). This clearly shows that childhood aggression can be considered a public health threat and a prominent marker for future problems, warranting considerable efforts in terms of prevention and treatment.

Despite all of this, there is also room for optimism. Fortunately, two thirds of aggressive children appear to grow out of their problems and achieve relatively good outcomes, while the other third follows the life-course persistent antisocial lifestyle with its associated low functioning (Odgers et al., 2008; Wertz, 2019). Although this is a positive message, it also complicates matters: which children with aggression will grow up to flourish and which will flounder? The presence of childhood aggression as a risk indicator should be expanded by additional characteristics that enables us to differentiate between those children that have bad prognoses and those that achieve relatively good functioning later on.

The ACTION (Aggression in Children: unraveling gene-environment interplay to inform Treatment and InterventiON strategies; http://www.action-

euproject.eu/) consortium was founded in 2014 to answer several aspects of this question. ACTION aims to study childhood aggression, focussing on a) clinical epidemiology and current classification and treatment problems; b) genetic epidemiology, including genome-wide association studies and epigenetics; c) gene-environment correlation and interaction; d) biomarkers and metabolomics. This thesis was written within the framework of the ACTION, and shared its aim to gain insight in the etiology, predictors, and outcomes of aggression and antisocial behavior. The first part of this thesis will focus on more conventional prediction of outcomes and continuation of aggression and antisocial behavior on the basis of the following constructs: parental psychopathology (**Chapter 2**), anxiety and depression (**Chapter 3**), and Oppositional Defiant Disorder symptoms (**Chapter 4**). The second part of this thesis focusses on novel biological markers of aggression and consists of a review on the genetics of aggression (**Chapter 5**), and a study on the metabolomics of aggression (**Chapter 6**).

THIS THESIS

Settings

The chapters in this thesis were based on multiple community and clinic-referred samples. In the **second and third chapters**, twins from the Child and Adolescent Twin Study in Sweden (CATSS) were used. The CATSS is a nationwide longitudinal study that targets all twins born in Sweden since July 1992 (Anckarsäter et al., 2011). Parents of twins were contacted by telephone in connection with the twins' ninth birthday (twins born from July 1, 1992 to June 30, 1995 were included at age 12 years). The families were contacted again in connection with the twins' 15th birthday and again at age 18. The follow-up at 15 years includes twins born in 1994 and onward, whereas the follow-up at 18 years includes twins born in 1992 and onward. At both follow-up assessments, at least one parent and both twins were invited to participate.

In the **fourth chapter** on Oppositional Defiant Disorder subtypes, clinic-referred 6- to 18-year-olds from Curium-LUMC, a center for child and adolescent psychiatry in the Netherlands, were studied. The data were collected as an integral part of a clinical protocol between October 2008 and October 2017. The sample consisted of children and adolescents between 5 and 18 years old who were consecutively referred for a diverse range of psychiatric problems. Parent, teachers, youths, and clinicians provided information.

The **fifth chapter** consists of a literature review. Specifically a review of reviews on aggression and a review of genome-wide association studies of aggression and antisocial behavior.

In the **sixth chapter**, again a clinic-referred sample from Curium-LUMC was studied. In contrast with the fourth chapter, these children were enrolled in the Biobank of Curium-LUMC and were aged 6- to 13-years. Children were included between February 2016 and January 2018. This Biobank targets 6- to 12-year-olds and collects, amongst others, morning urine and phenotypical data from the parents and teachers. In the same chapter Dutch twins of the same age from the longitudinal Netherlands Twin Register (NTR) were included (Boomsma et al., 2006; Ligthart et al., 2019).

Outline of the studies described

The second chapter focusses on the association between parental mental disorders and childhood disruptive behavior (DB), which includes aggression, and how both relate to adolescent outcomes. Although longitudinal outcomes of parental psychopathology and child DB have been extensively studied, up until now no study has focused on the combination of both constructs in a longitudinal setting spanning from childhood to adolescence. This chapter aims to investigate whether children with DB and parents with a mental disorder have worse outcomes in adolescence than children with DB and parents without a mental disorder.

The third chapter investigates the co-occurrence between DB, depression, and anxiety in childhood and adolescence. Symptoms of anxiety and depression are often seen in children with DB. However, it is not yet clear whether these associations are related to increased DB in adolescence. Furthermore, it is not clear whether cross-sectional and longitudinal associations are likely to be causal. To investigate whether causal relationships are likely, a discordant co-twin design is used to stringently control for genetic and environmental confounding.

The fourth chapter focusses on Oppositional Defiant Disorder (ODD) symptoms in a clinic-referred setting. ODD symptoms can be divided in irritable and oppositional symptoms and are correlated to different types of problems. Latent Class Analysis is used to investigate whether children and youths can be classified into separate classes on the basis of their parent- and teacher-reported ODD symptoms at referral. The clinical utility of the resulting classes is studied

by comparing these on clinically relevant outcomes at the end of referral, like psychiatric classifications, treatment outcome, and other psychological problems.

The fifth chapter gives a review of the molecular genetic research on aggressive and antisocial behavior, including genetic linkage, candidate gene, and genome-wide association studies. The behavior genetics literature is also covered, as well as the definition and measurement of aggression. This review furthermore includes the first application of a machine-learning-assisted literature search.

The sixth chapter covers the first metabolomics study targeting childhood aggression including both 6- to 12-year-old clinic-referred children and community-residing twins. Two platforms are used one targeting amines and a second targeting organic acids, as well as other biomarkers of larger molecular weight.

The seventh and final chapter will summarize and discuss the results of chapters two to six, provide key conclusions, clinical implications, and suggestions for future research.

CHAPTER 2

CHILDREN WITH EARLY-ONSET DISRUPTIVE BEHAVIOR: PARENTAL MENTAL DISORDERS PREDICT POOR PSYCHOSOCIAL FUNCTIONING IN ADOLESCENCE

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ABSTRACT

Objective: Parental mental disorders (MD) and child early-onset disruptive behavior (DB) are well-established risk factors for poor outcomes in adolescence. However, it is not clear whether parental MD increases risk of future maladjustment among children *who already display DB*.

Methods: Parents of 9-year-old children reported on child DB, while a patient registry was used to determine parental MD. At follow-ups at 15 (N = 6319) and 18 years (N = 3068) information about various problems were collected via registries, parent- and, self-reports.

Results: In the total sample, child DB was related to all outcomes (mean odds ratio [*OR*] = 1.18; range = 1.07-1.51; *p*s < .01), paternal MD to criminality, aggression, truancy, poor school performance, and a cumulative risk index of poor functioning, and maternal MD to peer problems, rule-breaking, and truancy (mean *OR* = 1.67; range = 1.19-2.71; *p*s < .05). In the subsample of children with DB, paternal MD predicted criminality, consequences of antisocial behavior, truancy, poor school performance, and cumulative risk, while maternal MD predicted peer problems (mean *OR* = 1.94; range = 1.30-2.40; *p*s < .05).

Conclusion: This study provides novel evidence that parental MD puts 9-year-olds with DB at risk for negative outcomes in adolescence. Additionally, paternal MD is a better predictor than maternal MD, regardless of child DB at age 9, suggesting that fathers should be given increased attention in future research. Treatment-as-usual of children with DB could be augmented with additional screening and, if necessary, treatment of mental health problems in their parents.

Lay summary: Children with behavioral problems experience on average more negative long-term outcomes than typically developing children, and more often have parents with psychiatric disorders. In this study we showed that Swedish twins who had behavioral problems and parents with a psychiatric disorder were more likely to experience bad outcomes in adolescence, such as criminal behavior and poor academic performance, than twins with behavioral problems only. Interestingly, paternal psychiatric disorders seemed far more predictive of negative outcomes than maternal psychiatric disorders. These findings suggest

that taking parents into account when treating children with behavioral problems would be promising for clinical practice and treatment effectiveness.

INTRODUCTION

It is well documented that children with early-onset disruptive behavior (DB), including oppositional defiant and conduct disorder symptoms, have a high risk for adverse psychosocial outcomes in adolescence and adulthood, such as school dropout, criminality, substance abuse, reduced social skills, and mental health problems (Baker, 2016; Bevilacqua, Hale, Barker, & Viner, 2017; Cleary & Nixon, 2012). There are also clear indications that parental mental disorders (MDs) are involved in the onset (Connell & Goodman, 2002) and maintenance (DeKlyen, Biernbaum, Speltz, & Greenberg, 1998; Odgers et al., 2007) of childhood DB and other negative psychosocial outcomes (Flouri & Ioakeimidi, 2017). Children with DB often cause emotional distress and discord in the family (George, Herman, & Ostrander, 2006), suggesting that childhood DB increase the risk for mental disorder in parents (Panico, Becares, & Webb, 2014). However, it is not well researched if parental MD increases the risk for poor psychosocial outcomes in children *who already display early-onset DB*, mainly because studies did not test interaction effects between child DB and parental MD in their total sample (Wertz et al., 2018) or did not test the prognostic usefulness of parental MD in a subsample of youth with DB (Network & Arsenio, 2004). This lack of research is surprising since parental MDs have been considered to constitute a major risk factor for treatment failure of childhood DB (Shelleby & Kolko, 2015).

We are aware of only one study that has addressed this topic. In a sample of 132 3-year-old preschoolers with DB, Breaux and colleagues (2014) showed that indices of maternal and paternal psychopathology were predictive of parent-ratings of child externalizing and internalizing problems and social skill deficits three years later (Breaux, Harvey, & Lugo-Candelas, 2014). These findings suggest that parental MD in children with DB is a risk factor of poor prognoses. Yet, the Breaux study (Breaux et al., 2014) had some notable limitations that must be addressed in future work on this topic. First, parents were the sole informants and this shared method variance increased the likelihood to reveal significant associations between parental psychopathology and child functioning. Second, parental psychopathology was assessed by means of dimensional measures and the findings, therefore, may not generalize to parents with clinical diagnoses. Third, Breaux et al. used a three-year follow-up interval to study outcomes of preschoolers with a MD. Therefore, it is uncertain if children with DB who have parents with MDs are at an increased risk for outcomes assessed in adolescence.

Both early-onset DB and parental MDs are risk factors for a variety of problems in adolescence. Therefore, we first tested the hypothesis that DB and parental MDs predict poor psychosocial functioning in the total sample of children. Crucially, our main aim was to investigate if 9-year-old children with DB are at a greater risk for maladjustment in middle (age 15) and late adolescence (age 18) when considering maternal and paternal MD status. As such, we hypothesized that prospective relations between parental MD and outcomes in a subsample of children with DB would emerge. In line with prior work (Wertz et al., 2018), we examined if child DB and parental MD predicted each outcome separately as well as a cumulative index of poor functioning.

METHODS

Participants

The Child and Adolescent Twin Study in Sweden (CATSS) is a nation-wide longitudinal study that targets all twins born in Sweden since July 1992 (Anckarsäter et al., 2011). Parents of twins were administered the Autism-Tics, AD/HD and other Comorbidities inventory (A-TAC) by telephone in connection with the twin's ninth birthday (twins born from July 1, 1992 to June 30, 1995 were included at age 12). The families were contacted again in connection with the twins' 15th birthday and again at age 18. The follow-up at 15 years includes twins born in 1994 and onwards, while the follow-up at 18 years includes twins born in 1992 and onwards. At both follow-up assessments, at least one parent and both twins were invited to participate.

At baseline (age 9), parents completed the A-TAC as described below (see Measures) for 8906 twins (born 1992-1999), of which 7105 participated at the first follow-up, and 4492 at the second follow-up. For the purpose of the present investigation participants were selected for whom outcome measures of interest were available at age 15 (first follow-up), resulting in a sample of 6319 children, and for whom outcome measures of interest were available at age 18 (second follow-up), resulting in a sample of 3068 children. A subsequent selection of children with DB resulted in a subsample of 2215 children at the first follow-up, and a subsample of 1190 children at the second follow-up. Descriptive information of all samples can be retrieved from Tables 1 and 2.

Baseline measures at Age 9

Parent-reported disruptive behavior (DB)

DB of the child was assessed using A-TAC, which consists of 96 questions covering common child and adolescent psychiatric disorders, including oppositional defiant disorder (ODD) and conduct disorder (CD; Hansson et al., 2005). The A-TAC ODD and CD subscales consist of five gate questions, each asking a parent about lifetime presence of ODD and CD symptoms in his/her child, respectively. The answering options are coded as 0 ("no"), 0.5 ("yes, to some extent"), or 1 ("yes"). All A-TAC questions can be retrieved from Table S1, available online.

Registered parental mental disorder

The presence of parental mental disorder (MD) was based on information retrieved from the National Patient Register (NPR). The NPR has been registering psychiatric inpatient admissions since 1973 and outpatient consultations since 2001. MDs are classified using the International Classification of Diseases (ICD) versions: eight (1969-1986), nine (1987-1996), or ten (1997-present). A parent was considered to have a MD if at least one of the following diagnoses had been assigned: substance use disorders, disorders with psychotic features, mood disorders, anxiety disorders, eating disorders, nonorganic sleep disorders, personality disorders, mental retardation, developmental disorders, and conduct disorders (specific ICD-codes are presented in Supplement 1, available online). In addition, the diagnosis had to be assigned before the child's tenth birthday. Prevalence of mothers and fathers within various disorder categories are presented in Tables S2 and S3, respectively, and are available online.

Parental education

The educational level of each parent was obtained during the telephone interview at baseline. First, education level was coded into three different categories: 1 (completed primary school or less (≤ 9 years of formal education)), 2 (completed a high school education (10-12 years)) and 3 (university studies or equivalent (≥ 13 years)). Next, education level of both parents were summed, resulting in a score ranging from 2 to 6. If information about the education of one parent was missing, the education level of the other parent with available data was imputed.

Outcome measures at age 15

Information was collected on various outcomes at age 15, relying on self- and parent-reports. *Reactive* (or impulsive) and *proactive* (or planned) *aggression* was assessed through a youth self-report questionnaire (Raine et al., 2006). *Criminality* was assessed with a self-report tool that assessed the frequency of violent and non-violent criminal acts (Ring, 1999). *Conduct problems* of the child were assessed using the Conduct Problems subscale of the Strengths and Difficulties Questionnaire's (SDQ) parent version (Goodman, 1997). *Alcohol misuse* was measured through self-report (Englund, 2016), and refers to frequent alcohol consumption and/or frequent alcohol intoxication. *Emotional problems*, *peer problems*, and *low prosocial behavior* were measured by means of the corresponding scales of the SDQ parent version. In line with prior work (Norén Selinus et al., 2015), self-reported *truancy* of the child was assessed using one item ("Did you ever skip school"). Details of these measures (including example items) can be retrieved from Supplement 2, available online.

Outcome measures at age 18

Information was collected on various outcomes at age 18, thereby, relying on self- and parent-reports, and a registry. *Aggression* was assessed using self-report (Coccaro, Berman, & Kavoussi, 1997) and parent-report questionnaires (Achenbach & Rescorla, 2003). *Criminality* was assessed with the same self-report tool which was used at age 15 to assess the frequency of violent and non-violent criminal acts. *Rule-breaking behavior* was assessed by the parent-reported Adult Behavior Checklist (ABCL; Achenbach & Rescorla, 2003) *Consequences of antisocial behavior* was assessed through a self-report questionnaire that taps social consequences (e.g., reprimands) caused by involvement in antisocial behaviour (Coccaro et al., 1997). The self-report Alcohol Use Disorders Identification Test (AUDIT; Saunders, Aasland, Babor, De La Fuente, & Grant, 1993) was used to assess alcohol consumption, drinking behavior (dependence), and alcohol-related problems. Gender-specific AUDIT cut-offs were used to define *Alcohol Misuse*. *Emotional problems* were assessed by the parent-reported Anxious/Depressed subscale of the aforementioned ABCL. *Truancy* was assessed as described earlier (Outcome Measures at Age 15). *Registered school performance* of the child was assessed using the sum of the final grades of 16 subjects (e.g., math, English) in primary school. The grades were obtained through the National School Registry. Details of these measures can be retrieved from Supplement 2, available online.

Cumulative poor functioning at ages 15 and 18

For each follow-up assessment, a cumulative risk index was computed by summing the times a child was above the cut-off used to define poor outcomes (See Statistical Analyses). The score for this index ranged from "0" (indicating that the child did not experience any of the poor outcomes measured at follow-up) to "10" (indicating that the child experienced all 10 poor outcomes measured at follow-up), for prevalences see Table 1 and 2. At both follow-ups, disruptive behavior subsamples had significantly higher prevalences of maternal mental disorder (follow-up 15 years: 6.4% vs. 5.0%; χ^2 (1, N = 6319) = 5.43, p <.05; 18 years: 5.4% vs. 4.5%; χ^2 (1, N = 3068) = 4.25, p <.05) and paternal mental disorder, (follow-up 15 years: 5.7% vs. 4.0%; χ^2 (1, N = 6319) = 10.20, p <.01; 18 years: 6.2% vs. 3.7%; χ^2 (1, N = 3068) = 4.75, p <.05) compared to non-disruptive behavior samples (follow-up 15 years: n = 4104; 18 years: n = 1878).

Statistical analyses

The continuous outcome variables were substantially skewed, even after data normalization transformations. Therefore, consistent with a large body of research (Côté, Tremblay, Nagin, Zoccolillo, & Vitaro, 2002; Kerr, Tremblay, Pagani, & Vitaro, 1997), dichotomized outcome variables were used. Specifically, echoing prior work (Bechtold, Hipwell, Lewis, Loeber, & Pardini, 2016; Kerr et al., 1997), all outcome measures, except self-reported crime, were dichotomized into high (i.e., the 30% highest scores, 1, which is indicative of low functioning) versus low (i.e., 70% lowest scores, 0). These cutoffs were also used because Swedish norms were unavailable for the majority of the outcome measures. Because high scores on prosocial behavior and grades indicate a high level of functioning, these were dichotomized differently, with a low level of functioning corresponding with the 30% lowest scores (indicated by a score of 1), and high functioning corresponding with the 70% highest scores (score of 0). Table S4, available online, describes with which raw score the dichotomization cutpoints correspond. In line with prior research on the prediction of criminal outcomes (Camp, Skeem, Barchard, Lilienfeld, & Poythress, 2013; Colins, Andershed, & Pardini, 2015), we used dichotomized variables (0 offenses vs. 1 ≤ offenses) to define future violent and non-violent criminality.

Table 1 Descriptive Statistics for Children with Complete Data at Baseline and Age 15 Years

		Total sample (N = 6319)		Disruptive behavior subsample (n = 2215)	
Variable function and child age at assessment	Variable	Mean (*SD*)	Range	Mean (*SD*)	Range
Predictors at 9 years	Disruptive behavior (PR)	0.62 (1.34)	0-21	1.78 (1.75)	0.5-21
	Maternal mental disorder (Reg.) [n(%)]	345 (5.5%)	0-1	141 (6.4%)	0-1
	Paternal mental disorder (Reg.) [n(%)]	290 (4.6%)	0-1	127 (5.7%)	0-1
Covariates at 9 years	Parental education level (PR)	4.80 (1.00)	2-6	4.13 (1.00)	2-6
	Maternal age at childbirth (PR)	31.02 (4.52)	16-50	30.82 (4.47)	16-46
	Paternal age at childbirth (PR)	33.45 (5.74)	17-65	33.33 (5.75)	18-65
	Child's gender male (PR) [n(%)]	2885 (45.7%)	0-1	1120 (50.6%)	0-1
Outcomes at 15 years	Nonviolent crime (SR)	0.57 (1.86)	0-52	0.70 (1.94)	0-37
	Violent crime (SR)	0.45 (1.23)	0-36	0.48 (1.29)	0-20
	Proactive aggression (SR)	0.70 (1.53)	0-24	0.87 (1.74)	0-16
	Reactive aggression (SR)	4.55 (3.50)	0-22	5.21 (3.80)	0-22
	Truancy (SR)	0.52 (0.98)	0-4	0.65 (1.12)	0-4
	Frequent alcohol consumption (SR) [n(%)]	1046 (16.6%)	0-1	387 (17.5%)	0-1
	Frequent alcohol intoxication (SR)	1.09 (1.53)	0-5	1.16 (1.58)	0-5
	Conduct problems (PR)	1.73 (1.43)	0-10	2.02 (1.56)	0-10
	Emotional problems (PR)	2.85 (2.23)	0-10	2.96 (2.33)	0-10
	Peer problems (PR)	1.76 (1.57)	0-9	1.96 (1.65)	0-9
	Prosocial behavior (PR)	7.06 (1.69)	0-10	6.89 (1.79)	0-10

Table 1 Continued.

		Total sample (N = 6319)		Disruptive behavior subsample (n = 2215)	
Variable function and child age at assessment	Variable	Mean (*SD*)	Range	Mean (*SD*)	Range
	Cumulative risk index [n(%)]		0-10		0-10
	0 poor outcomes	1391 (22.0)		380 (17.2)	
	1 poor outcome	1488 (23.5)		452 (20.4)	
	2 poor outcomes	1142 (18.1)		386 (17.4)	
	3 poor outcomes	796 (12.6)		304 (13.7)	
	4 ≤ poor outcomes	1502 (23.8)		693 (31.3)	

Note. PR = parent-reported; Reg. = registry; SR = self-reported.

Table 2 Descriptive Statistics for Children with Complete Data at Baseline and Age 18 Years

		Total sample (N = 3068)		Disruptive behavior sample (n = 1190)	
Variable function and child age at assessment	Variable	Mean (SD)	Range	Mean (SD)	Range
Predictors at 9 years					
	Disruptive behavior (PR)	0.72 (1.43)	0-13	1.85 (1.79)	0.5-13
	Maternal mental disorder (Reg.) [n(%)] [a]	159 (5.2%)	0-1	64 (5.4%)	0-1
	Paternal mental disorder (Reg.) [n(%)] [a]	134 (4.4%)	0-1	74 (6.2%)	0-1
Covariates at 9 years					
	Parental education level (PR)	4.80 (1.01)	2-6	4.78 (1.01)	2-6
	Maternal age at childbirth (PR)	30.68 (4.59)	19-56	30.46 (4.67)	16-46
	Paternal age at childbirth (PR)	33.05 (5.62)	16-46	32.77 (5.59)	19-56
	Child's gender male (PR) [n(%)]	1331 (43.4%)	0-1	584 (49.1%)	0-1
Outcomes at 18 years					
	Nonviolent crime (SR)	1.57 (3.18)	0-37	2.02 (3.76)	0-37
	Violent crime (SR)	0.95 (1.97)	0-20	1.27 (2.32)	0-20
	Aggression (SR)	6.62 (5.07)	0-25	7.67 (5.39)	0-25
	Consequences of antisocial behavior (SR)	0.51 (1.32)	0-14	0.71 (1.64)	0-14
	Truancy (SR)	1.45 (1.48)	0-4	1.68 (1.53)	0-4
	Alcohol misuse (SR)	4.74 (4.16)	0-34	5.12 (4.51)	0-34
	Rule-breaking behavior (PR)	13.68 (1.73)	6-34	14.02 (2.19)	6-34
	Aggression (PR)	18.60 (3.01)	8-40	19.40 (3.51)	8-34

Table 2 Continued.

		Total sample (N = 3068)		Disruptive behavior sample (n = 1190)	
Variable function and child age at assessment	Variable	Mean (SD)	Range	Mean (SD)	Range
	Emotional problems (PR)	16.01 (3.21)	7-39	16.57 (3.65)	7-39
	School performance (Reg.)	236.11 (55.32)	0-320	228.15 (56.91)	0-320
	Cumulative risk index [n(%)]		0-10		0-10
	0 poor outcomes	423 (13.8)		105 (8.8)	
	1 poor outcome	622 (20.3)		166 (13.9)	
	2 poor outcomes	562 (18.3)		207 (17.4)	
	3 poor outcomes	438 (14.3)		185 (15.5)	
	4 ≤ poor outcomes	1023 (33.3)		527 (44.3)	

Note. PR = parent-reported; Reg. = registry; SR = self-reported.

Generalized linear mixed models (GLMMs) for logistic regression, unless otherwise specified, were conducted, using a binomial distribution with a logit link. GLMMs combine both linear mixed models and generalized linear models, and enable the introduction of random effects. The introduction of a random effect (i.e., twins nested within families) is needed in this study to correct for dependency of observations (i.e., one parent reporting on the behavior of two twins). In this study, a robust estimator (Huber/White/sandwich estimation) was used to estimate the covariance. This estimator corrects for the dependence of observations and other departures from normality, like under- and overdispersion. Wald Chi-square tests were used to test the fixed effects. For the fixed effects corresponding odds ratio's (*OR*s) and 95% confidence intervals (CIs) were computed and reported.

Poisson or negative binomial models can accommodate non-normality without having to resort to dichotomizing outcomes. However, the appropriateness of these models varied across outcome measures. Therefore, logistic regression models also helped to test all outcome measures uniformly. Yet, when appropriate, we ran negative binomial and/or Poisson regressions analyses and found that the pattern of the findings were substantially similar to the results of the logistic regression analyses.

Specifically, using GLMM for logistic regression, four models were tested. The first model was a crude effects model consisting of child DB (continuous), paternal MD (dichotomous), or maternal MD (dichotomous) together with four theoretically relevant control variables: parental education level (Evans, 2004), maternal age at childbirth (Chang et al., 2014), paternal age at childbirth (Janecka et al.), and gender of the child (Zahn-Waxler, Shirtcliff, & Marceau, 2008). In the second model, child DB, paternal MD, and maternal MD were included simultaneously in an adjusted model, together with the aforementioned control variables. These latter two models were run in the total sample to assess the influence of parental MD and child DB in middle and late adolescence. However, to test if parental MD is a risk factor of future maladjustment among children who already display DB, both models (being referred to as Models 3 and 4, respectively) were repeated in a subsample of children who displayed at least some DB (i.e., a raw DB score of 0.5 or higher). Of note, GLMMs were conducted separately for individuals with outcome data at age 15 years (N = 6319; DB sample n = 2215, 35.1% of total sample at age 15) and for individuals with outcome data at age 18 years (N = 3068; elevated DB sample n = 1190, 38.8% of

total sample at age 18) for two reasons. First, there was a relatively low number of children for whom data were available for both follow-up assessments (n = 1696) and only 126 of these children had a parent with a MD. Therefore, it was not tenable to run the GLMMs. Second, different outcome measures were used across the follow-up assessments, limiting the possibility to test stability and change from age 15 to age 18 without introducing measurement bias. When using CD and ODD symptoms as separate predictors instead of combining CD and ODD in an omnibus variable (i.e., DB), results remained substantially similar. Details are available upon request from the first author. The analyses were performed in SPSS version 23, using the IBM SPSS MIXED function. We used $p < .05$ as an indicator of statistical significance. Sequential Bonferroni was used to adjust for multiple comparisons. Two-tailed tests were used in all analyses.

Attrition

At age 15, there were 1680 out of 7999 children who were not included in the analyses due to some degree of missing data. These children did not differ from children without missing data at age 15 years in terms of maternal and paternal age at childbirth. However, children with (versus without) missing data were more often boys (45.3% vs. 60.2%, $p <.001$) and had parents with lower levels of education ($p <.001$). At age 18 years 3305 out of 6373 children were excluded due to missing data. Significant differences emerged between children with and without missing data in terms of age of the mother at birth ($M = 30.4$, $SD = 4.72$ vs. 30.7 years, $SD = 4.56$, $p = .023$, $d = 0.07$), percentage of boys (48.3% vs. 60.2%, $p < .001$), and parental education level ($p < .001$), but not in terms of paternal age at childbirth.

RESULTS

Outcomes at age 15

Total sample

First, crude models were run for the predictors, child DB, paternal MD, and maternal MD separately (Table 3: Model 1). Child DB was significantly positively associated with all outcome measures at age 15 years ($p < .01$). Paternal MD was significantly positively associated with self-reported violent crime, nonviolent crime, reactive aggression, truancy, and the cumulative risk index. Maternal

MD was significantly positively related to parent-reported peer problems and self-reported truancy.

Next, adjusted models, in which all three predictors were included simultaneously (Table 3: Model 2) showed that child DB remained significantly associated with all outcomes. Paternal MD remained significantly positively related to violent crime, nonviolent crime, truancy, and the cumulative risk index, though the prospective association with reactive aggression was no longer statistically significant. Maternal MD remained positively associated to peer problems, but not to truancy at age 15.

Subsample of children with disruptive behavior

Paternal MD was not predictive of any of the outcomes, while maternal MD was positively associated to peer problem in both the crude model (*OR* = 1.64; 95% CI = 1.13; 2.38) and the adjusted model (*OR* = 1.62; 95% CI = 1.12; 2.34). For details see: Table S5, available online.

Outcomes at Age 18

Total sample

Child DB was associated with all outcomes at age 18 years (p <.01) in the crude model (Table 4) Paternal MD was positively associated with self-reported nonviolent crime, aggression, consequences of antisocial behavior, truancy, registered school performance, and the cumulative risk index of poor functioning. Maternal MD was significantly positively related to only one outcome, being parent-reported rule-breaking behavior. These prospective relations between paternal MD and future outcomes remained significant in the adjusted models (Model 2), though maternal MD was no longer related to rule-breaking behavior in Model 2.

Subsample of children with disruptive behavior

Paternal MD was prospectively related to self-reported nonviolent crime, consequences of antisocial behavior, truancy, registered poor school performance, and the cumulative risk index, both in the crude (Model 3) and adjusted models (Model 4) (Table 5). Maternal MD was not predictive of any of the outcomes in Models 3 and 4. It could also be the case that the "what-question" (i.e., Is there an internalizing or externalizing MD present in the parents?) might be more important than the "who-question" (i.e., Does the mother or the father have a MD?), especially since a higher prevalence of externalizing disorders in fathers than in mothers might explain why paternal MD was most often related to the reported antisocial outcomes in the subsample of children with DB at age 18. We addressed this issue in Supplement 3, and in Tables S6 and S7, available online. In short, the outcomes of these analyses suggest that the "what-" and "who-question" are equally important.

Table 3 Odds ratios and 95% Confidence Intervals of the Fixed Part of the Crude and Adjusted Main Effects Models at Follow-up 15 Years

		Criminality		Aggression		Problems						
		Violent (SR)	Nonviolent (SR)	Proactive (SR)	Reactive (SR)	Conduct (PR)	Emotional (PR)	Peer (PR)	Alcohol (SR)	Truancy (SR)	Low prosocial (PR)	Cumulative risk[a]
Predictor	Model	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)
Child DB	1	**1.19****	**1.18****	**1.15****	**1.24****	**1.23****	**1.11****	**1.17****	**1.07****	**1.14****	**1.08****	**1.10****
		[1.13, 1.25]	[1.07, 1.17]	[1.10, 1.21]	[1.18, 1.30]	[1.17, 1.29]	[1.06, 1.17]	[1.12, 1.22]	[1.03, 1.12]	[1.08, 1.19]	[1.03, 1.13]	[1.08, 1.11]
	2	**1.19****	**1.12****	**1.15****	**1.24****	**1.23****	**1.11****	**1.17****	**1.07****	**1.13****	**1.08****	**1.09****
		[1.13, 1.25]	[1.06, 1.17]	[1.10, 1.20]	[1.18, 1.30]	[1.17, 1.29]	[1.06, 1.16]	[1.12, 1.22]	[1.03, 1.12]	[1.08, 1.19]	[1.03, 1.13]	[1.08, 1.11]
Paternal MD	1	**1.65****	**1.40***	1.18	**1.38***	1.28	1.16	1.10	1.31	**1.67****	0.96	**1.19****
		[1.22, 2.23]	[1.04, 1.89]	[0.83, 1.67]	[1.02, 1.87]	[0.96, 1.70]	[0.85, 1.57]	[0.83, 1.45]	[0.96, 1.79]	[1.26, 2.21]	[0.70, 1.32]	[1.06, 1.34]
	2	**1.59****	**1.36***	1.12	1.29	1.19	1.11	1.02	1.29	**1.58****	0.93	**1.17***
		[1.16, 2.17]	[1.00, 1.84]	[0.78, 1.60]	[0.94, 1.78]	[0.89, 1.61]	[0.82, 1.50]	[0.77, 1.34]	[0.94, 1.77]	[1.19, 2.11]	[0.67, 1.29]	[1.03, 1.30]
Maternal MD	1	0.84	0.98	1.09	1.10	1.09	1.16	**1.41****	0.97	**1.33***	1.05	1.06
		[0.60, 1.19]	[0.75, 1.29]	[0.78, 1.53]	[0.84, 1.44]	[0.84, 1.42]	[0.87, 1.56]	[1.10, 1.80]	[0.72, 1.31]	[1.01, 1.74]	[0.78, 1.41]	[0.95, 1.19]
	2	0.77	0.93	1.04	1.02	1.02	1.12	**1.36***	0.94	1.25	1.03	1.02
		[0.54, 1.09]	[0.71, 1.22]	[0.74, 1.46]	[0.77, 1.35]	[0.78, 1.33]	[0.83, 1.51]	[1.06, 1.73]	[0.70, 1.26]	[0.95, 1.64]	[0.78, 1.38]	[0.92, 1.14]

Note. N = 6319. Model 1 = crude model including the control variables: gender child, parental education level, maternal age at birth, and paternal age at birth; Model 2 = adjusted model including child DB, paternal MD, maternal MD, and the same control variables as model 1. CI = confidence interval; DB = disruptive behavior; MD = mental disorder; *OR* = odd ratio; PR = parent-reported; SR = self-reported.

[a] Cumulative risk was predicted through negative binomial regressions.

**$p < .01$ *$p < .05$.

Table 4 Odds ratios and 95% Confidence Intervals of the Fixed Part of the Crude and Adjusted Main Effects Models at Follow-up at 18 Years

		Criminality		Aggression		Problems						
		Violent (SR)	Nonviolent (SR)	Aggression (SR)	Aggression (PR)	Conseq. of antisocial behavior (SR)	Rule-break. (PR)	Emotional (PR)	Alcohol (SR)	Truancy (SR)	Poor school performance (Reg.)	Cumulative risk[a]
Predictor	Model	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)
Child DB	1	**1.22****	**1.13****	**1.22****	**1.51****	**1.14****	**1.33****	**1.30****	**1.08***	**1.19****	**1.16****	**1.12****
		[1.15, 1.30]	[1.06, 1.21]	[1.15, 1.29]	[1.41, 1.62]	[1.08, 1.21]	[1.25, 1.42]	[1.23, 1.39]	[1.01, 1.14]	[1.12, 1.27]	[1.09, 1.24]	[1.11, 1.14]
	2	**1.22****	**1.13****	**1.22****	**1.51****	**1.14****	**1.33****	**1.30****	**1.07****	**1.18****	**1.16****	**1.12****
		[1.15, 1.30]	[1.07, 1.21]	[1.14, 1.29]	[1.40, 1.62]	[1.07, 1.21]	[1.24, 1.42]	[1.22, 1.39]	[1.01, 1.14]	[1.11, 1.26]	[1.08, 1.24]	[1.10, 1.14]
Paternal MD	1	1.44	**1.87****	**1.72****	1.45	**1.85****	1.17	1.01	1.38	**2.71****	**1.89****	**1.34****
		[0.96, 2.15]	[1.23, 2.83]	[1.14, 2.59]	[0.94, 2.24]	[1.24, 2.77]	[0.68, 2.03]	[0.70, 1.73]	[0.88, 2.16]	[1.72, 4.26]	[1.20, 2.99]	[1.16, 1.55]
	2	1.38	**1.90****	**1.68***	1.33	**1.77****	1.01	0.99	1.34	**2.60****	**1.76***	**1.29****
		[0.92, 2.07]	[1.25, 2.89]	[1.10, 2.58]	[0.82, 2.16]	[1.18, 2.66]	[0.57, 1.79]	[0.61, 1.59]	[0.86, 2.11]	[1.66, 4.07]	[1.11, 2.79]	[1.12, 1.49]
Maternal MD	1	1.12	0.83	0.99	1.20	1.25	**1.66***	1.44	1.14	1.32	1.44	1.13
		[0.75, 1.68]	[0.54, 1.29]	[0.63, 1.55]	[0.83, 1.73]	[0.80, 1.95]	[1.04, 2.64]	[0.97, 2.13]	[0.71, 1.84]	[0.80, 2.19]	[0.86, 2.41]	[0.97, 1.31]
	2	0.98	0.72	0.84	0.96	1.09	1.45	1.28	1.07	1.09	1.26	1.03
		[0.65, 1.48]	[0.46, 1.13]	[0.52, 1.34]	[0.64, 1.44]	[0.70, 1.71]	[0.89, 2.37]	[0.85, 1.93]	[0.66, 1.73]	[0.66, 1.80]	[0.75, 2.12]	[0.89, 1.20]

Note. N = 3068. Model 1 = crude model including the control variables: gender child, parental education level, maternal age at birth, and paternal age at birth; Model 2 = adjusted model including child DB, paternal MD, maternal MD, and the same control variables as model 1. CI = confidence interval; Conseq. = consequences; DB = disruptive behavior; MD = mental disorder; *OR* = odd ratio; PR = parent-reported; Reg. = registry; Rule-break. = rule-breaking; SR = self-reported.

[a] Cumulative risk was predicted through negative binomial regressions.

**$p < .01$ *$p < .05$.

Table 5 Odds ratios and 95% Confidence Intervals of the Fixed Part of the Crude and Adjusted Main Effects Models at Follow-up 18 years in a Subsample of Children with Disruptive Behavior

		Criminality		Aggression		Problems						
		Violent (SR)	Nonviolent (SR)	Aggression (SR)	Aggression (PR)	Conseq. of antisocial behavior (SR)	Rule-break. (PR)	Emotional (PR)	Alcohol (SR)	Truancy (SR)	Poor school performance (Reg.)	Cumulative risk[a]
Predictor	Model	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)	*OR* (95%CI)
Paternal MD	3	1.59 [0.93, 2.71]	**2.03*** [1.07, 3.84]	1.51 [0.92, 2.47]	1.13 [0.67, 1.90]	**2.24**** [1.30, 3.87]	1.15 [0.59, 2.25]	0.90 [0.49, 1.64]	1.75 [0.96, 3.21]	**2.40**** [1.33, 4.33]	**2.02*** [1.11, 3.67]	**1.30**** [1.12, 1.51]
	4	1.50 [0.87, 2.58]	**2.05*** [1.08, 3.90]	1.53 [0.91, 2.56]	1.11 [0.65, 1.89]	**2.13**** [1.22, 3.72]	1.12 [0.58, 2.17]	0.82 [0.45, 1.51]	1.70 [0.93, 3.11]	**2.22*** [1.21, 4.08]	**2.01*** [1.09, 3.72]	**1.28**** [1.09, 1.49]
Maternal MD	3	1.55 [0.91, 2.65]	1.04 [0.55, 1.98]	0.98 [0.54, 1.78]	1.11 [0.71, 1.75]	1.57 [0.85, 2.90]	1.24 [0.69, 2.24]	1.63 [0.96, 2.76]	1.33 [0.70, 2.53]	1.87 [0.96, 3.64]	1.13 [0.56, 2.31]	1.18 [1.00, 1.39]
	4	1.46 [0.85, 2.53]	0.94 [0.49, 1.80]	0.92 [0.49, 1.71]	1.10 [0.69, 1.74]	1.40 [0.75, 2.62]	1.22 [0.68, 2.19]	1.68 [0.97, 2.89]	1.23 [0.65, 2.33]	1.67 [0.83, 3.33]	1.03 [0.49, 2.16]	1.14 [0.96, 1.36]

Note. n = 1190. Model 1 = crude model including the control variables: gender child, parental education level, maternal age at birth, and paternal age at birth; Model 2 = adjusted model including paternal MD, maternal MD, and the same control variables as model 1. CI = confidence interval; Conseq. = consequences; MD = mental disorder; *OR* = odd ratio; PR = parent-reported; Reg. = registry; Rule-break. = rule-breaking; SR = self-reported.

[a] Cumulative risk was predicted through negative binomial regressions.

$^{**}p < .01$ $^{*}p < .05$.

DISCUSSION

The main aim of this study was to test whether 9-year-old children with disruptive behavior (DB) who have a parent with a mental disorder (MD) display increased maladjustment in adolescence compared to children with DB whose parents were without a MD. Overall, the current findings partially support this hypothesis. Specifically, maternal MD was a risk factor for peer problems at age 15, a finding that adds to prior work showing that maternal MD, but not paternal MD, was predictive of reduced social skills in preschoolers with DB (Breaux et al., 2014). This difference could be explained by gender-specific parenting behavior, with fathers tending to focus on promoting their child's exploratory behavior and rough-and-tumble play, while mothers are more focused on social-affective behaviour (Feldman, 2012). Furthermore, paternal MD was a risk factor for children with DB to show higher levels of nonviolent crime and truancy, to experience more negative consequences (e.g., school suspensions) of antisocial behaviour, to perform worse at school at age 18, and to experience multiple poor outcomes (cumulative risk index). Importantly, nonviolent crime in adolescence has been demonstrated a risk factor for reoffending (Piquero, Jennings, & Barnes, 2012), while truancy in adolescence is also a risk factor for later crime (Loeber & Farrington, 2000), mental health problems (Dembo et al., 2012), and academic underachievement (Bridgeland, DiIulio Jr, & Morison, 2006). In addition, poor school performance in adolescence increases the risk for later health problems (Lleras-Muney, 2005), reliance on government assistance, illicit substance use, arrest, and being fired (Lansford, Dodge, Pettit, & Bates). Therefore, paternal MD may not only jeopardize the transition from childhood to adolescence (this study), but also a successful transition from adolescence to adulthood, a possibility that is in need of empirical evaluation.

Despite the aforementioned findings in partial support of our hypothesis, it cannot be disregarded that in children with DB, parental MD was more often unrelated to the majority of outcomes at ages 15 and 18, including the ones that most clearly affect society as a whole, being aggression and violent criminality. Intriguingly, in the total sample, main effects were revealed for paternal MD as predictor of these latter two indices of severe antisocial behaviour, suggesting that parental MD has more prognostic value if one does not specifically focus on 9-year-old children who already display DB. Although replication is warranted, we should note that these findings dovetail with prior work in criminology

showing that well-established risk factors of first-time offending are less useful to predict reoffending (Cottle, Lee, & Heilbrun, 2001). Also, it should be noted that most prospective associations between parental MD and poor outcomes, including the cumulative risk index, in children with DB were not significant at age 15, but were at age 18. Possibly, children at age 18 become increasingly independent from their parents in various areas of life. This developmental transition increases differences between individuals (which may be reflected in the larger standard deviation in outcome measures at age 18 than at age 15, see Tables 1 and 2), and, therefore, also the likelihood to find significant associations in late as compared to middle adolescence.

Notwithstanding that this study's main focus was on the effects of parental MD among children with DB, our findings also contribute substantially to the literature on outcomes of children of parents with a MD. Crucially, fathers have been understudied compared to mothers in studies linking parental MD and child maladjustment (Breaux et al., 2014). The current investigation provides evidence that paternal MD is predictive of various outcomes at age 15 and 18, suggesting that mothers are not the only parent of interest when examining the prognostic value of parental MD. In fact, maternal MD merely predicted increased peer problems at age 15 years, a finding that is surprising in the light of evidence that maternal MD is a risk factor for a variety of poor psychosocial outcomes in offspring (S. H. Goodman et al., 2011). Nevertheless, there is some prior evidence to suggest that paternal MD is a stronger risk factor for emotional and behavioral problems in older children and adolescents compared to younger children, while maternal MD has a larger impact on younger children (Connell & Goodman, 2002). Also, prior work suggests that paternal MDs are more strongly associated with child behavioral problems than child internalizing problems (Narayanan & Nærde, 2016; Trautmann-Villalba, Gschwendt, Schmidt, & Laucht, 2006). This is consistent with our results showing that paternal MD in the total sample was associated with increased rates of antisocial behavior (e.g., aggression, crime, and truancy) but not once to emotional problems. Furthermore, our findings are also consistent with evidence that fathers have a larger effect on the development of delinquency in their offspring than mothers (Hoeve et al., 2009), and suggest that the impact of paternal MD extends well into middle and late adolescence.

This study also contributes to the broader literature on early-onset DB, generally showing that conduct problems in children younger than 10 (Fergusson et al., 2005; Wertz et al., 2018) are significant predictors of antisocial behavior,

psychopathology, and school drop-out in adolescence and adulthood. The current study confirmed these findings, with early-onset DB predicting worse outcomes on all measures at age 15 and 18 years, even after controlling for paternal and maternal MD. Importantly, parent-reported DB was not only predictive of parent-reported outcomes (e.g., conduct problems, emotional problems and low prosocial behaviour) at both follow-up intervals but also of negative outcomes that were based on youth self-report (e.g., proactive aggression, violent criminality, and alcohol use) and information from registries (i.e., poor school performance), showing that the prognostic usefulness of child DB was not solely caused by shared-method variance. To bolster what is known about parental MD as predictor of poor outcomes in children with DB, we focused on the presence of DB. Consequently, the vast majority of children with DB in our subsample likely does not display severe DB and does not meet criteria to warrant a formal ODD or CD diagnosis. Future research is warranted to see if our findings can be replicated in children with severe DB or with formal disruptive behaviour disorder diagnoses.

This study has several strengths, including its longitudinal design and the use of a well-described sample, the availability of multiple information sources, and the variety of outcomes across various domains which were assessed at two different follow-ups (Anckarsäter et al., 2011). Evidently, the findings should be interpreted in the context of various limitations. First, the present study used lifetime prevalence of parental MD and child DB, which implies that it is uncertain whether parental MD occurred before, at the same time, or after the onset of child DB. This might have hampered the likelihood to find significant main effects of parental MDs in children with DB, especially since there is some evidence that the timing of exposure of parental MD matters when studying outcomes in children (Kim-Cohen, Moffitt, Taylor, Pawlby, & Caspi, 2005). Second, specific mental disorders in parents have been associated with different child outcomes (Connell & Goodman, 2002; Ramchandani & Psychogiou), and there is some evidence to suggest that relations between specific parental mental disorders with future child outcomes is influenced by the gender of the parent (Breaux et al., 2014). Notwithstanding that our exploratory post hoc analyses (with the broad disorder categories externalizing and internalizing disorder as predictors) support these findings to some extent, prevalence issues hampered us to test the effect of specific MDs (e.g., substance use disorder, major depressive disorder) in parents in general, or in mothers and fathers separately.

Also, regardless of the large number of children and parents included in this study, the number of children with DB who had parents with a MD was rather low. Thus, prevalence issues may also have hampered the likelihood to find significant effects of parental MDs in children with DB, whereas interactions between maternal and paternal MD were not possible to study because the number of dyads in which both parents were diagnosed with a MD ranged from 14 (subsample with DB at age 18) to 39 (total sample at age 15). Third, even though statistical testing for continuous outcomes was not an option (see Methods section), it can be argued that dichotomizing our outcome variables may have decreased the power to reveal significant prospective relations. However, the use of logistic regression and dichotomization of outcome variables has benefits as well (Farrington & Loeber, 2000), and enhances comparison with prior work that used distribution-based cut-offs (Bechtold et al., 2016; Côté et al., 2002; Kerr et al., 1997). Fourth, officially recorded parental mental disorders are most likely an underestimation of the true extent of parental mental disorders, suggesting that future research also need to rely on diagnostic interviews with parents.

This study supports the importance of treating parental MD, in both children with and without early-onset DB. Although screening for parental MD in the general adult population would be time consuming and costly, relatively easy gains could be made in children that are already in treatment for DB. Treatment-as-usual of the child could be augmented with additional screening and, if necessary, treatment of mental health problems in its parents improvement of parental mental health has been associated with better child outcomes (Wesseldijk et al., 2018). Furthermore, the current study clearly underscores the urgency to screen for early-onset child DB in the community, since an accurate identification of these children may eventually increase the likelihood that intervention programs might mitigate or even prevent a developmental pathway towards disruptive behavior disorders and maladjustment in adolescence and adulthood (Kyranides, Fanti, Katsimicha, & Georgiou, 2018).

In conclusion, the results strongly suggest that fathers must be considered when studying prospective associations between parental MD and offspring psychosocial functioning. Crucially, we provided novel evidence that children with early-onset DB who had a parent with (versus without) a registered MD were at an increased risk of poor psychosocial functioning in middle and late adolescence.

CHAPTER 3

ASSOCIATIONS BETWEEN ANXIETY, DEPRESSION, AND DISRUPTIVE BEHAVIOR SPANNING CHILDHOOD AND ADOLESCENCE

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IN PREPARATION

ABSTRACT

Objective: To investigate whether childhood anxiety and depression are predictive of DB in adolescence, above and beyond childhood DB, and whether these prospective relations are subject to environmental and genetic confounding.

Methods: Parents of 9-year-old twins reported on depression, anxiety, and DB (N = 19,347). At follow-ups at ages 15 (n = 3,852) and 18 (n = 786) years, information about DB was collected via parent- and self-reports. The relationships between anxiety, depression, and DB at baseline, as well as DB at both follow-ups were quantified by negative binomial regressions. Next, to control for genetic and environmental confounding, co-twin control analyses were performed in monozygotic (McLaughlin et al.) and dizygotic (DZ) twin pairs discordant for anxiety (MZpairs = 91, DZpairs = 287) and depressive disorders (MZpairs = 104, DZpairs = 330), as well as affected twin cases and unrelated, unaffected twin controls ($n^{anxiety}$ = 908, $n^{depression}$ = 1018).

Results: Anxiety (Incidence Rate Ratio (IRR) = 1.07; 95% CI: 1.07, 1.08) and depression (IRR = 1.22; 95% CI: 1.21, 1.23) showed cross-sectional co-occurrence in childhood (p's < .001). Longitudinally, childhood anxiety and depression predicted adolescent DB, but these associations became non-significant when controlling for childhood DB. Cross-sectional co-twin control analyses in childhood showed moderate relations between anxiety, depression, and DB in unrelated cases and controls ($d^{anxiety}$ = 0.59, $d^{depression}$ = 0.65), which were attenuated to small effect sizes in DZ and MZ twin pairs ($d^{anxiety}$ = 0.28, 0.26; $d^{depression}$ = 0.43, 0.30). Notably, in MZ twins, when controlling for comorbid depression, anxiety lost its association with DB, while depression retained its association with DB when controlling for comorbid anxiety.

Conclusion: In childhood, depression has a more robust association with DB than anxiety. However, neither childhood anxiety nor depression predict adolescent DB, suggesting fleeting and short-term relations at most.

Children with early-onset disruptive behavior (DB), like oppositional defiant and conduct disorder symptoms, often experience comorbid internalizing problems such as anxiety and depression (Bartels, Hendriks, Mauri, Krapohl, Whipp, Bolhuis, Conde, Luningham, Fung Ip, et al., 2018; Marshall, Arnold, Rolon-Arroyo, & Griffith, 2015). For example, children with conduct problems meet criteria for an anxiety disorder in 22-33% of community samples and 60%-75% of clinical samples (Granic, 2014). There are several explanations for this overlap between DB, anxiety, and depression. Some hypothesize depression to be a consequence of DB, because DB predisposes an individual to depression-invoking negative experiences, like peer rejection or academic failure (Patterson & Capaldi, 1990; Wolff & Ollendick, 2006). Conversely, the "acting out" hypothesis suggests that depression can also be expressed as DB, with depressive symptoms like irritability or hopelessness increasing the chances of engaging in DB (Kasen et al., 2001). The relation between anxiety and DB is more complicated, with anxiety being attributed with both increased and decreased DB (Cunningham & Ollendick, 2010; Granic, 2014; Klingzell et al., 2016; Raine, 2013). Anxiety is hypothesized to inhibit DB through increased sensitivity to social punishments and rewards (Cunningham & Ollendick, 2010), while a lack of anxiety or fear is associated with increased involvement in DB (Klingzell et al., 2016; Raine, 2013). High levels of anxiety are also believed to escalate into DB in case of defensive reactive aggression, and more indirectly through ego depletion (Granic, 2014). Although differentiation of internalizing problems in anxiety and depression could potentially provide valuable insights into its co-morbidity with DB, literature on the long-term influence of anxiety and depression on the development of DB from childhood into late adolescence is sparse and inconclusive.

These diverse roles of anxiety and depression on DB in youths are mostly based on longitudinal research focussing on either childhood (Fanti et al., 2018), or adolescence (Fanti, Colins, & Andershed, 2019; Fontaine et al., 2019), while not much is known about how anxiety and depression relate to the development of DB from childhood into late adolescence. Specifically, some studies report predictive value of anxiety and depression on later DB in the community (Jolliffe et al., 2019 Loeber, Ahonen & Palacios, 2019; Reinke & Ostrander, 2008). There are also indications of considerable cross-sectional comorbidity, but a lack of (Leadbeater, Thompson, & Gruppuso, 2012), or reverse longitudinal relationships (Burke, Loeber, Lahey, & Rathouz, 2005). Even so, other research suggests a cascade of

increasing DB and increasing anxiety and depression (Thompson, Leadbeater, & Ames, 2015). Although there are a number of studies focusing on anxiety and depression as outcomes of DB, these do not include anxiety and depression as predictors at baseline (for an overview: Reising, Ttofi, Farrington, & Piquero, 2019). All in all, the available literature on DB, anxiety, and depression covering both childhood and adolescence is both sparse and conflicting.

One reason for these inconsistencies in literature could be that the co-occurrence between anxiety, depression, and DB is not necessarily causal, with confounding by environmental and genetic factors explaining some of the inconsistent relationships. For instance, although increased neighbourhood disadvantage is correlated with increased DB, only specific acts of DB (i.e., nonviolent criminal acts) are actually influenced by neighbourhood disadvantage (Burt, Klump, Gorman-Smith, & Neiderhiser, 2016). Additionally, putative causal relations between anxiety, depression, and DB could also be confounded by genetic pleiotropy, since different phenotypes (e.g., anxiety, depression, and DB) are influenced by the same genes (Ligthart & Boomsma, 2012). Put differently, differences between groups of genetically unrelated individuals who also differ in environments could be substantially inflated. This raises the question whether intercorrelations among anxiety, depression, and DB are actually indicative of causal relationships or epiphenomenal.

Fortunately, the co-twin control method enables researchers to account for a greater extent for environmental and genetic confounding compared to more conventional case-control designs, enabling researchers to give a more robust statement on the potential causality of associations (Lichtenstein et al., 2002 Svartengren, Svedberg & Pedersen, 2002). This is because both monozygotic and dizygotic twin pairs share the same prenatal and rearing environment. In addition, dizygotic twins share 50% of their genetic makeup, while monozygotic twins are genetically (nearly) identical. Because of these genetic and environmental similarities, monozygotic and dizygotic twin pairs who are discordant on one trait allow for a stringent within-pair comparison of another trait that is hypothesized to be causally associated with the discordance. In other words, in a discordant twin pair the unaffected co-twin can function as a well-matched control for the affected twin. If there is a causal relation, we expect twins affected by anxiety or depression to exhibit higher levels of DB than their non-affected counterpart. Therefore, selection of twin pairs that are discordant on depression or anxiety (e.g., one twin has an anxiety disorder, the other co-twin has not; one twin has a

depressive disorder, the other co-twin has not) has great value to study putative causal relationships between, anxiety, depression, and DB. To our knowledge no twin study to date has focussed on the association between anxiety, depression, and DB covering childhood and adolescence, whilst using a co-twin design.

This study will investigate (1) if childhood anxiety and/or depression are cross-sectionally associated with DB (2) and predictive of DB in middle and late adolescence. If significant associations are found, (3) co-twin control analyses will be used to investigate whether the co-occurrence of DB, anxiety, and depression is likely to be causally related or due to genetic or environmental confounding. We expect anxiety and depression to be significantly related to DB, although depression to a larger extent. Furthermore, we expect co-twin analyses consisting of twin pairs that are discordant on anxiety and twin pairs that are discordant on depressive disorders to gauge putative causal relationships with DB.

METHODS

Participants

The Child and Adolescent Twin Study in Sweden (CATSS) is a nationwide longitudinal study that targets all twins born in Sweden since July 1992. Parents of twins were administered the Autism-Tics, AD/HD and other Comorbidities inventory (A-TAC; Anckarsäter et al., 2011), the Screen for Child Anxiety Related Disorder (SCARED; Birmaher et al., 1997), and the Short Mood and Feelings Questionnaire (SMFQ; Angold, Costello, Messer, & Pickles, 1995) by telephone in connection with the twins' ninth birthday. The families were contacted again in connection with the twins' 15th birthday and at age 18. The follow-up at 15 years includes twins born in 1994 and onward, whereas the follow-up at 18 years includes twins born in 1992 and onward. At both follow-up assessments, at least one parent and both twins were invited to participate.

At baseline (age 9), parents completed the A-TAC, SCARED, and SMFQ as described below (see Measures). At baseline, for 19,347 twins data were available. Of these twins 4,540 participated at the first follow-up and 1,286 at the second follow-up. Because the SMFQ and the SCARED were later included in the CATSS study, considerably less data was available at follow-ups as compared to baseline. For the present longitudinal analysis, participants were selected for whom measures of interest were available at age 9 (baseline) and age 15 (first follow-up), resulting in a sample of 3,852 children, and for whom outcome

measures of interest were available at age 9 (baseline) and age 18 (second follow-up), resulting in a sample of 786 children.

For the co-twin control analyses three samples were formed. The first sample consisted of unrelated individuals, which were twins with parent-reported depressive disorder and/or an anxiety disorder and an identical number of unaffected, unrelated twin controls with neither depression nor anxiety (anxiety: 454 cases vs. 454 controls; depression: 509 cases vs. 509 controls). The second sample consisted of dizygotic twin pairs discordant for depression or anxiety (i.e., one twin has depression/anxiety – the other twin has neither depression or anxiety (anxiety = 287 pairs; depression = 330 pairs). Third, a sample of monozygotic twin pairs discordant for depression or anxiety was selected (anxiety = 91 pairs; depression = 104 pairs).

Baseline measures at age 9 years

Parent-reported disruptive behavior

Parent-reported disruptive behavior (DB) of the twin was assessed using the A-TAC (Anckarsäter et al., 2011), which consists of 96 questions covering symptoms of common child and adolescent psychiatric disorders, including oppositional defiant disorder (ODD) and conduct disorder (CD). The A-TAC ODD and CD subscales consist of five gate questions, each asking a parent about lifetime presence of ODD and CD symptoms in his/her child, respectively. The answering options are coded as 0 ("No"), 0.5 ("Yes, to some extent"), or 1 ("Yes"). All A-TAC questions are included in Supplement 1, available online.

Parent-reported anxiety

Anxiety of the twin was assessed using the parent-version of the SCARED, which consists of 38 items, and is aimed at screening for signs of anxiety disorders in children (Birmaher et al., 1997). The questionnaire covers the following disorders and problems: panic, social anxiety, separation, and generalized anxiety disorders, as well as school avoidance and somatic problems. Answers are given on a 3 point scale, and are coded as: 0 ("Not True or Hardly Ever True"), 1 ("Somewhat True or Sometimes True"), or 2 ("Very True or Often True"). Scores of 25 or higher are indicative of an anxiety disorder. Continuous scores were used in the initial cross-sectional and longitudinal analyses. In the twin control analyses, a clinical cutoff indicating the presence of an anxiety disorder was used to define case or control status (i.e., 1 = anxiety disorder present; 0 = anxiety disorder absent).

Parent-reported depression

Depression of the twin was assessed using the parent-version of the SMFQ, which consists of 13 items, and measures depression in childhood and adolescence (Angold et al., 1995). Answers are given on a 3 point scale, and are coded as: 0 ("No"), 1 ("Yes, to a certain agree"), or 2 ("Yes"). Scores of 8 or higher are indicative of a depressive disorder. Continuous scores were used in the initial cross-sectional and longitudinal analyses. In the twin control analyses, a clinical cutoff indicating the presence of a depressive disorder (i.e., 1 = depressive disorder present; 0 = depressive disorder absent), was used to define case and status.

Parental education

The educational level of each parent was obtained during the telephone interview at baseline. First, education level was coded into three different categories: 1 (completed primary school or less [9 years of formal education]); 2 (completed a high school education [10-12 years]); and 3 (university studies or equivalent [>13 years]). Next, educational level of both parents were summed, resulting in a score ranging from 2 to 6. If information about the education of one parent was missing, the educational level of the other parent with available data was imputed.

Disruptive behavior at age 15 years

Information on various forms of DB were collected at age 15 years, relying on self- and parent-reports. Reactive (or impulsive) and proactive (or planned) aggression were assessed through a youth self-report questionnaire (Raine et al., 2006). Criminality was assessed with a self-report tool that assessed the frequency of violent and nonviolent criminal acts (Ring, 1999). Conduct problems of the twin were assessed using the Conduct Problems subscale of the Strengths and Difficulties Questionnaire (SDQ) parent version and self-report version. Bullying perpetration was measured by the Revised Olweus Bully/Victim Questionnaire (OBVQ; Olweus, 1996). Details of these measures (including example items) are provided in Supplement 1, available online.

Similar to prior research (Roetman et al., 2019), a cumulative index was computed by summing the times that a twin was above the cut-off used to define involvement in DB on several variables measuring different aspects of DB (see Analyses). The score for this index ranged from "0" (indicating that the twin exhibited no disruptive behaviors measured at follow-up) to "7" (indicating that the twin engaged in all DB behaviors at the follow-up at 15 years for prevalences, see Table 1).

Disruptive behavior at age 18 years

Information was collected on various forms of DB at age 18 years, relying on self- and parent-reports. Aggression was assessed using self-report (Coccaro et al., 1997) and parent-report questionnaires (Achenbach & Rescorla, 2003). Nonviolent and violent criminality were assessed with the same self-report tool that was used at age 15. Rule-breaking behavior was assessed by the parent-reported Adult Behavior Checklist (ABCL; Achenbach & Rescorla, 2003). Consequences of antisocial behavior were assessed through a self-report questionnaire that taps social consequences (e.g., reprimands) caused by involvement in antisocial behaviour (Coccaro et al., 1997). Details of these measures are given in Supplement 1, available online.

Also for this follow-up a cumulative index was computed by summing the times that a twin was above the cut-off. This index ranged from "0" (no disruptive behaviors measured at follow-up at 18 years) to "6" (the twin engaged in all DB behaviors at the follow-up at 18 years; for prevalences, see Table 1)

Analyses

The various continuous variables tapping DB at ages 15 and 18 years were substantially skewed, even after data normalization transformations. Therefore, consistent with a large body of research, dichotomized variables were used for the DB measures at both follow-ups (Bechtold et al., 2016; Kerr et al., 1997). Specifically, all DB measures, except self-reported crime and bullying perpetration, were dichotomized into high (i.e., the 30% highest scores, 1, which is indicative of low functioning) versus low (i.e., 70% lowest scores, 0). These cut-offs were implemented because Swedish norms were unavailable for the majority of the DB measures. In line with prior research on the prediction of criminal outcomes (Camp et al., 2013; Colins et al., 2015), we used dichotomized variables (no offenses versus one or more offenses) to define violent and nonviolent criminality, while for bullying perpetration reliable cut-offs were available. Consequently, all these dichotomized DB variables were summed to form disruptive behavior scores at ages 15 and 18 years, respectively (see Disruptive Behavior Scores at Ages 15 and 18 Years). Furthermore, to ease interpretability and to uniformly conduct negative binomial regressions, presence of DB at age 9 years was treated as a count variable, with scores of 0.5 ("Yes, to some extent") and 1 ("Yes") being coded to 1 (i.e., this disruptive behaviour is present), while a score of 0 indicated the absence of a DB.

Table 1 Descriptive Statistics for Children with Complete data at Baseline, and Age 15 Years, and Baseline and 18 Years

		Total sample at 9 years (N = 19,706)		Total sample at 15 years (N = 3,852)		Total sample at 18 years (N = 786)	
Variable function and child age at assessment	Variable	Mean (*SD*)	Range	Mean (*SD*)	Range	Mean (*SD*)	Range
Variables at 9 years	Disruptive behavior (PR)	1.40 (2.30)	0-14	1.04 (1.90)	0-13	1.08 (1.98)	0-13
	Anxiety (PR)	5.96 (7.03)	0-74	4.72 (5.68)	0-50	4.72 (5.49)	0-47
	Anxiety disorder classification (PR) [n(%)]	523 (2.7%)	0-1	51 (1.3%)	0-1	10 (1.3%)	0-1
	Depression (PR)	0.99 (2.42)	0-25	0.79 (1.93)	0-24	0.66 (1.81)	0-24
	Depressive disorder classification (PR) [n(%)]	577 (2.9%)	0-1	62 (1.6%)	0-1	7 (0.9%)	0-1
	Parental education level (PR)	4.72 (0.97)	2-6	5.39 (0.89)	2-6	5.35 (0.92)	2-6
	Child's gender male (PR) [n(%)]	9901 (50.2%)	0-1	1749(45.7%)	0-1	360 (45.8%)	0-1
Disruptive behavior at follow-up	Cumulative disruptive behavior index [n(%)]				0-7		0-6
	0 behaviors			1598 (41.5%)		181 (23.0%)	
	1 behavior			873 (22.7%)		168 (21.4%)	
	2 behaviors			550 (14.3%)		171 (21.8%)	
	3 behaviors			368 (9.5%)		116 (14.7%)	
	4 ≤ behaviors			463 (12.0%)		150 (19.1%)	

Note. PR = parent-reported; SR = self-reported.

Cross-Sectional and longitudinal associations between anxiety, depression and DB

Generalized linear mixed models (GLMMs) for negative binomial regression were used to investigate cross-sectional and longitudinal relations between anxiety, depression and DB at ages 15 and 18 years. GLMMs combine both linear mixed models and generalized linear models, and enable the introduction of random effects. The introduction of a random effect (i.e., twins nested within families) is needed to correct for dependency between twins and dependency of observations (i.e., one parent reporting on the behavior of two twins). In this study, a robust estimator (Huber/White/sandwich estimation) was used to estimate the covariance. This estimator corrects for the dependence of observations and other departures from normality, such as under- and overdispersion. Wald χ^2 tests were used to test the fixed effects. For the fixed effects corresponding incidence rate ratios (IRRs) and 95% confidence intervals (CIs) were computed and reported.

Three different models were run to test cross-sectional and longitudinal associations between anxiety, depression, and DB. The first model was a crude effects model consisting of one predictor at baseline (i.e., depression, or anxiety in both cross-sectional and longitudinal analyses, DB at baseline was added as an additional predictor in the longitudinal analyses to predict DB at follow-ups 15 and 18 years), together with two theoretically relevant control variables: parental education level and sex of the child. In the second model, depression and anxiety at baseline were included simultaneously in an adjusted model, together with the same covariates. In the longitudinal models DB at baseline was included as an additional predictor to predict DB at the follow-ups. The third model included two interaction terms, one including DB and anxiety, and a second including DB and depression.

Co-twin control analyses

The co-twin control design was used to further investigate significant relationships between anxiety, depression, and DB. Regular case-control studies of unrelated individuals can result in overestimation of effects between exposure and outcome, because these designs are less able to control for confounding due to unmeasured environment (e.g., low SES is driving the association between internalizing problems and DB) and genetic background of individuals (e.g., the development of internalizing problems is a simple co-occurrence to DB). Co-twin designs enable researchers to control for both confounders to some extent because twins, especially children, share a substantial part of their (rearing) environment and have substantial (i.e., dizygotic twins share 50% of their genetic makeup)

or complete genetic overlap (monozygotic share nearly 100% of their genetic makeup; Lichtenstein et al., 2002).

In line with prior work (Dinkler et al., 2017; Stubbe, de Moor, Boomsma, & de Geus, 2007), this co-twin control design was put into practice by comparing twins on the basis of depressive and anxiety cases separately, with case status defined by the clinical cut-offs of the MFQ (i.e., depression) and the SCARED (i.e., anxiety), respectively. First, comparisons between an even number of unrelated twin cases and unaffected twin controls were performed, simulating a conventional case-control design. A significant association in this comparison can indicate a causal relationship, but fails to control for unmeasured environment and genetic background. This is because cases and controls are genetically unrelated and do not share (rearing) environments. Second, comparisons were made within dizygotic twin pairs discordant for the presence of anxiety and depression, (i.e., one twin has an anxiety disorder/depression, the other twin has neither an anxiety disorder or depression). This comparison allows to control for shared environment, because twin pairs grow up in the same environment, and controls for genetic confounding to some extent (i.e., dizygotic twins share 50% of their genetic makeup). A significant association in this stage of the analyses indicates that effects in case-control studies are due to unmeasured environmental factors. Third, comparisons were made within monozygotic twin pairs discordant for anxiety and depression, respectively. The identical genetic makeup of monozygotic twins allows to control for genetic confounding. Importantly, a significant association between DB and anxiety and/or depression gives stronger support for a causal effect.

Because of the use of count data, the first comparison was made using the Mann-Whitney test (i.e., two independent samples of cases and controls), while the second and third comparisons were made by means of the Wilcoxon signed-rank test (i.e., dependent samples of dizygotic and monozygotic twin cases and co-twin controls). We used $p < .05$ as an indicator of statistical significance. Two-tailed tests were used in all analyses.

Attrition

At age 15 years, there were 1583 out of 5435 children who were not included in the analyses because of some degree of missing data. Children with (versus without) missing data were more often boys (45.4% versus 47.4%, $p < .05$) and had parents with lower educational levels ($p < .001$). No differences were found in

baseline levels of anxiety, depression, and DB. At age 18 years, 1034 out of 1820 children were excluded because of missing data. Significant differences emerged between children with and without missing data in terms of parental education ($p < .001$), while no differences in sex, anxiety, depression, and DB were found.

RESULTS

Anxiety, depression, and DB

At baseline 9, crude negative binomial regression models indicated that anxiety (IRR = 1.07; 95% CI: 1.07, 1.08) as well as depression (IRR = 1.22; 95% CI: 1.21, 1.23), were significantly related to DB (p's < .001). When included simultaneously in an adjusted model, both anxiety (IRR = 1.05; 95% CI: 1.04, 1.05) and depression (IRR = 1.15; 95% CI: 1.14, 1.16) retained their associations with DB (p's < .001).

Longitudinally, crude negative binomial regression models indicated that DB at 9 years was predictive (p's < .001) of DB at 15 (IRR = 1.11; 95% CI: 1.09, 1.13) and 18 years (IRR = 1.17; 95% CI: 1.11, 1.24). Similar crude models indicated significant predictive effects for depression on DB at 15 (IRR = 1.04; 95% CI: 1.03, 1.06; $p < .001$) and 18 years (IRR = 1.05; 95% CI: 1.02, 1.09; $p = .002$). For anxiety, a significant effect on DB was found at 15 years (IRR = 1.01; 95% CI: 1.00, 1.02; $p = .001$), though not at 18 years.

When DB, depression, and anxiety at 9 years were included simultaneously in one negative binomial regression model, DB retained its predictive associations (p's < .001) with DB on 15 years (IRR = 1.11; 95% CI: 1.09, 1.13), and 18 years (IRR = 1.09; 95% CI: 1.06, 1.12). Both depression and anxiety lost their associations with DB on age 15 and 18 years. Interaction models did not indicate significant interactions between DB and depression and anxiety at 15 years, and 18 years. Similar analyses were also conducted with dichotomous anxiety and depression measures, which were based on the presence or absence of an anxiety or depressive disorder. These analyses yielded identical results (see Supplement 2, available online).

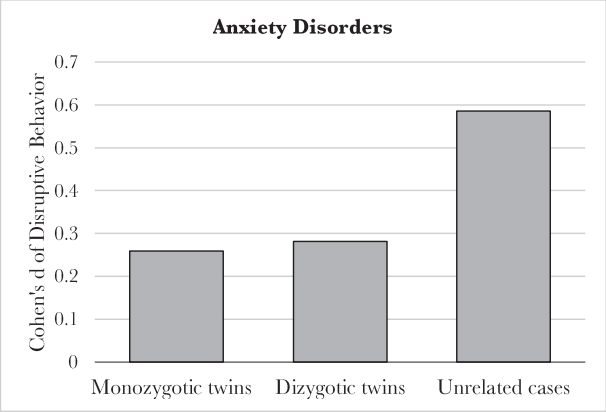
Co-twin control analyses

Although anxiety and depression did show very clear cross-sectional relations with DB at 9 years, longitudinal relations when controlling for baseline DB were non-existent. Therefore co-twin analyses were not performed on the longitudinal

data. To gain a deeper understanding of the causal relations between DB and depression and anxiety, and in line with prior work (Dinkler et al., 2017; Stubbe et al., 2007), we conducted co-twin control analyses at baseline in childhood.

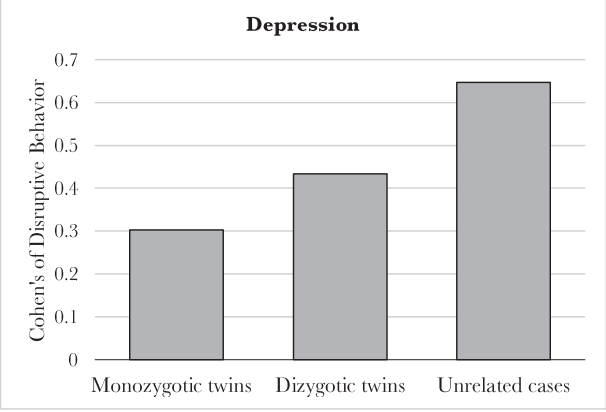
For both anxiety and depression, monozygotic twins, dizygotic twins, and unrelated cases differed significantly from their unaffected co-twin or unaffected, unrelated controls in terms of DB (p's < .001; Figures 1 and 2). Effect sizes were attenuated from medium to small in monozygotic and dizygotic twin pairs, as compared to the analyses in unrelated cases. Furthermore, effect sizes of monozygotic twins and dizygotic twins did not differ considerably. See Tables 2 and 3 for descriptives of the anxiety and depression co-twin samples, respectively.

Figure 1



MZ twins = 182; DZ twins = 574; Unrelated Cases and Controls = 908.

Figure 2



MZ twins = 208; DZ twins = 660; Unrelated Cases and Controls = 1018.

Table 2 Descriptive Statistics for the Anxiety Co-twin Control Analyses at Baseline

	Monozygotic twins		Dizygotic twins		Unrelated cases	
	Anxiety cases (n = 91)	Anxiety controls (n = 91)	Anxiety cases (n = 287)	Anxiety controls (n = 287)	Anxiety cases (n = 454)	Anxiety controls (n = 454)
Variable	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Disruptive behavior (PR)	3.79 (3.39)	2.62 (3.25)	4.80 (3.78)	2.75 (3.13)	4.65 (3.68)	0.88 (1.53)
Anxiety (PR)	31.76 (6.86)	13.01 (7.12)	33.34 (8.54)	9.52 (6.57)	33.14 (8.39)	5.07 (5.57)
Anxiety disorder classification (PR) [n(%)]	91 (100%)	0 (0%)	287 (100%)	0 (0%)	454 (100%)	0 (0%)
Depression (PR)	5.84 (5.11)	1.90 (2.86)	6.71 (6.11)	2.12 (3.38)	6.73 (5.97)	0.00 (0.00)
Depressive disorder classification (PR) [n(%)]	29 (31.9%)	4 (4.4%)	106 (36.9%)	19 (6.6%)	171 (37.7%)	0 (0%)
Parental education level (PR)	4.44 (1.14)	-	4.52 (0.90)	-	4.48 (1.00)	4.79 (0.97)
Child's gender male (PR) [n(%)]	32 (25.2%)	-	141 (59.1%)	149 (51.9%)	214 (47.1%)	229 (50.4%)

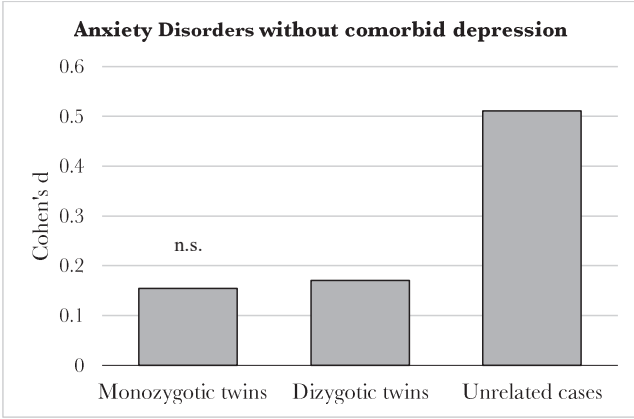
Note. PR = parent-reported; SR = self-reported.

Table 3 Descriptive Statistics for the Depression Co-twin Control Analyses at Baseline

	Monozygotic twins		Dizygotic twins		Unrelated cases	
	Depression cases (n = 104)	Depression controls (n = 104)	Depression cases (n = 330)	Depression controls (n = 330)	Depression cases (n = 509)	Depression controls (n = 509)
Variable	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Disruptive behavior (PR)	4.70 (3.63)	3.07 (3.03)	5.51 (3.80)	2.51 (2.87)	5.38 (3.81)	0.88 (1.52)
Anxiety (PR)	19.55 (14.17)	11.40 (9.94)	19.88 (13.95)	9.41 (8.65)	20.02 (13.79)	3.76 (4.54)
Anxiety disorder classification (PR) [n(%)]	104 (100%)	0 (0%)	330 (100%)	0 (0%)	509 (100%)	0 (0%)
Depression (PR)	10.57 (3.18)	2.30 (2.36)	11.77 (4.02)	1.88 (2.12)	11.62 (3.96)	0.00 (0.00)
Depressive disorder classification (PR) [n(%)]	104 (100%)	0 (0%)	330 (100%)	0 (0%)	509 (100%)	0 (0%)
Parental education level (PR)	4.54 (1.09)	-	4.54 (0.94)	-	4.53 (0.98)	4.79 (0.99)
Child's gender male (PR) [n(%)]	45 (43.3%)	-	192 (58.2%)	161 (48.8%)	284 (55.8%)	258 (50.7%)

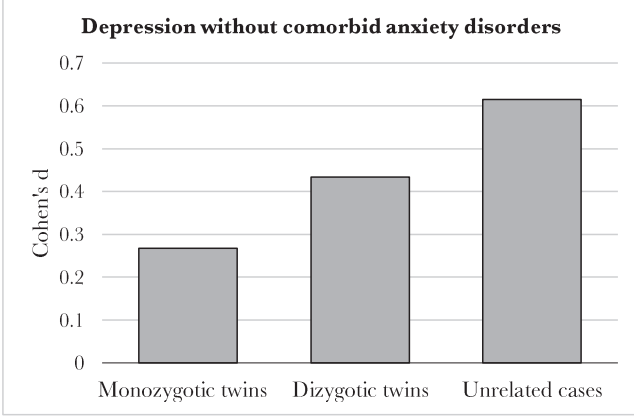
Note. PR = parent-reported; SR = self-reported.

Figure 3



MZ twins = 122; DZ twins = 348; Unrelated Cases and Controls = 674. n.s. = not significant.

Figure 4



MZ twins = 138; DZ twins = 428; Unrelated Cases and Controls = 566.

Because comorbidity between anxiety and depression is common (Cummings, Caporino, & Kendall, 2014), the same co-twin control analyses were conducted in cases with anxiety disorders without comorbid depression, and in cases with depression without comorbid anxiety disorders. These analyses resulted in attenuation of the relationships in unrelated cases and controls and the discordant dizygotic twin pairs, all (p's $\leq .001$; Figures 3 and 4). But more importantly, in monozygotic discordant twin pairs the relation between anxiety disorders and DB became non-significant, while the relation between DB and depression

remained significant (d = 0.27, p = .002). This indicates that the association between anxiety and DB is explained by comorbid depression, with the relationship between anxiety and DB being completely explained by confounding when controlling for this comorbidity. In the end, these results suggest that the relationship between DB and depression is less sensitive to environmental and genetic confounding than anxiety.

DISCUSSION

This study aimed to investigate cross-sectional relations between childhood anxiety, depression and disruptive behavior (DB), and whether childhood anxiety and depression were predictive of DB in adolescence. Furthermore, significant relationships were subjected to a co-twin control analysis to gauge the extent of environmental and genetic confounding.

In accordance to our hypotheses as well as previous research (Bartels, Hendriks, Mauri, Krapohl, Whipp, Bolhuis, Conde, Luningham, Ip, et al., 2018; Granic, 2014; Marshall et al., 2015), cross-sectional relationships were found between anxiety, depression, and DB; with depression showing a stronger relation to DB than anxiety. This likely reflects the observation that, regardless of the direction of effect, depression is uniformly associated with increased levels of DB (Kasen et al., 2001; Patterson & Capaldi, 1990; Wolff & Ollendick, 2006), while anxiety is attributed with both increased and decreased DB (Cunningham & Ollendick, 2010; Granic, 2014; Klingzell et al., 2016; Raine, 2013). In contrast to our expectations, longitudinal effects of childhood anxiety and depression on adolescent DB were not found; although both were predictive of adolescent DB in crude models, these lost significance when controlling for childhood DB at baseline. This finding potentially indicates that DB is the driving factor behind its comorbidity with anxiety and depression and not the other way around. It is already widely known that childhood DB is related to a wide variety of poor outcomes in adolescence, ranging from poor school performance to substance abuse (e.g., Colins, Fanti, & Andershed, 2020; Roetman et al., 2019), and also shows higher stability than anxiety and depression (de la Vega, Piña, Peralta, Kelly, & Giner, 2018; Hannigan, Walaker, Waszczuk, McAdams, & Eley, 2017; Nivard et al., 2015).

A co-twin control design was used to assess whether the cross-sectional associations between anxiety, depression, and DB in childhood were attributable

to environmental and genetic confounding. Although, cross-sectional comorbidity between anxiety, depression, and DB is widely reported in the literature (Bartels, Hendriks, Mauri, Krapohl, Whipp, Bolhuis, Conde, Luningham, Ip, et al., 2018; Granic, 2014; Marshall et al., 2015), these analyses indicated that associations between anxiety and DB could be completely attributed to confounding, while the associations between depression and DB, albeit small (d = 0.27), withstood this stringent test. In combination with the non-significant longitudinal associations spanning into adulthood, this means that based on the current data it is highly likely that anxiety is not causally related to DB, and depression very probably as well. In case of depression, another less likely possibility could be that DB influences depression or vice versa, but that these effects are transient and do not influence DB in the long-term (Thompson et al., 2015). Unfortunately, this hypothesis could not be tested because follow-up measurements took place many years after baseline (6 and 9 years) and spanned very different developmental timeframes (i.e., middle childhood and late adolescence).

This study has considerable strengths; we used a large community sample of twins spanning childhood and late adolescence, containing both twin- and parent-reported measures. As always, this study had several limitations. The baseline measurement in childhood and the follow-up measurements in adolescence were relatively far apart, which could be problematic if interrelations between anxiety, depression, and DB are transient or cascading. Attrition between baseline and follow-ups were substantial. However, the fact that measures for anxiety and depression were introduced later during this study also substantially contributed to the differences in sample size. Measures of DB varied across baseline and follow-ups. However, it should also be noted that DB at 9 years can be expressed very differently than DB in adolescence (e.g., Tremblay, 2014; Vitaro et al., 2006). Although we had a substantial number of twin pairs which were discordant for anxiety and depressive disorders, these clinical classifications were based on parent-report, not by mental health professionals. Furthermore, although childhood DB in this study consisted of oppositional defiant and conduct disorder symptoms, in the overwhelming majority of cases requirements were not met for diagnoses of oppositional defiant disorder or conduct disorder. Future research should be conducted to investigate whether these findings hold up in children who have severe DB or with formal disruptive behavior disorder diagnoses.

In sum, although anxiety and depression show considerable comorbidity with DB and cause significant distress, these associations are likely secondary

to DB and not causally related. This study suggests that treatment of DB should be the main focus of clinicians in case of comorbidity with internalizing problems, because of DB's severity and associations with a multitude of other worse outcomes. This study also emphasizes the need for extensive control for confounding, be it through inclusion of additional measures or behaviour genetic designs (e.g., twin, adoption).

CHAPTER 4

CLASSES OF OPPOSITIONAL DEFIANT DISORDER BEHAVIOR IN CLINIC-REFERRED CHILDREN AND ADOLESCENTS: CONCURRENT FEATURES AND OUTCOMES

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ABSTRACT

Objective: Oppositional Defiant Disorder (ODD) consists of irritable and oppositional behaviors, both of which are associated with different problems. However, it is unclear whether irritability and oppositionality enable classification of clinic-referred children and adolescents into mutually exclusive groups (e.g., high in oppositionality, low in irritability), and whether this classification is clinically meaningful.

Methods: As part of a clinical protocol, ODD behaviors were assessed at referral through a comprehensive diagnostic interview and questionnaire. Parent- and teacher-reported ODD of 2185 clinic-referred 5- to 18-year-olds (36.9% females) were used in latent class analysis. Resulting ODD classes were compared, concurrently at referral, and, longitudinally at the end of the diagnostic and treatment process, on various clinically relevant measures that were completed by various informants, including mental health problems, global functioning, and DSM classifications.

Results: Three classes emerged with high, moderate, and low levels of both irritability and oppositionality. At referral, the High class experienced the highest levels of mental health problems and DSM classifications. Importantly, all ODD classes defined at intake were predictive of diagnostic and treatment outcomes months later. Notably, the High class had higher rates of clinician-based classifications of ODD and Conduct Disorder, and the lowest levels of pre- and posttreatment global functioning. Additionally, the Low class exhibited higher rates of Generalized Anxiety Disorder and fear disorders.

Conclusion: Irritability and oppositionality co-occur in clinic-referred youths to such an extent that classification based on these behaviors does not add to clinical inference. Instead, findings suggest that the overall ODD severity at referral should be used as a guidance for treatment.

INTRODUCTION

DSM-defined Oppositional Defiant Disorder (ODD) is characterized by a pattern of problem behaviors ranging from anger and temper tantrums to arguing and vindictiveness (American Psychiatric Association, 2013). In addition to this heterogeneity in ODD symptomatology, children with ODD differ greatly in co-occurring mental health problems and prognosis (Biederman et al., 2008; Copeland, Shanahan, Costello, & Angold, 2009; Harpold et al., 2007). In order to gain further insight into this heterogeneity, efforts to distinguish between types of ODD behavior have shown that a differentiation can be made between at least two dimensions: an irritable dimension, consisting of touchy and angry behavior, and an oppositional dimension, consisting of hurtful and headstrong behaviour (Burke et al., 2014; Hukkelberg & Ogden, 2018). Irritability is mainly associated with affective problems, especially depression and anxiety (Hipwell et al., 2011; Vidal-Ribas, Brotman, Valdivieso, Leibenluft, & Stringaris, 2016), whereas oppositionality is correlated with symptoms of Attention Deficit Hyperactivity Disorder (ADHD) and Conduct Disorder (CD), as well as violent and non-violent delinquency (Hipwell et al., 2011). Some evidence suggests that the oppositional dimension can be divided further into a hurtful dimension, consisting of vindictive and spiteful behaviors, and a headstrong dimension, characterized by arguing, defiance, blaming, and annoying behaviour (Stringaris & Goodman, 2009b). Yet, it is still unclear which dimensional approach (i.e., differentiating between two or three dimensions) is most useful for applied clinical purposes.

Crucially, it remains unclear to what extent distinct ODD dimensions enable classification of clinic-referred children and adolescents into mutually exclusive groups (e.g., children who are only high in one ODD dimension versus children who are high in two or three ODD dimensions). The majority of prior studies explored this issue in community samples (Althoff, Kuny-Slock, Verhulst, Hudziak, & van der Ende, 2014; Boylan et al., 2017; Herzhoff & Tackett, 2016; Kuny et al., 2013; Wesselhoeft et al., 2019), with three notable exceptions. One study used latent class analysis (LCA) to assign 177 7- to 12-year-old clinic-referred boys to separate classes on the basis of parent-reported ODD symptoms (Burke, 2012). Based on this data-driven analysis, three classes emerged; one class comprised of boys low in oppositionality and irritability (Low ODD class); a second class high in oppositionality, but low in irritability (Oppositional ODD class); and a third class high in both oppositionality and irritability (Combined

ODD class). The prognostic usefulness of the classes was also supported; the Combined ODD class had the highest levels of future self-reported anxiety and depression in adolescence, and was highest in adult neuroticism and depression. Unfortunately, differences between the Oppositional ODD and the Low ODD class were not reported (Burke, 2012). A second study performed LCA in a sample of 158 detained male juvenile offenders (Aebi, Barra, et al., 2016), a population hallmarked by severe psychopathology (Beaudry, Yu, Långström, & Seena Fazel, 2020; Colins et al., 2010). Besides the aforementioned classes, a fourth class was revealed, characterized by substantial irritability, but low oppositionality (Irritable ODD class). Cross-sectionally, the Irritable and Combined ODD classes were related to suicidality and comorbid affective/anxiety disorders. The Irritable ODD class was at risk of criminal reoffending, even when controlling for CD (Aebi, Barra, et al., 2016). The third study used theory-driven classifications to assign 1,160 6- to 18-year-old clinic-referred youths to angry/irritable symptoms (AIS), primarily non-compliant symptoms (NS), and control groups (Drabick & Gadow, 2012). The AIS group showed the highest levels of concurrent parent- and teacher-reported anxiety, mood, and conduct symptoms, while the NS and control groups showed moderate and low levels of symptoms, respectively. In sum, prior work consistently shows that children and adolescents in the Combined ODD class experience substantial concurrent problems, while the differentiating capabilities of the Oppositional and Irritable classes are less clear. Furthermore, several important aspects which determine the clinical usefulness of these classes, like outcomes of the diagnostic process (e.g., clinician-based DSM classifications) or treatment, have not been studied.

This is the first study to investigate the viability of ODD classes for actual clinical inference; using data that were collected as part of a clinical protocol, starting at time of referral, and spanning the diagnostic process and treatment. Also, whereas prior work with community and clinic-referred samples merely considered the presence of ODD symptoms, this study will be the first to account for DSM-defined criteria of duration (≥ 6 months) and impairment in developmental contexts (e.g., family, friends). To facilitate comparison with most prior work (Aebi, Barra, et al., 2016; Althoff et al., 2014; Burke, 2012; Herzhoff & Tackett, 2016; Kuny et al., 2013; Wesselhoeft et al., 2019), LCA was used to assign children and adolescents to ODD classes. This data-driven analytical approach enabled us to investigate differences in ODD symptom profiles without committing ourselves to a priori choices about the number

(two or three) and the content (e.g., non-compliance only) of ODD dimensions. Contrary to prior work that relied on relatively small samples (Aebi, Barra, et al., 2016; Burke, 2012), the current study used a large sample of clinic-referred children and adolescents (N = 2,185), guaranteeing optimal model estimation (Wurpts & Geiser, 2014). We broadly expect to identify Low, Oppositional, and Combined ODD classes, with youths in the latter class exhibiting the lowest level of concurrent and future functioning. Yet, we do not rule out the existence of an Irritable ODD class (Aebi, Barra, et al., 2016). An Oppositional class would show substantial rates of conduct problems as well as ADHD, but relatively low levels of affective problems. Conversely, an Irritable class would show considerable levels of affective problems, but low conduct problems and rates of ADHD.

METHODS

Participants and procedure

This study used data that were collected as an integral part of a clinical protocol at a center for child and adolescent psychiatry between October 2008 and October 2017. The center is located in a predominantly urban area with moderate to high SES in the western Netherlands. The sample consisted of 5- to 18-year-old youths of predominantly Dutch European descent who were referred for various psychiatric problems, spanning from anxiety and depression to neurodevelopmental disorders. Youths with suspected low intelligence were referred to other institutions. Parents and youths were informed that their anonymized data could be used for scientific purposes at time of admission. To be eligible for admission and subsequent aftercare, parents and, if applicable, teachers were required to complete the Development And Well-Being Assessment at referral (DAWBA; see Measures; Goodman, Ford, Richards, Gatward, & Meltzer, 2000). The care provided was diverse, ranging from diagnostics, to various inpatient and outpatient treatment programs.

For 3362 youths DAWBA-reports were available from parents or teachers. Because diagnostic assessment of youths emphasizes information from multiple informants (Colins, Vermeiren, Schuyten, Broekaert, & Soyez, 2008; Handwerk, Larzelere, Soper, & Friman, 1999), only youths for whom DAWBA ODD parent- or teacher information was available were selected (excluding 387 youths). Next, we excluded 790 participants for whom parents did not report on all ODD symptoms (because they did not reach the DAWBA ODD screening threshold;

see Measures). Thus, in total, 2,185 youths (36.9% female) between the ages of 5 and 18 years (M = 9.96, SD = 3.22) were included. Due to missing values, the number of participants used for group comparisons will be slightly lower (2041 ≤) than those in the model-based clustering analyses (N = 2185).

Measures

Clustering variables

DSM-IV defined ODD behaviors or symptoms were measured by the Dutch parent and teacher versions of the DAWBA, a widely-used computerized diagnostic interview (Goodman et al., 2000). The Dutch DAWBA version separates the DSM symptom "vindictive and spiteful" into two different questions (see Table S1), resulting in a total of nine ODD symptoms. According to the DSM, we focused on clinically significant levels of the nine ODD symptoms, meaning we considered symptoms which are oft-occurring ("occurs a lot more than in other children"), persistent ("present for 6 months or longer"), and cause functional impairment in one or more developmental contexts. Finally, the nine DAWBA ODD symptoms will be used as clustering variables in LCA to assign youths to mutually exclusive classes. Consistent with recommendations to use multiple informants (American Psychiatric Association, 2013), the highest score from the parent and teacher for each ODD symptom were used (Piacentini, Cohen, & Cohen, 1992). This means that if at least one informant indicated an ODD symptom to be present, persistent, and impairing, the ODD symptom was indicated as present. Details about the use of the DAWBA ODD symptoms are found in Supplement 1.

Variables for cluster comparisons at referral

Parent, teachers, and if applicable, youths completed the Strengths and Difficulties Questionnaire (SDQ) as an index of dimensionally assessed mental health problems (Emotional Problems, Hyperactivity) and other problems (Peer Problems, and Prosocial Behavior; Goodman, 1997). Additionally, and in line with recommendations (Goodman, Heiervang, Collishaw, & Goodman, 2011) and prior work (Colins et al., 2008), we used the *DAWBA computer-generated DSM disorder categories* "depressive disorders" (referring to the presence of major depressive disorder, dysthymic disorder, and/or depressive disorder not otherwise specified) and "fear disorders" (referring to the presence of separation anxiety disorder, panic disorder agoraphobia specific, and/or social phobia).

Variables for longitudinal cluster comparisons

As an index of categorically assessed mental health problems, we relied on diagnoses of DSM-IV-defined psychiatric disorders that were determined by a multidisciplinary team at the end of a diagnostic process, conform clinical diagnostic guidelines. A main advantage of clinical classifications by a multidisciplinary team over parent- and teacher-reported classifications is the ability of clinicians to weigh several constellations of symptoms against one another to establish which symptoms (i.e., clinical classification[s]) are likely to be the main problem. Another important advantage is their ability to pick up symptoms that are difficult to detect (e.g., autistic symptoms) by non-trained raters (e.g., parents and teachers). These multidisciplinary evaluations took place on average 3.81 months (SD = 3.34) after referral. Any clinical classification, not just primary classifications, were included in the analyses. We also collected DSM-based Global Assessment Functioning (GAF) scores at the beginning and end of treatment, as an index of clinician-rated global functioning. See Supplement 1 for details.

Data analyses

Table 1 provides descriptive information for all variables. According to most prior work on ODD subtypes (Aebi, Barra, et al., 2016; Althoff et al., 2014; Burke, 2012; Herzhoff & Tackett, 2016; Kuny et al., 2013; Wesselhoeft et al., 2019), latent class analysis (LCA) was performed, using the nine ODD symptoms as clustering variables. LCA is a data-driven model-based clustering technique enabling differentiation between classes of youths with various constellations of ODD symptoms. Specifically, LCAs provide a probability of endorsement of an ODD symptom within a class, with a value of 1 indicating a 100 percent probability of item endorsement (e.g., youths in this class are always reported to have temper tantrums), while a 0 indicates a 0 percent chance of endorsement. LCA also provides per individual the most probable class to which he or she belongs. In the LCA it was assessed whether gender and/or age should be included as covariates. These covariates were deemed important because of gender (Nock, Kazdin, Hiripi, & Kessler, 2007) and developmental differences (e.g., ODD rarely develops after early adolescence; Rowe, Costello, Angold, Copeland, & Maughan, 2010). To test if ODD classes differed in dimensionally and categorically assessed variables, analyses of variance (ANOVAs) and logistic regressions were performed. Finally, to examine if ODD classes differed in pre-

and posttreatment functioning repeated measures ANOVAs were performed, with pre- and posttreatment GAF scores as within-subjects factor and ODD class as between-subjects factor. To account for multiple testing, we used $p < .01$ as an indicator of statistical significance. Cohen's *d's* were calculated for continuous measures. Two-tailed tests were used in all analyses. LCAs were conducted in Mplus version 8 (Muthén & Muthén, 2016), all other analyses in SPSS version 25 (IBM, 2017).

Table 1 Descriptive Statistics for Youths with Parent- and Teacher-reported Oppositional Defiant Disorder Data

	Variable	Mean (*SD*)	Range
Latent class analysis data (N = 2185)	Youth's gender male (PR) [n(%)]	1378 (63.1%)	0-1
	Age in years (PR)	9.96 (3.22)	5-18
	ODD criteria (PR, TR)	3.29 (3.30)	0-9
	Irritable ODD criteria (PR, TR)	1.25 (1.27)	0-3
	Oppositional ODD criteria (PR, TR)	2.03 (2.20)	0-6
Cross-sectional data (n = 2164)	Strengths and difficulties questionnaire scales (PR, TR, SR)		
	Total problems	20.30 (5.30)	3-38
	Emotional problems	5.81 (2.54)	0-10
	Conduct problems	4.22 (2.00)	0-10
	Hyperactivity	7.12 (2.40)	0-10
	Peer problems	3.97 (2.25)	0-10
	Prosocial behavior	7.05 (1.99)	0-10
	DAWBA computer-generated DSM classifications (PR, TR, SR)		
	Oppositional Defiant Disorder [n(%)]	959 (44.3%)	0-1
	Conduct disorder [n(%)]	219 (10.1%)	0-1
	ADHD [n(%)]	848 (39.2%)	0-1
	Depressive disorders [n(%)]	333 (15.4%)	0-1
	Generalized anxiety disorder [n(%)]	355 (16.4%)	0-1
	Fear disorders [n(%)]	451 (20.8%)	0-1
	Autism spectrum disorder [n(%)]	99 (4.6%)	0-1
Longitudinal data (n = 2041)	Multidisciplinary team-based DSM classifications (CR)		
	Oppositional Defiant Disorder [n(%)]	177 (8.7%)	0-1
	Conduct disorder [n(%)]	69 (3.4%)	0-1
	ADHD [n(%)]	755 (37.0%)	0-1

Table 1 Continued.

	Variable	Mean (*SD*)	Range
Latent class analysis data (N = 2185)	Youth's gender male (PR) [n(%)]	1378 (63.1%)	0-1
	Depressive disorders [n(%)]	137 (6.7%)	0-1
	Generalized anxiety disorder [n(%)]	92 (4.5%)	0-1
	Fear disorders [n(%)]	61 (3.0%)	0-1
	Autism spectrum disorder [n(%)]	486 (23.8%)	0-1
	Global Functioning (CR)		
	Global Assessment Functioning pretreatment[a]	52.49 (6.66)	6-80
	Global Assessment Functioning posttreatment[b]	54.58 (7.32)	5-80

Note. ADHD = attention deficit hyperactivity disorder; CR = clinician-rated; DAWBA = Development and Well-being Assessment; DSM = Diagnostic and Statistical Manual of Mental Disorders; ODD = oppositional defiant disorder; PR = parent-reported; SR = self-reported; TR = teacher-reported. [a] n = 1997; [b] n = 1630, pairwise n = 1628.

RESULTS

Identification of classes

Table S4 shows that the LCA indicated a 3-class solution to be the best fit (see Supplement 2 for details)[1]. Additional analyses revealed it was unnecessary to control for age and gender (Supplement 2 and Table S5). Figure 1 shows that participants were assigned to one class high in both oppositionality and irritability with a high probability of ODD (High ODD class; 25.8% of total sample), one class low in both behaviors and a low probability of ODD (Low ODD class; 34.7%), and one class with moderate levels of oppositionality and irritability and a moderate probability of ODD (Moderate ODD class; 39.4%).

1 To facilitate comparison with prior work, especially with community samples, we also ran a LCA using a "symptom approach", meaning that a very minimal threshold was used for an ODD symptom (i.e., "A little more than others") to be present, without additional requirements for persistence and impairment. In short, this LCA solution, although stable across gender, did not result in very distinct ODD symptom profiles and was unstable across age (see Supplement 2; Table S5). Descriptive information and results from group comparisons of this LCA solution are available upon request.

Figure 1 Three-class DSM Solution for Parent- and Teacher-reported Oppositional Defiant Behavior of the Development and Well-Being Assessment (DAWBA)

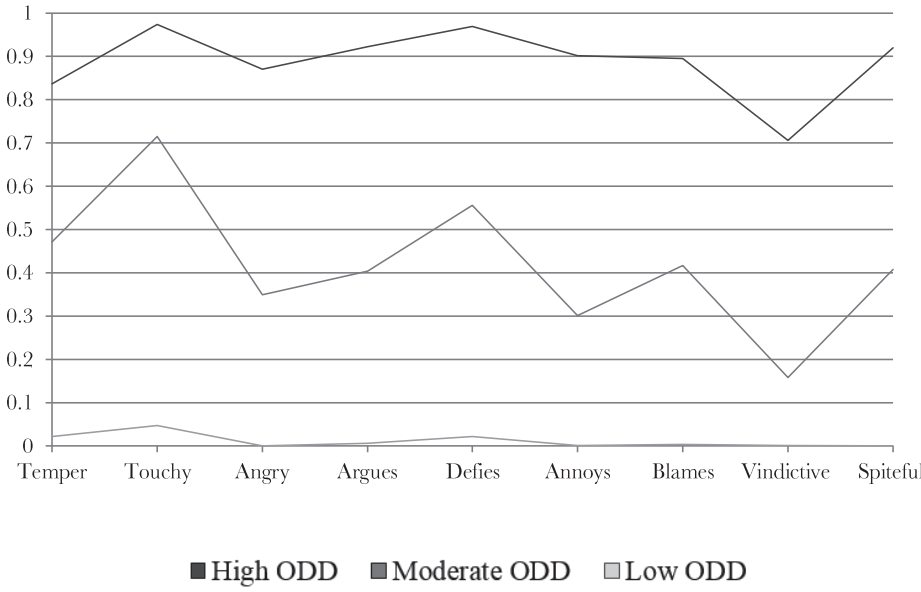

Figure 1. N = 2185. High ODD = 576 (26.4%); Moderate ODD = 698 (31.9%); Low ODD = 911 (41.7%). ODD = Oppositional Defiant Disorder.

Class comparisons: concurrent features at referral

Dimensionally assessed mental health and other problems

Figure 2 shows that participants in the High ODD class had significantly higher levels of total, hyperactivity, and peer problems, and lower levels of prosocial behavior than the two other classes (range *d*: 0.17-1.00) with the exception of emotional problems. Furthermore, the Moderate class functioned worse than the Low ODD class in terms of total problems, hyperactivity, peer problems, and prosocial behavior (range *d*: 0.23-0.47), but had comparable levels of emotional problems (see Table S6 for descriptives).

Figure 2 Differences of the Oppositional Defiant Disorder Classes on Highest Prevailing Parent-Self- and Teacher-reported Strength and Difficulties Questionnaire Scores

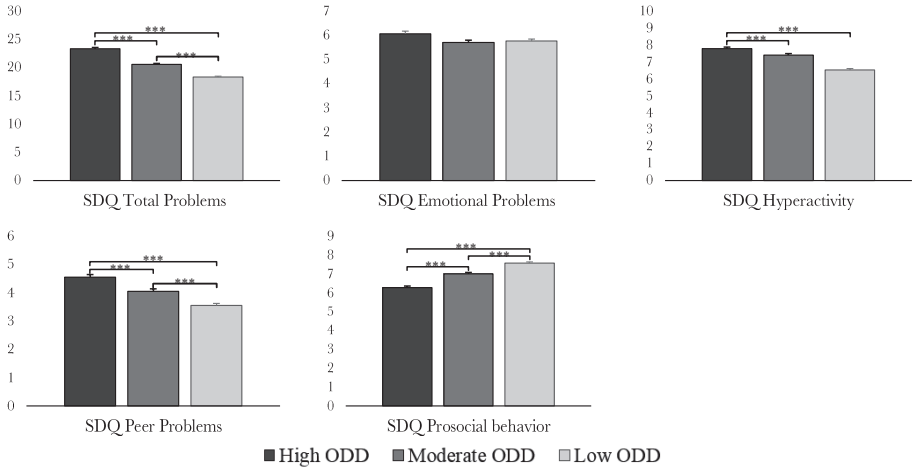

Figure 2. N = 2164. ***p <.001**p <.01.

Categorically assessed mental health problems

Figure 3 shows that the rates of DAWBA computer-generated classifications of ODD, CD, and ADHD were higher in the High ODD class as compared to the other two (ASD) and GAD than the Low ODD class, while both classes did not differ in depressive and fear disorders. The Moderate ODD class was higher than the Low ODD class in ODD, CD, ADHD, and ASD, but were equal in terms of internalizing disorders (i.e., GAD, depression, and fear disorders).

Figure 3 Prevalence of DAWBA Classifications and Differences Between Parent- Self- and Teacher-reported Oppositional Defiant Disorder Classes

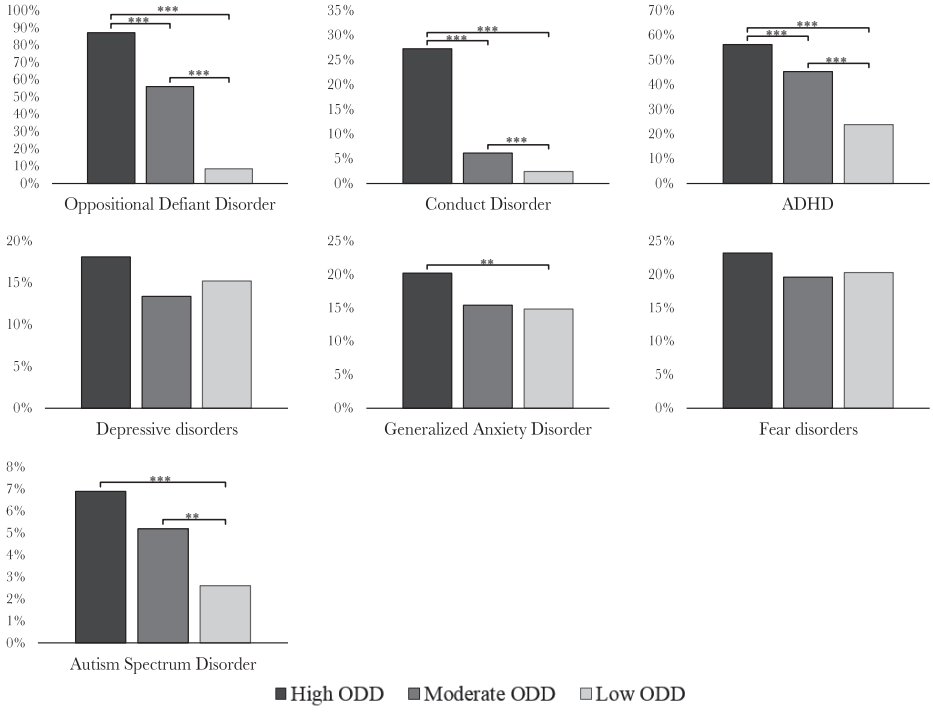

Figure 3. N = 2164. ADHD = attention deficit hyperactivity disorder. The y-axis indicates the disorder rate in each respective class. ***p <.001**p <.01.

Class comparisons: longitudinal features

Categorically assessed mental health problems

In terms of multidisciplinary team-based classifications, the High ODD class had significantly higher rates of ODD and CD than the two other ODD classes (Figure 4; see Table S8 for descriptives). Further, compared to the Low ODD class, both the High and Moderate ODD classes had significantly lower rates of GAD, the High ODD class had a lower rate of fear disorders, whereas the Moderate ODD class had a higher rate of ODD than the Low ODD class. No class differences emerged in rates of ADHD, depressive disorders, and ASD.

Figure 4 Prevalence of Clinical Classifications and Differences Between the Oppositional Defiant Disorder Classes

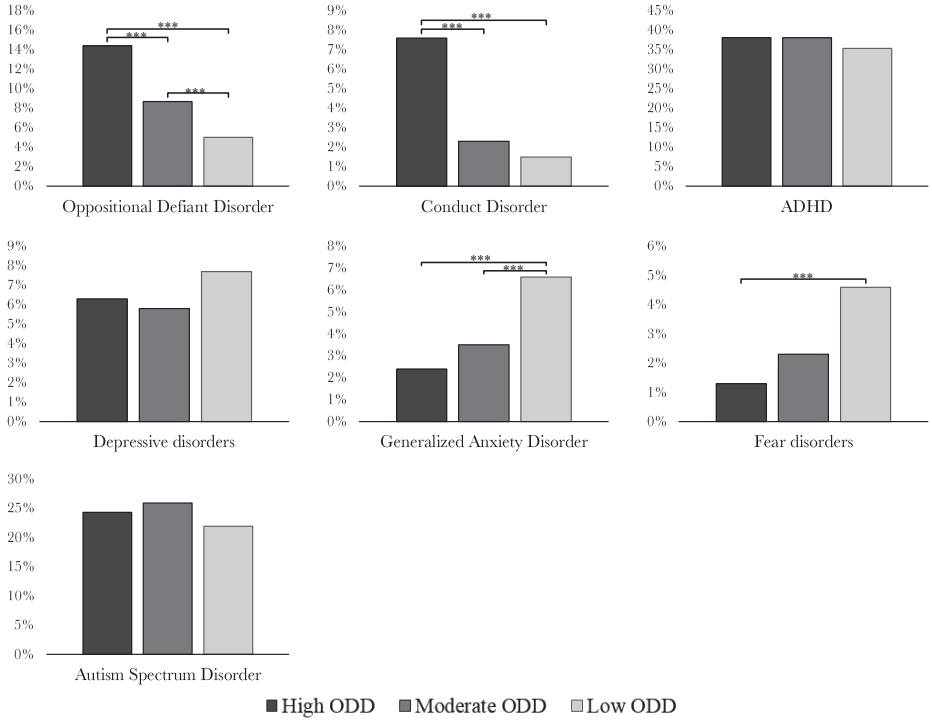

Figure 4. Note. N = 2041. ADHD = attention deficit hyperactivity disorder. The y-axis indicates the disorder rate in each respective class. ***p <.001**p <.01.

Pre- and posttreatment functioning

The three ODD classes differed in terms of clinician-rated GAF scores at both the beginning F(2, 1994) = 19.58, $p \leq .001$, range d: 0.35-0.15, and end of treatment, F(2, 1627) = 22.22, $p \leq .001$, range d: 0.43-0.18, with the High ODD class showing the highest impairment (start of treatment: M = 51.14, SD = 6.02; end of treatment: M = 52.85, SD = 6.42), followed by the Moderate (start of treatment: M = 52.39, SD = 6.30; end of treatment: M = 54.44, SD = 7.80), and Low classes (start of treatment: M = 53.43, SD = 7.14; end of treatment: M = 55.81, SD = 7.25). All classes increased in functioning during treatment, F(1, 1625) = 207.56, $p \leq .001$, $\eta p^2 = .11$, though these changes were independent of class membership F(2, 1625) = 1.20, $p = .30$].

DISCUSSION

Model-based clustering analyses in clinic-referred youths showed three distinct ODD (Oppositional Defiant Disorder) classes: High ODD (high in irritability and oppositionality), Moderate ODD (moderate levels of irritability and oppositionality), and Low ODD (low in irritability and oppositionality). We could not find children and adolescents who were solely high in oppositionality (Oppositional ODD class), or solely high in irritability (Irritable ODD class). Instead, the overall severity of the ODD symptoms differentiates between individuals, suggesting that classification of clinic-referred youths based on ODD typologies, whether it be oppositionality and irritability or headstrong, hurtful, and irritable behavior, is unrealistic. Furthermore, in contrast to considering the mere presence of ODD symptoms, an approach which incorporated ODD symptom severity, duration, and impairment resulted in a viable class differentiation, that proved stable across age and gender, suggesting that these can be identified through childhood and adolescence, and in girls and boys.

There are several, partially overlapping, explanations why the present study failed to find ODD classes which were solely high in irritability (Irritable ODD class) or solely high in oppositionality (Oppositional ODD class). First, data-driven studies in clinic-referred boys(Burke, 2012) and detained male adolescents (Aebi, Barra, et al., 2016), which found Oppositional and Irritable ODD classes, were relatively underpowered for the LCAs performed (Burke, 2012). Hence, it cannot be excluded that these classes emerged as a chance finding. Second too many patients may display irritability (e.g., those with Major Depressive Disorder), oppositionality (e.g., those with ASD), or both (e.g., those with ODD), thereby restricting the likelihood to find Irritable ODD and Oppositional ODD classes. Third, the strong correlation between irritability and oppositionality in our study (r = .62, see Supplement 1) might explain why only classes of increasing severity emerged.

Importantly, this overall increase in ODD symptom severity also indicates that other proposed subtyping approaches of ODD (Burke, Hipwell, & Loeber, 2010; Stringaris & Goodman, 2009b), including the DSM's differentiation between angry/irritable mood, defiant/headstrong behavior and vindictiveness (American Psychiatric Association, 2013), as well as the ICD's distinction between ODD with chronic irritability-anger and ODD without chronic irritability-anger (World Health Organization, 2018), are unsuitable to classify

individuals into mutually exclusive groups or classes. In addition, our results also deny the existence of a theoretically proposed ODD class comprised of youths with predominantly non-compliant symptoms and without anger and irritability (Drabick & Gadow, 2012). However, aside from classification, the ODD dimensions' distinct correlates can still provide some clinical relevance. For example, irritability is mainly associated with affective problems, while oppositionality correlates with Attention Deficit Hyperactivity Disorder (ADHD), Conduct Disorder (CD), and delinquency (Hipwell et al., 2011; Vidal-Ribas et al., 2016). In sum, our results do raise the question to what extent distinct diagnostic groups in a psychiatric setting can be found that merely display one type of ODD behavior.

Rather, we found indications that besides serving as a differentiating characteristic, overall ODD symptom severity may serve as a guidance for ODD treatment. The High ODD class, overall, showed the highest levels of concurrent parent-, teacher- and/or self-reported hyperactivity, peer, and total mental health problems, and lower levels of prosocial behavior, followed by the Moderate and Low classes. With regard to DAWBA computer-generated classifications at referral, the High ODD class showed higher rates of ODD, CD, and ADHD than the two other classes, and higher rates of GAD and ASD than the Low ODD class. Though fewer differences emerged between Moderate and Low ODD classes, youths in the Moderate class were more troubled at referral in terms of dimensionally and categorically assessed mental health, and other problems. Altogether, the High ODD class constitutes the smallest class (26.4% of our sample), but appears to be the most troubled group at referral.

Importantly, the SDQ and computer-generated DAWBA classifications simply count the presence of problem behavior, and cannot explain why symptoms occur (e.g., ODD symptoms as a manifestation of ODD or as a consequence of ASD). Clinicians are able to oversee different co-occurring symptoms and weigh their relative importance to one another. Therefore, it is crucial to test if ODD classes differ in a meaningful manner when considering the clinician-rated and multidisciplinary team-based classifications at the end of the diagnostic process. Findings indicated higher rates of ODD and CD in the High ODD class compared to the other classes, which is not surprising since the ODD classes are based on ODD symptoms, while CD frequently co-occurs with ODD (Frick & Nigg, 2012; Rowe, Maughan, Pickles, Costello, & Angold, 2002). The High ODD class also had the lowest levels of posttreatment functioning as measured by the

GAF, followed by the Moderate and Low classes. Finally, the Low ODD class had the highest rate of clinician-rated GAD classifications compared to the High and Moderate ODD classes, and a higher rate of fear disorders compared to the High ODD class. Overall, this pattern of findings at the end of the diagnostic process contrasts with those at referral. This discrepancy may suggest that clinicians consider externalizing problems, like ODD or CD, to be the main problems of youths in the High ODD class. However, the discrepancy also indicates that, although externalizing problems are deemed the main problem in the High ODD class, affective problems are very prevalent. In sum, findings indicate that ODD classes based on low-cost questionnaires at referral, are clearly predictive of clinically relevant outcomes as rated by clinicians months later. Interestingly, this study also shows that less severe ODD features at referral already bear prognostic usefulness. To illustrate, the Moderate ODD class, consisting of youths with modest levels of ODD behaviors, showed considerable worse functioning compared to the Low ODD class.

This study has several strengths: its large clinical sample, reliance on cross-sectional and longitudinal data that were collected for applied clinical purposes, and its use of multiple informants. As always, there are several limitations. First, a part of the clinic-referred sample had no ODD-report available (790 excluded vs. 2185 included). Therefore, we cannot exclude a minor selection bias, for example, some parents did not meet the screening thresholds for the ODD questionnaire. This could make it relatively difficult to detect groups with one type of ODD behavior, like the Irritable and Oppositional classes. Nevertheless, considerable higher rates of ODD reports were available (73.4%) than regular referral rates because of behavioral problems (50%; Hubbard, McAuliffe, Morrow, & Romano, 2010; Sytema et al., 2006). Hence, we likely included the vast majority of youths with behavioral problems. Second, treatments were quite heterogenous, and we were unable to collect reliable data on treatment engagement, intensity, and effectivity. Third, although our data-driven analytical approach greatly enables comparison with prior work, we did not explicitly test theory-driven approaches to account for heterogeneity among youths with ODD symptoms (e.g., Drabick & Gadow, 2012). Fourth, the data in this study were already available for a large sample. Clinicians who deal with children and their families at referral need to estimate to what ODD class a youth belongs, long before data are available for analyses within one's own institution.

CONCLUSION

This study indicates that youths who were high in irritability and oppositionality, were overall, most affected in terms of global functioning, concurrent and later mental health, and other problems. In contrast with prior work, our findings suggest that irritability and oppositionality in clinic-referred children and adolescents go hand in hand, making it improbable to assign individuals to classes which are only high in one of these behaviors.

CHAPTER 5

GENOMICS OF HUMAN AGGRESSION: CURRENT STATE OF GENOME-WIDE STUDIES AND AN AUTOMATED SYSTEMATIC REVIEW TOOL

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PSYCHIATRIC GENETICS (2019)

ABSTRACT

Objectives: There are substantial differences, or variation, between humans in aggression, with its molecular genetic basis mostly unknown. This review summarizes knowledge on the genetic contribution to variation in aggression with three foci: 1) a comprehensive overview of reviews on the genetics of human aggression, 2) a systematic review of genome-wide association studies (GWASs), and 3) an automated tool for the selection of literature based on supervised machine learning.

Methods: The phenotype definition "aggression" (or "aggressive behaviour", or "aggression-related traits") included anger, antisocial behaviour, Conduct Disorder, and Oppositional Defiant Disorder. The literature search was performed in multiple databases, manually and using a novel automated selection tool, resulting in 18 reviews and 17 GWASs of aggression.

Results: Heritability estimates of aggression in children and adults are around 50%, with relatively small fluctuations around this estimate. In 17 GWASs, 817 variants were reported as suggestive ($P \leq 1.0E^{-05}$), including 10 significant associations ($P \leq 5.0E^{-08}$). Nominal associations ($P \leq 1E^{-05}$) were found in gene-based tests for genes involved in immune, endocrine, and nervous systems. Associations were not replicated across GWASs. A complete list of variants and their position in genes and chromosomes is available online. The automated literature search tool produced literature not found by regular search strategies.

Conclusion: Aggression in humans is heritable, but its genetic basis remains to be uncovered. No sufficiently large genome-wide association studies have been carried out yet. With increases in sample size, we expect aggression to behave like other complex human traits for which GWAS has been successful.

INTRODUCTION

Aggression is a common type of human behaviour (Tuvblad & Baker, 2011) and is considered a characteristic that is shared by all humans (Veroude, Zhang-James, et al., 2016). The propensity for aggression, however, varies considerably between individuals. This paper addresses the question to what extent the variation that is seen for aggression has a genetic cause. Broadly, aggression can be defined as a behaviour that intends to cause physical or emotional harm to others (Anderson & Bushman, 2002). High levels of aggression are also seen in individuals with severe mental disorders (e.g., autism, bipolar disorder, schizophrenia) as well as in patients with (rare) Mendelian disorders (Zhang-James et al., 2019). Because of the large impact of aggression on the affected individual, their families, their environment, and society as a whole, there is a substantial interest in studying aggression from a wide range of disciplines. In this context, one goal is to unravel the aetiology of aggression by identifying environmental exposures and biomarkers, including genetic factors, epigenetic marks, and metabolites, that could function as predictors of (excessive) aggression (Boomsma, 2015b).

Research often focuses on the pathological aspects of aggressive behaviour, while aggression does not solely have negative consequences or outcomes. Under certain circumstances, aggressive behaviour is beneficial to individuals, for example when competing for limited resources, like food or mates (Lindenfors & S.Tullberg, 2011), or achieving social dominance (Little et al., 2007). Aggression can further be a powerful deterrent against aggressive behaviour from others. Because both high and low levels of aggression can be detrimental to survival and procreation, it has been postulated that aggression is under stabilizing selection, implying that variation in aggression should show significant heritability. Substantial heritability estimates have indeed been reported in animals (Anholt & Mackay, 2012) and humans, as reviewed below.

Benefits of aggressive acts depend on the type of aggression, its success, environmental circumstances and also vary across cultures (Rubin, Bukowski, & Laursen, 2011). For example, predatory goal-oriented aggression has been associated with social dominance in some instances (Dodge, Lochman, Harnish, Bates, & Pettit, 1997; Hawley & Vaughn, 2003; Voulgaridou & Kokkinos, 2015), but this association seems to vary between groups that are more prosocial and groups that consist predominantly of individuals with disruptive behaviour problems (Wright, Giammarino, & Parad, 1986). A decrease in social status

can also result from aggression, in particular from reactive aggression, which is an uncontrolled type of aggression stemming from internal or external frustration. In reverse, after a conflict, proactive aggression is increased in the victorious party while the losing party is less likely to engage in another aggressive act (Penn, Zito, & Kravitz, 2010; Polman, Orobio de Castro, Koops, van Boxtel, & Merk, 2007). To differentiate between different outcomes of aggression, researchers have distinguished aggression subtypes (e.g., reactive vs. proactive; overt vs. covert), developmental stages (childhood vs. adolescent onset), and comorbidities (e.g., with internalizing problems or with attention deficit hyperactivity disorder (ADHD)). In summary, the outcomes and types of aggressive acts can differ greatly between persons and circumstances, and need not always be dysfunctional.

At the start of the 1990s, research on aggressive behaviour was given a new impulse by a seminal paper of Brunner et al. (1993), in which a Dutch pedigree was described where men exhibited impulsive aggression, arson, violence and borderline mental retardation. The family appeared to have a rare point mutation in the structural gene for monoamine-oxidase-A (MAOA) – which codes for an enzyme that is involved in the oxidative deamination of neurotransmitters like dopamine, serotonin and norepinephrine – resulting in a deficiency of the MAOA enzyme. A study, by Caspi et al. (2002), compared variants of the MAOA gene in children who experienced maltreatment and showed that children with the variant resulting in lower levels of the MAOA enzyme were more likely to develop antisocial behaviour. Efforts to replicate the latter finding have been contradictory, either without replication (Haberstick et al., 2005; Young et al., 2006) or with replication (Foley et al., 2004; Kim-Cohen et al., 2006; Nilsson, Åslund, Comasco, & Oreland, 2018). Nevertheless, the studies of Brunner and Caspi stressed the importance of biological factors in the development of aggression and antisocial behaviour. This instigated extensive efforts to study the genetic basis of aggression.

Enormous progress has been made with respect to technology in molecular biology and large-scale genotyping, as well as in the development of statistical methods for genetic association studies and polygenic scores for individual risk assessment, once sufficiently large genetic-association studies are available (Dudbridge, 2016). Costs for genotyping and sequencing of DNA, the epigenome and of RNA, and biomarker assessment, such as metabolomics, have steadily decreased, allowing for large studies, relating aggressive behaviour to genome,

epigenome, transcriptome and other biomarkers (Hagenbeek et al., 2016). Progress also has been made in characterizing the exposome, which reflects the totality of a person's environmental exposures in space and time (Wild, 2005).

Genome-wide association studies (GWAS) provide a conceptual framework to examine whether individual differences in aggression are associated with allelic differences in millions of single nucleotide polymorphisms (SNPs) across the genome (Visscher et al., 2017). Because a GWAS targets the entire human genome, it enables a data-driven approach to identify loci of interest. This hypothesis-free approach could potentially help researchers to overcome limits imposed by multifactorial nature of a trait and incomplete understanding of its physiological basis.

Here we synthesise knowledge deriving from studies on genetics of human aggression and variance in liability to aggression-related traits. Our review has three foci: 1) to give a comprehensive overview of reviews already done on genetics of human aggression, 2) to carry out a systematic review of GWAS studies on human aggression, and 3) to introduce an automated systematic review for the selection of relevant literature based on supervised machine learning. For consistency, in this review we will use the general term "aggression" (or "aggressive behaviour", or "aggression-related traits") to refer to the terminologies used by different authors (see Supplement S1), including anger, hostility dimensions, parent-reported child aggressive behaviour, physical aggression, antisocial behaviour (ASB), violent offending, conduct disorders (CD), oppositional defiant disorder (ODD), and antisocial personality disorder (ASPD).

METHODS

To optimize detection of the relevant literature for our review, we incorporated two strategies:

a) A "traditional" (manual) search strategy where search terms were used to extract the relevant articles from literature databases.
b) An automated screening with Automated Systematic Review Software (ASR) where relevant articles were detected via the utilization of machine learning algorithms and a software development platform.

Traditional approach

Search strategy

Search terms were developed by the authors based on prior literature and discussions with an expert librarian (J.W.S) from the LUMC. A literature search was performed in PubMed, Embase, Web of Science, Cochrane library, PsychInfo and Academic Search Premier with a comprehensive list of general search terms and medical subject headings (Supplement S2). Searches were conducted separately for reviews/meta-analyses and GWA studies. Searches included literature without a specific time limit and were conducted in mid-April 2019.

Selection criteria

A selection was made from all titles and abstracts that were found in the databases using pre-specified inclusion and exclusion criteria (see Table 1). Articles were included if they (1) were written in English and (2) focused on human aggression. Studies were excluded if (1) they focused on animals, or (2) general terms linked to "aggression/violent etc." did not refer to a psychological/ psychiatric perspective but rather to characteristics of disease (e.g., aggressive cancer), or (3) articles discussed only a single gene. Psychiatric disorders which incorporate acts of aggression and are highly correlated to aggression and antisocial lifestyles, like Oppositional Defiant Disorder (ODD), Conduct Disorder (CD), and Antisocial Personality Disorder (ASPD) were included. Papers referring to associations between genetic data and other (neuro)psychiatric disorders as main outcome (e.g., psychosis, borderline personality disorders, schizophrenia, bipolar disorder, anxiety, major depression, intellectual disability, Alzheimer's disease, autism, ADHD, addictions) as main outcome were excluded. This increased the probability that the genetic profile that we examined was not confounded due to high comorbidity of aggression with other psychiatric disorders. Papers referring to aggression from the perspective of victimization and bullying were excluded. The publications were reviewed independently by 2 authors (V.V.O and P.J.R.), and when in doubt other co-authors were consulted until consensus on inclusion was reached.

Selection procedure and analyses

The search on review/meta-analyses resulted in 1,713 records (see Figure 1). Duplicate entries were removed (N=27). Next, 1,660 records were excluded based on screening the titles and abstracts. In total, 26 potentially relevant

reviews were retrieved for a full-text screening. Studies that did not fulfil or only partially fulfilled our criteria were excluded from the analysis (N=12), leading to the inclusion of 14 articles. Four additional reviews were added through the automated selection, leading to a total of 18 articles – 13 targeted and 5 systematic reviews. These were organized into the following categories: review type (targeted or systematic), definition of aggression, type of reviewed studies (heritability, candidate gene, GWAS), population (children, adolescents, adults), quantity and period of the publications included in the reviews (parameters are made on the basis of reference lists with inclusion of publications on the aggression-related traits), described genes and main conclusions.

Table 1 Inclusion and exclusion criteria for the systematic review

Selection Criteria	Inclusion Criteria	Exclusion Criteria
Language	English	Non-English
Population	Human studies (all ages)	Animal studies
Use of term "aggression"	Psychological/psychiatric	Disease characteristics (e.g., aggressive cancer, aggressive form of somatic diseases etc)
		Victimization, victims of bullying
Psychiatric disorders	ODD, CD, ASPD	Other neuropsychiatric and psychiatric disorders (e.g., psychosis, anxiety etc)
Discussion of genes	At least 2 genes associated with aggression*	No genetic methods and information on genes associated with aggression

*This was done to exclude reviews focussing on a single candidate gene.

The search for GWASs on aggression resulted in 356 records. A total of 331 were excluded based on screening of the titles and abstracts. This led to the retrieval of 25 potentially relevant studies for full-text screening. Studies that did not fulfil or only partially fulfilled our criteria were excluded (N=8), leading to the inclusion of 17 GWAS articles. Three additional studies were selected from the automated selection, including 1 SNP-heritability and 2 linkage studies. The studies were analysed by phenotype, sample characteristics, SNPs or genetic variants associated with aggression-related traits at $p < 1E^{-05}$, genetic variants position in genes and chromosomes.

Several GWAS papers report findings on multiple (stratified) GWASs. Tielbeek et al. (2017) adjusted for the fact that they performed three genome-wide association meta-analyses (GWAMA) by setting the genome-wide significance

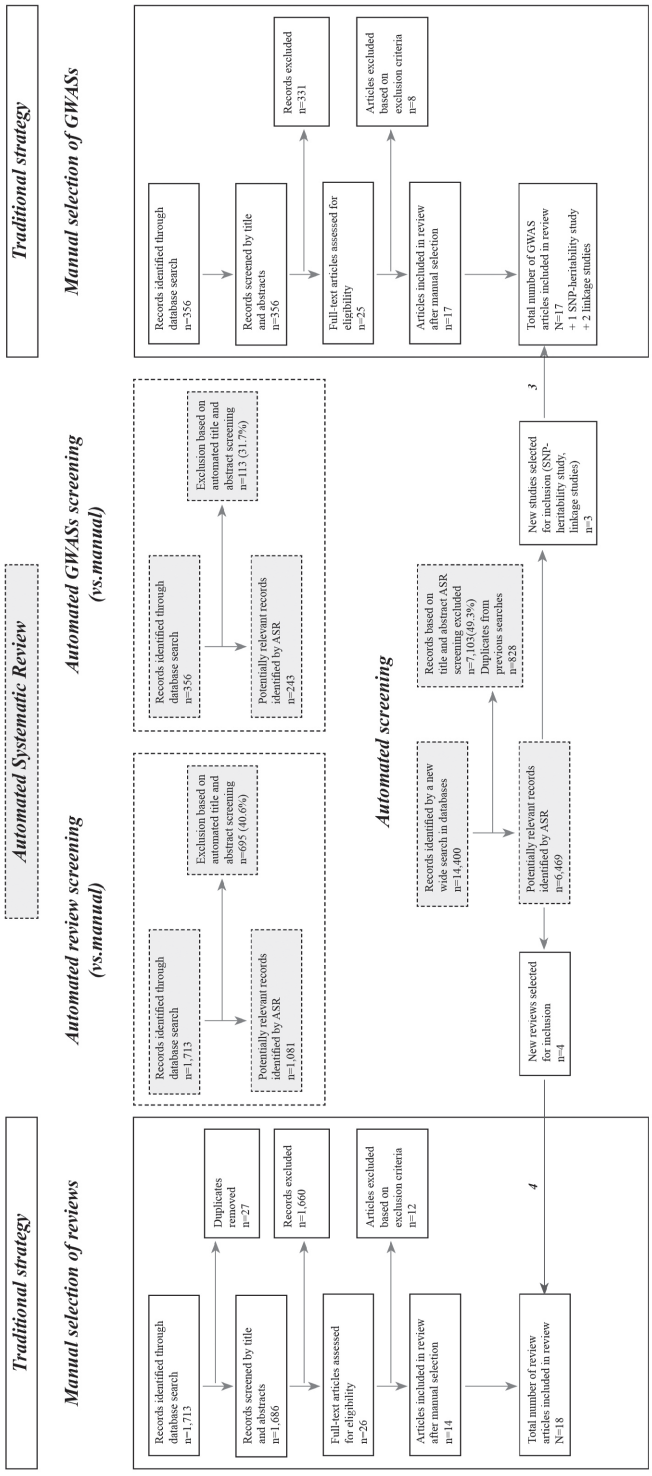
threshold at $p = 1.67E^{-08}$, whereas others did not apply such a correction. This threshold might be overly conservative as the GWAMAs are stratified, which makes the p-values non-independent across GWAMA. Therefore, we maintained a significance threshold of $p = 5.0E^{-08}$ for all studies, and denote any SNP with a p-value below this threshold as genome-wide significant. While the traditional threshold might be too lenient in this context, we note that, when discussing GWASs, the p-value of a SNP in any given study is of less relevance than replication across GWASs.

Automated titles and abstracts screening

In parallel with the manual selection of titles and abstracts, another selection was made with the use of an automated selection tool "Automated Systematic Review" (ASR) – software hosted at https://github.com (*Automated systematic reviews by using Deep Learning and Active Learning*, 2019). This software allows for automated in- and exclusion of articles for systematic reviews based on the titles and abstracts of articles. This enabled a comparison between "traditional" manual selection and the automated screening on performance characteristics (e.g., time spent on selection, false negative results). Furthermore, an additional selection was performed with the ASR on a large dataset of references to retrieve any new additional papers to our review, which would have been missed in the traditional search strategy (see Supplement S3).

We trained a model using ASR. To do so, the model requires a training set based on expert knowledge, consisting of papers that are either labelled relevant or non-relevant (labels 1 = included , 0 = not) (see Supplement S3: Figure S3.1). To study the operating characteristics of the ASR, we used a dataset (N=2,955) consisting of relevant and non-relevant papers on the genetics of human aggression, as labelled by researchers. From this labelled dataset of N=2,955, 500 records were repeatedly drawn at random as training sets. The number of relevant records in the training sets varied between 10 and 80 (e.g., 10 relevant records versus 490 non-relevant records), in increments of 10. These sets were used to train models to include relevant records and exclude non-relevant records. For each model we computed Receiver Operating Characteristic (ROC) parameters that were then used to select the optimal model (see Supplement S1: Table S3.1, Figure S3.2).

Figure 1 Flow diagram of literature selection



We selected the model that returned the lowest false positive rate (FPR) while allowing for a maximum false negative rate of FNR = 0.03 at most. Note that FNR = 0.03 corresponds with a true positive rate of TPR = 0.97.

We applied the optimal model to predict classification in different searches: (1) reviews of genetics of human aggression (1,713 records); (2) GWASs on human aggression (356 records); (3) searches 1 and 2 combined (2,069 records) to analyse parameters of automated selection in comparison to manual selection.

Training sets were provided to the ASR for the reviews on aggression (26 relevant records out of 1,713 [1.5%]) and the GWASs on aggression (25 relevant records out of 356 [7.0%]) (see Supplement S3: Table S3.2). The automated selection predicted 1,018 records out of 1,713 (59.4%) as relevant for reviews (including all pre-labelled positives: TPR = 1.0; FPR = 0.59) and 243 records out of 356 (68.3%) for GWAS (including 24 pre-labelled positives: TPR = 0.96; FPR = 0.66). Automated selection predicted 1,261 records out of 2,069 (60.9%) as important (including 50 pre-labelled positives: TPR = 0.98; FPR = 0.60). The workload for manual selection was ~60 hours. This means that for the applied model and these set(s), the reduction in workload is expected to be ~23.5 hours. By allowing for a higher FNR in model selection, the workload could be reduced even further, although at the expense of missing more true positives.

Our automated selection repeated the traditional manual search with inclusion rates (100% for reviews [58.8% false positives], 96.0% for GWASs [66.2% false positives], 98.4% for reviews and GWASs combined [60.0% false positives]), 0 cases were false negatives for reviews, 1 case for GWASs, and 1 case for reviews and GWASs combined.

A new search on "human aggression genes" was performed in the same databases without additional search terms and time limitation (14,400 records) to detect new contributions to the systematic review, resulting in 55.8% included records. Exclusion of duplicate records resulted in 6,469 records. From these, four reviews were added to the overview of reviews on aggression, and one SNP-heritability and two linkage studies were added to the GWASs review as additional information for the interpretation of GWAS findings. These seven studies were detected only by the ASR approach and did not appear in the traditional approach.

RESULTS

We included 18 reviews on the genetics of human aggression in our analyses, each covering different periods and including varying numbers of studies (see Table 2). The reviews cover more than 2,000 studies on aggression.

What is considered to be aggression?

Reviews indicate that the phenotypic definitions of aggression vary considerably, and heterogeneity of the phenotypic definition is mentioned as a major hurdle in aggression research by multiple papers. Definitions of aggression, as well as the focal points of reviews, range from broadly-defined externalizing and antisocial behaviours (see Supplement S1), which also include potentially non-aggressive behaviours like rule-breaking behaviour (Fernàndez-Castillo & Cormand, 2016), to a narrow focus on chronic physical aggression (Tremblay, Vitaro, & Côté, 2018). Other reviews and studies focus more explicitly on psychiatric classifications like Oppositional Defiant Disorder (ODD), Conduct Disorder (CD), and Antisocial Personality Disorder (ASPD), which encompass aggressive acts and are correlated to antisocial behaviour (ASB; Raine, 2019; Veroude, Zhang-James, et al., 2016)One review incorporated the analysis of genetics of aggression in suicidal behaviour (Baud, 2005). Classifications which are useful in clinical practice (e.g., American Psychiatric Association, 2013), tend to consist of constellations of heterogeneous antisocial behaviours (e.g., "often initiates physical fights" vs. "is often truant from school") and personality characteristics (e.g., "having difficulty sustaining long-term relationships" vs. "lacks concern, regret or remorse about other people's distress").

Table 2 Reviews on genetics of human aggression

Review	Type of studies included	N papers with trait-related studies	Taxonomy of aggressive behaviour (phenotype)	Samples
Baud (2005)	heritability studies, CGS	91	limited discussion of genetics studies of aggression, impulsivity and anger-related traits in suicidal behaviour	humans
Moffitt (2005)	Heritability studies (twins, adoption, family)	117	antisocial behaviour	children, adolescents, adults
Craig and Halton (2009)	heritability studies, CGS, GWAS	117	human aggressive behaviour; instrumental (proactive) and reactive	humans
Tuvblad and Baker (2011)	heritability studies (twin and adoption studies), CGS	138	human aggressive behaviour	children, adolescents, adults
Anholt and Mackay (2012)	CGS, GWAS	127	aggression as quantitative trait, pathological aggression (in substance abuse, psychiatric disorders, Alzheimer), externalizing behaviour	humans and animals
Vassos et al. (2014)	CGS	185	aggression and violence (categorical and continuous outcomes)	general population and specific subgroups

Discussed genes and polymorphisms in association with aggressive behaviour	Main conclusions
TPH, MAOA, COMT polymorphism	Aggression and unprovoked anger could be associated with intronic polymorphism in the *TPH* gene, VNTR regulatory polymorphism in the promoter region of the gene for *MAO-A*. The *COMT* genotype could differentially affect outwardly and inwardly directed aggressive behaviour.
MAOA, 5-HTTLPR polymorphisms	Environmental and genetic causes are equally important for antisocial outcomes. Heritability estimates form a curve with its peak at 50%, and small tails to the left (0% h^2) and right (80% h^2). Candidate genes should be chosen for GxE research based on a biologically plausible hypothesis that gene moderates responses to an environmental risk.
DGKA (DAGK1), GRIA3, CAG repeats, *MAOA, MAOB, SLC6A4, TPH1, TPH2, 5HT2A*, G861C, T102C, C-1021T polymorphisms, *COMT, ADRB1, NET1, SLC6A2, SLC2A1, NOS1, AVPR1A*	Genetic factors and common environment are equally important in childhood, heritability became more prominent in adulthood. Male heritability is slightly higher than that for females that implies specific genes on the X and/or Y chromosome. Genes do not operate independently, but function against a background in which other genetic and environmental factors are crucial.
MAOA, SLC6A4 (5HTTLPR), DRD2, DRD3, DRD4, DAT1, COMT, VNTR alleles of 5HTTLPR, SNPs of epinephrine and norepinephrine	About half (50%) of the variance in aggressive behaviour is explained by genetic influences in both males and females, 50% is explained by nonshared environmental factors. Form of aggression, method of assessment, and age of the subjects seem to be significant moderators. Study design and sex seem to be not significant moderators. Identification of genetic risks at the level of specific genes will reflect only an increased (probabilistic) risk and not a biological determinism.
apolipoprotein E e4 allele, tryptophan hydroxylase, serotonin 5HT-2A and 5HT-2C receptors and serotonin transporter, *COMT, MAOA, SLC6A4, DRD4, NOS-I, NOS-III*	Aggression is a quantitative trait, the manifestation of which is attributable to multiple segregating genes that are sensitive to the environment. Aggression is under stabilizing selection. It is difficult to discriminate correlations with disease status from causality in the aggressive phenotype. Polymorphisms in genes encoding the serotonin transporter and MAOA have been definitevly implicated in predisposition to aggression.
HTR1B, SLC6A4 (5HTTLPR), 5HTT-VNTR, *BDNF, COMT, SLC6A3, DRD4, MAOA-F, MAOA-M, TPH1, AR* (CAG), *DRD2*	No strong associations between selected polymorphisms and aggression outcomes are found. The candidate gene approach has not succeeded in identifying genes associated with aggression.

Table 2 Continued.

Review	Type of studies included	N papers with trait-related studies	Taxonomy of aggressive behaviour (phenotype)	Samples
Provencal et al. (2015)	heritability studies, CGS, GWAS, EWAS	176	chronic physical aggression	humans and animals
Zhang-James and Faraone (2016)	CGS	524 OMIM records	aggressive and antisocial behaviour, conduct disorder	humans
Fernandez-Castillo and Cormand (2016)	CGS, GWAS, pathways and functions	198	aggressive behaviours including aggression traits (aggressiveness, impulsive aggression, anger, externalizing behaviour, violence, delinquency or criminality) or diagnostic categories (OD, CD, ASPD, CU, and psychopathy)	humans
Veroude et al. (2016)	heritability studies, animal models, CGS, GWAS	378	RDoC nomenclature: frustrative non-reward, defensive and offensive (or proactive) aggression. ODD, CD, APD	humans (children, adolescents, adults) and animals

Discussed genes and polymorphisms in association with aggressive behaviour	Main conclusions
5-HT, MAOA, DRD2, SLC6A4, methylation patterns of *NR3C1*, *PCDH*, *SLC6A4*, *GR* and *CRH* genes, *AVPR1A*, *HTR1D*, HPA-regulating genes (*NR3C1*, *CRHBP*) and others	The response to early-life social adversity and aggression has an immune component. The immune system and the brain are interconnected through the hypothalamic-pituitary-adrenal (HPA) axis and the 5-HT system, and might play a role in the response to social adversity and in the development of chronic physical aggression through epigenetic mechanisms. T-cells could be useful to investigate.
Genes associated with aggressive behaviours in human (n=86)	A list of human disorder (n=95) have documented aggressive symptoms in at least one individual with a well-defined genetic variant; 86 causal genes were retreived.
Genes of dopamine and serotonin neurotransmission, hormone regulation and others in CGS. *BDNF, CAMK2A, DYRK1AFYN, ILVBL(FLJ39061), KIRREL3, LOC729257, LRRC7, MYRFL(c12orf28), NTRK2, PAWR, RBFOX1(A2BP1), RGL1, SHISA6* and others in GWASs.	Most CGS have identified associations with genes involved in dopaminergic and serotonergic neurotransmission and in hormone regulation. GWAS have not yet identified genome-wide significant associations, but top nominal findings are related to several signalling pathways, such as axon guidance or estrogen receptor signalling, and to neurodevelopmental processes and synaptic plasticity.
5HTT, 5HTTLPR, A2BP1, ABCG1, ADH1C, AKAP5, androgen receptor haplotype, *ANK3, AVP, AVPR1A, AVPR1B, BDNF, CAMK2A, COMT, DRD2, DRD4, DYRK1A, ESR1, FYN, HTR1B, ILVBL (FLJ39061), KIRREL3, MAOA, MFHAS1, MYRFL, NTRK2, OXTR, PAWR, PURG, RBFOX1, RIT1, ROBO2, SHISA6, SLC6A1* and others	Both CGS and GWAS approaches have identified potential susceptibility genes for aggressive behaviour. CGS have focused mainly in dopaminergic and serotonergic genes. GWAS, although not reaching genome-wide significance, have highlighted genes involved in neurodevelopmental processes and synaptic plasticity.

Table 2 Continued.

Review	Type of studies included	N papers with trait-related studies	Taxonomy of aggressive behaviour (phenotype)	Samples
Waltes et al. (2016)	heritability studies, animal models, CGS, GWAS, EWAS	248	human aggressive behaviour, reactive (impulsive) and proactive (pre-mediated) aggression	humans
Manchia and Fanos (2017)	CGS, GWAS, epigenetic, metabolomic, microbiomic association studies	87	aggression in mental illness	humans
Zhang-James et al. (2018)	GWAS	9	aggression	children, adults
Beaver et al. (2018)	heritability studies, CGS, GWAS	40	antisocial behaviour, aggression, violence	humans
Tremblay et al., (2018)	heritability studies (twin studies, adoption studies), CGS, epigenetic studies	123	physical aggression	human (children, adolescents) and animals

Discussed genes and polymorphisms in association with aggressive behaviour	Main conclusions
ABCG1, APOE, AR, AVPR1A, AVPR1B, BDNF, COMT, CRHR1, DRD1, DRD2, DRD3, DRD4, ESR1, HTR1A, HTR1B, HTR2A, MAOA, NOS1, NOS3, NR3C2, OXTR, SLC6A3, SLC6A4, TPH1, TPH2.	Heritability estimates from twin studies are highly variable. Several CTG are related to the monoaminergic neurotransmitter systems, genes regulating the HPA axis, and hormone pathways. Targeted analysis of genes known to be associated with aggressive behaviour suggests the epigenetic modulations.
ADNP2, BDNF, HTR2A, ITGB3, MTHFR, NRGN, PARD6G-AS1, TPH1, TRPS1	Specific genetic signatures of aggressive behaviour are present, which might result in substantial neurobiological alteration predisposing to behavioural dysregulation, particularly in individuals with severe mental illnesses. Environmental moderators act on the predisposing liability threshold set by genetic factors altering the expression of specific genes through, but not exclusively, changes in DNA methylation.
ACHE, ALDH5A1, ALK, AVPR1A, CACNB3, CADM1, CHMP2B, CRHR1, DNAJB5, EN2, ERBB4, FGF14, GRIA3, HDAC4, KCNJ18, LAMA2, LRRC7, MAOA, MECP2, NFKB1, OSMR, PRNP, RBFOX1, SERPINI1, WDR62	Among the top enriched pathways, several were previously well-known pathways for aggression (the dopamine, serotonin, glutamate, and GABA signalling pathways). The adult and child GWAS sets had six genes in common: *ALK, LAMA2, NFKB1, OSMR, RBFOX1*, and *WDR62*. Ranked gene list highlights 40 top genes, involved in neurotransmission, axon guidance, synaptic plasticity, learning and memory, neuronal development, or hormone signalling.
COMT, DAT1, DRD2/ ANKK1, DRD3, DRD4, DRD5, MAOA; 5HTTLPR, 5HTR2A, 5HTR1B, 5HTR2C polymorphisms, SNPs located in *C1QTNF7, DYRK1A, CDH13*	The heritability of antisocial behaviour is approximately 50%. Nonshared environmental influences account for the overwhelming majority of all environmental variance. Genetic polymorphisms involved in neurotransmission have most frequently been connected to antisocial phenotypes. Genetic and environmental influences frequently interact to predict variation in antisocial outcomes.
MAOA, DRD2, 5-HTT, SLC6A4. Methylation of glucocorticoid receptor gene and serotonergic system genes.	The development of chronic physical aggression is generally influenced by genetic and environmental factors through numerous interrelated bio-psycho-social channels from conception onwards. Involved genes vary with age and interact with the environment.

Table 2 Continued.

Review	Type of studies included	N papers with trait-related studies	Taxonomy of aggressive behaviour (phenotype)	Samples
Davydova et al. (2018)	Heritability studies, CGS, GWAS	78	aggressive behaviour	children, adults
Salvatore and Dick (2018)	Heritability studies, CGS, linkage, GWAS, GxE studies, rGE studies, epigenetics	96	conduct disorder	humans
Gard et al (2018)	heritability studies, CTG, GWAS (metaanlyses)	56	antisocial behaviour, including aggression, violence and rule-breaking	humans

CGS=candidate gene studies, GWAS = genome-wide association study, EWAS=epigenome-wide association study, GxE=genome-environment interaction, rGE=genome-environment correlation, VNTR=variable number tandem repeat, Chr=chromosome
Genes are sorted in alphabetic order. When gene name has a new name in HUGO, the old name used in the article is given in brackets.

Discussed genes and polymorphisms in association with aggressive behaviour	Main conclusions
AR, AVPR1A, AVPR1B, BAXBDNF, CASP3, COMT, DRD4, ESR1 (ER1), HTR2A, MAOA, OXT, OXTR, SLC6A, SLC6A4, TNR2A, TPH1, TPH2	Genes involved in cell adhesion, synaptic plasticity, and neurogenesis as key processes in development of aggressive phenotype may be considered as potential genetic markers for further research of aggressive behaviour
A2BP1, AVPR1A, ILVBL (FLJ39061), GABRA2, KIRREL3, LOC729257, LRRTM4/SNAR-H, MAOA, MYRFL (c12orf28), PAWR, PKD1L2, PKD1L3, RGL1, SLC6A4	Linkage studies identified regions of interest in different chromosomes, but few regions reach conventional thresholds. There is little consistency among regions identified across samples, with the exception of the region on chromosome 2. Suggestive evidence was found for SNP rs11126630 and between conduct disorder related phenotypes and *GABRA2, MAOA, SLC6A4*, and *AVPR1A* across independent samples.
Dopamine genes *DRD4, D4, DAT1, DRD2, DRD5, D5.* Serotonin genes 5-HTTLPR in *SLC6A4.* Catecholamine catabolism genes *MAOA, COMT.* Chr 1, 4, 7, 11, 13, and X. *ABCB1, C1QTNF7, LRRTM4/SNAR-H.*	The current body of work is limited by single candidate gene and GxE interaction studies that often utilize small sample sizes and imprecise measures of ASB. GWAS has not been able to identify any single gene(s) linked to ASB, emphasizing the need to look for biological substrates through which genes may indirectly impact ASB. Novel approaches, including neurogenetics and GxE studies, represent exciting potential avenues to better understanding the mechanisms of ASB.

Several reviews proposed a focus on more homogeneous or dimensional constructs of aggression (Fernàndez-Castillo & Cormand, 2016; Tremblay et al., 2018). A dimensional construct is in line with the conceptualization that pathological aggression is situated on the extreme ends of a normal distribution (Veroude, Zhang-James, et al., 2016). Some authors see a risk in the dimensional approach and note that findings might become predominantly driven by variations within normal, adaptive levels of aggression (Ferguson, 2010). However, if pathological levels of aggression are indeed the extreme end of a continuous phenotype, the same genetic and environmental factors should apply to both the normal range and extremes of the distribution.

In the end, concerns regarding heterogeneity and the impact of different phenotype definitions are empirical questions, which are currently also being asked in other GWASs of psychiatric disorders such as depression (Cai et al., 2018). Such questions can be resolved, once well-powered GWASs are available, by estimation of genetic correlations among different phenotype definitions of aggression and can also be addressed through genetic modelling of twin and family data. For example, Hendriks et al. (2020) analysed twin data collected by multiple instruments, commonly employed to measure aggression in children. While phenotypic correlations between different aggression scales could be low, a genetic multivariate analysis of these data showed high genetic correlations among different instruments. Such observations mean that different instrument tap into the same genetic liability and could be analysed simultaneously in GWAS.

Reviews that propose some sort of differentiation among aggressive behaviours, often return to a distinction between reactive and proactive aggression. Reactive aggression is commonly described as impulsive and defensive, while proactive aggression is considered predatory and premeditated. Both types of aggression may involve similar biological systems. The aminergic systems (e.g., serotonergic, dopaminergic) have been proposed as likely to regulate both forms of aggression (Waltes, Chiocchetti, & Freitag, 2016). Interestingly, Runions and colleagues (2019) argue that researchers studying reactive and proactive forms of aggression have conflated motivation (aversive vs. appetitive) and implementation (impulsive vs. premeditated) and propose that predatory aggression can also be impulsive in nature, defined as recreation instead of rage, while reactive aggression could also be delivered after a longer period of time, referring to reward instead of revenge.

The developmental aspect of aggression is a major theme in reviews (Davydova, Litvinov, Enikveeva, Malykh, & Khusnutdinova, 2018; Moffit, 2005; Provencal, Booij, & Tremblay, 2015; Tuvblad & Baker, 2011; Veroude, Zhang-James, et al., 2016; Waltes et al., 2016). Age of onset is often mentioned as an important differentiating factor for subtypes of antisocial behaviour, with aggression usually already present in early childhood, while rule-breaking behaviour and delinquency usually develop during adolescence. Tremblay (2010) proposes a developmental framework of aggression among a covert/overt axis and a second destructive/non-destructive axis as the most viable constructs to subtype disruptive behaviour (aggression, opposition-defiance, rule breaking, and stealing-vandalism). Children who display destructive and overt disruptive behaviours, especially those exhibiting chronic physical aggression, experience more risk factors early in life, engage in aggression from a young age, and have a more persistent developmental course of aggression and antisocial behaviour. A differentiation on age of onset is considered especially relevant in reviews which include epigenetics. Epigenetic changes may be triggered by early life adversity (Curry, 2019; Manchia & Fanos, 2017; Provencal et al., 2015; Tremblay et al., 2018), although variation in epigenetic marks can also reflect influences of DNA polymorphisms (van Dongen et al., 2016).

In research, aggressive behaviour often is measured by questionnaires, such as the Achenbach System of Empirically Based Assessment scales (ASEBA; Achenbach, Ivanova, & Rescorla, 2017), the Strengths and Difficulties Questionnaire (SDQ; Goodman, Lamping, & Ploubidis, 2010), or the Buss Durkee Hostility Inventory (BDHI; Buss & Durkee, 1957). Aggression scales in such instruments may include items which reflect behaviour that is related to aggression, but would not be considered aggression based on item content. For example, the ASEBA Aggressive Behaviour scale of for children contains items like “Argues a lot” or “Gets in many fights”, but also “Unusually loud” or “Suspicious”. Measures can also derive from observational studies, especially in younger children, and some experimental paradigms are available to measure aggression in across wider age ranges. Such experiments can, however, not cover the full spectrum of aggressive behaviour and, perhaps even more critically, cannot be applied in epidemiological samples.

There is a divergence between measurement of aggression in research projects compared to how (pathological) aggression is defined in clinical practice. Questionnaires are used as tools by clinicians, but the presence of these

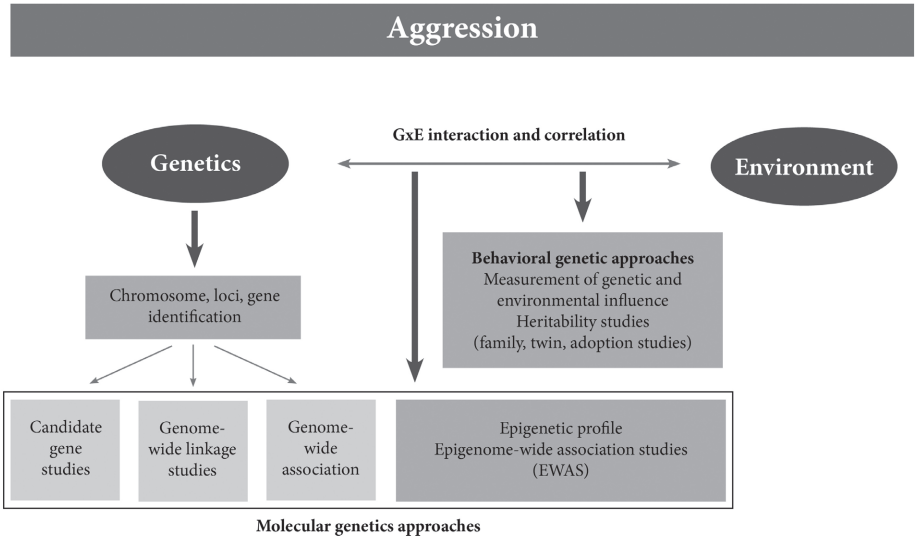
behaviours is mostly determined by interviews with the patient, and others who know the person (e.g., parents, teachers), by observation, and by the patient's (criminal) records. Psychiatric disorders that include aggressive behaviours or disorders which are correlated to aggressive and antisocial lifestyles, are dependent on classification systems like the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Diseases (ICD). In these classifications a dichotomy is applied in which a disorder is either present or absent, largely ignoring the dimensional nature of human behaviour. In genetic studies, a focus on the dichotomy rather than on continuous variation, may lead to a loss of statistical power (Van der Sluis, Posthuma, Nivard, Verhage, & Dolan, 2013).

Another important question, especially in clinical settings, is when aggression becomes pathological. Some aggressive behaviours are clearly defined as pathological, like aggressive behaviours that define Conduct Disorder (e.g., "Has used a weapon that can cause serious physical harm to others), or Antisocial Personality Disorder (e.g., "Irritability and aggressiveness, as indicated by repeated physical fights or assaults"). In contrast, other aggressive behaviours are less clearly considered pathological, because they occur to some extent in all individuals, like anger or hostility. This even is the case for some aggressive behaviours which are part of disruptive behaviour disorders (e.g., ODD: often argues with authority figures). For aggression to be pathological, it is essential that aggressive behaviours cause clinically significant impairment in social, academic, or occupational functioning.

Approaches in genetics of aggression studies and the current status quo

There are several designs to study the genetic aetiology of aggression, with the two major ones being genetic epidemiological / behavioural genetic approaches on the one hand and molecular genetic approaches on the other (see Figure 2). Behavioural genetic studies have a long and successful history (Loehlin, 2009). More recently, molecular genetic studies have seen enormous breakthroughs with the development of techniques like GWASs (Visscher et al., 2017).

Figure 2 Interplay of genetic, epigenetic, and environmental factors in behaviour and genetic studies of aggression



Behavioural genetic approaches

Numerous studies focused on explaining the aetiology of aggression and antisocial behaviour through family, twin, and adoption studies, which can disentangle genetic and environmental influences. Twin models enable researchers to divide the variance for a trait, or the liability to a disorder, into genetic and non-genetic components. The genetic variance component often is defined as the additive (A) effects of many genes. Environmental variance components consist of environmental influences common to siblings from the same family (C), creating resemblance of family members through environment rather than through genetics, and a unique or non-shared environmental component (E). Unique environmental influences affect family members in different ways (Boomsma, Busjahn and Peltonen, 2002). Unsystematic influences such as measurement error also are included in the E component, unless explicitly modelled. In general, reviews indicate that additive genetic factors explain around 50% of the variability of aggressive behaviour (Craig & Halton, 2009; Fernàndez-Castillo & Cormand, 2016; Rhee & Waldman, 2002; Tuvblad & Baker, 2011). The estimate varies around 50% across studies, with some reviews reporting somewhat higher heritability estimates (65%) and others giving estimates for aggression and

antisocial behaviour that vary more (e.g., 38%-88%; Veroude, 2016; 28%-78%; Tuvblad & Baker, 2011). Physical aggression seems to show larger heritability estimates (65%) than reactive (20-43%) and proactive aggression (32-48%), while rule-breaking behaviour, which is often aggregated with aggression indices, also shows a heritability around 50% (Gard, Dotterer, & Hyde, 2019; Waltes, Chiocchetti, & Freitag, 2015). Heritability estimates of aggressive behaviour were higher in children with stable callous unemotional traits (81%) compared to children low in callous unemotional traits (30%; Gard et al., 2019). This suggests a larger influence of genes on children with more severe aggressive tendencies (Gard et al., 2019). Contributions of shared environment are relatively small and decrease with age, with the vast majority of adult studies not reporting any shared environmental influences (Tuvblad & Baker, 2011; Veroude, Zhang-James, et al., 2016; Waltes et al., 2015). Thus, research in behaviour genetics clearly indicates that there is a substantial genetic component to aggressive behaviour in humans. In longitudinal studies, heritability estimates of aggression and antisocial behaviour increase somewhat from childhood through adulthood (Tuvblad & Baker, 2011; Veroude, Zhang-James, et al., 2016; Waltes et al., 2015). Genetic factors also contribute to the stability of aggressive behaviour during preschool and school age, and puberty (Porsch et al., 2016; Waltes et al., 2015). Measurement instrument, and also rater seem to influence heritability estimates, with heritability based on parent-report and teacher-report estimated as higher than those based on self-report and observational studies. Studies based on self-report tend not to find any shared environmental influences (Tuvblad & Baker, 2011), but such studies are not available for younger children. Unlike parent or teacher reports, observational studies more often give an assessment of aggression at one particular moment in time only. Parent- and teacher-reports tend to provide phenotype information that is more averaged over longer periods of time and are similar in terms of heritability estimates. Parent-report leads to higher estimates of shared environmental influences than teacher-report, when parental characteristics that influence ratings of multiple children (e.g., twins or siblings) are not taken into account. When twins have different teachers, similarities between them tend to decrease. This may reflect actual differences in aggressive behaviour with different teachers and/or different settings, but may also reflect teacher characteristics that influence assessments of multiple children.

In summary, heritability is estimated consistently around 50%, with some variation that may be due to different conceptualization of aggressive and

antisocial behaviours, with more severe types of aggression showing higher heritability.

Heritability estimates of aggression and antisocial behaviour may differ between environments suggesting an interaction between genes and environment (GxE). Proposed putative environmental moderators are familial adversity (e.g., maltreatment, parental delinquency), social disadvantage (e.g., poverty, bad neighbourhoods), violent media exposure, and alcohol use. Tuvblad and Baker (2011) argue that, compared to genetic factors, environmental influences are relatively more pronounced for antisocial behaviours in the presence of high environmental risk and disadvantaged environments. Conversely, genetic influences will be more pronounced when environmental risk factors are absent or less prominent. In one study, the moderating effects of neighbourhood seemed to be specific to the heritability of nonaggressive antisocial behaviour, while heritability estimates of aggressive antisocial behaviour were not influenced by neighbourhood disadvantage (Burt et al., 2016). Such findings underscore the differential influence of environmental adversity on certain types of antisocial behaviour, with aggressive behaviour showing less sensitivity to environmental influences than other types of antisocial behaviour. Later reviews, however, indicate mixed findings. Some reported an increase in genetic variance in the presence of environmental risk. To illustrate, when young children were subjected to high levels of maternal disengagement, genetic factors explained more variance in later conduct problems (Boutwell, Beaver, Barnes, & Vaske, 2012; Waltes et al., 2015). An increase in heritability of externalizing disorders was also found when young adults were exposed to a combination of risk factors (e.g., antisocial or lack of prosocial peers, relationship problems with parents (e.g., antisocial or lack of prosocial peers, relationship problems with parents; Hicks, South, DiRago, Iacono, & McGue, 2009; Veroude, Zhang-James, et al., 2016).

Depending on the type of aggression, mean levels of aggression often are higher in males than in females. Differences in heritability estimates, however, between males and females are modest or absent. According to Tuvblad (2011) heritability did not differ significantly between genders across different twin studies, either quantitatively or qualitatively (see also: Vink et al., 2012). These studies mainly included mother-reports of childhood aggression and heritability estimates were higher in males than in females when self-report data were analysed (Waltes et al., 2015). It has been suggested that gender differences in heritability become more pronounced from adolescence, which could be

indicative of the "Young Male Syndrome", in which the onset of puberty and increasing levels of testosterone are related to increases in aggression in 12- to 25-year-old males (Craig & Halton, 2009). This would also suggest a possible role of genes related to androgen synthesis and function in the development of aggression from puberty onwards.

In summary, twin studies highlight the importance of genetic influences, with estimates of the heritability of aggression and antisocial behaviour often reported to be around 50% (Moffitt, 2005), without much evidence for sex differences in heritability estimates. Such significant heritability is a first requirement for initiating studies that aim to find molecular signatures in the DNA sequence that are associated or causally related to the phenotype.

Integrating data on genetics of aggression from molecular genetic studies

Genetic linkage and candidate gene studies

Molecular genetic studies include genetic linkage and association studies, either genome-wide or with a focus on a limited number of candidate genes or candidate regions. In *linkage studies*, DNA markers are assessed in related individuals to investigate the inheritance of markers with known chromosomal locations together with aggression in pedigrees. Sometimes candidate regions to be investigated are suggested from studies in other species. With the arrival of large scale association studies, linkage studies, which require family-based designs, have become less common, but early studies have suggested regions on three chromosomes that could be associated with aggression. Dick et al. (2004) analysed retrospectively reported childhood conduct disorder in an adult sample from COGA (Collaborative Study on the Genetics of Alcoholism). Regions on chromosomes 19 and 2 may contain genes associated with risk of CD. The same region on chromosome 2 has been linked do alcohol dependence in this sample. Criado et al. (2012) in a linkage study of cortical even-related oscillations associated with ASPD and CD suggested that chromosome 1 may contain a genetic locus for ASPD/CD.

Genetic association studies initially were *candidate gene studies*. These require *a priori* knowledge of or hypotheses about which genes are implicated in the aetiology of the trait of interest.

For aggression, associations were considered for genes from the serotoninergic [*5-HTTLPR* (5-hydroxytryptamine (serotonin) receptors), *SLC6A4* (solute carrier family 6 member 4)], dopaminergic [dopamine receptors genes *DRD4, DRD2, DRD5*, and *SLC6A3* (solute carrier family 6 member 3)] and GABAergic systems [e.g., genes that code GABA (gamma-aminobutyric acid) receptors, like *GABRA2* (gamma-aminobutyric acid type A receptor alpha2 subunit)], as well as genes related to catecholamine catabolism [*MAOA* (monoamine oxidase A), *COMT* (catechol-O-methyltransferase)] (Davydova et al., 2018; Fernàndez-Castillo & Cormand, 2016; Gard et al., 2019; Provencal et al., 2015; Veroude, Zhang-James, et al., 2016). Other studies focused on associations with the genes involved in stress response pathways (Craig & Halton, 2009; Waltes et al., 2015); hormone regulation (e.g., *AVPR1A* (argenine vasopressin receptor 1A)) (Fernàndez-Castillo & Cormand, 2016; Salvatore & Dick, 2018; Veroude, Zhang-James, et al., 2016; Waltes et al., 2015); hypoglycaemia and insulin secretion (Craig & Halton, 2009); and neuronal transcripts and brain expression patterns (Anholt & Mackay, 2012; Craig & Halton, 2009; Gard et al., 2019; Waltes et al., 2015). Candidate gene studies have been criticised (e.g., Duncan and Keller, 2011), since it became clear that findings for candidate genes are often not replicated in well-powered genome-wide association studies (e.g., Bosker et al., 2011; Luo et al., 2016). It is likely that this also extends to studies of aggression, but the status of the candidate genes for aggression must await well-powered GWASs.

Many reviews agree that aggression is a polygenic trait influenced by many genes, that each explains a small proportion of the phenotypic differences. There may however be an overlap between genes of large effect underlying monogenic disorders and those affecting continuous variability of related quantitative traits. Extending the idea of a shared genetic basis between Mendelian disorders and polygenic traits, one alternative approach based on the search for genes for aggression in studies of rare, functional genetic variants associated with aggression phenotypes catalogued in Online Mendelian Inheritance in Man (OMIM; Zhang-James & Faraone, 2016). Most of these genes had not been implicated in human aggression before, but the most significantly enriched pathways (e.g., serotonin and dopamine signalling) had been previously implicated in aggression. Among these genes, only two were previously related to aggression (*MAOA*, *GRIA3* (glutamate ionotropic receptor AMPS type subunit 3). New associations were found with genes [e.g., *CAMTA1* (calmodulin binding transcription activator 1), *APBB2* (amyloid beta precursor protein binding family

B member 2), *DISC1* (DISC1 scaffold protein) and others], which are implicated in cell-to-cell signalling and interaction, nervous system development and function, and behaviour. The novel genes and pathways identified in this study suggested additional mechanisms underlying aggression.

Genome-wide association studies

Genome-wide association studies (GWASs*)* investigate millions of Single Nucleotide Polymorphisms (SNPs), under a continuous or dichotomous, case/control, model. The result is a list that, for every variant, indicates the expected increase in a trait (continuous) or genetic liability (dichotomous) for every copy of an effect allele. Due to the large number of tests, the genome-wide significance level is set at $p = 5.0E^{-08}$ (Sham & Purcell, 2014), to properly control for the type I error rate. This adjusted threshold already considers the fact that neighbouring SNPs are not inherited independently from one another. However, the non-independent inheritance of SNPs indicates that association tests between non-causal SNPs and the trait of interest contain a part of the polygenic signal (Bulik-Sullivan et al., 2015). As such – even when only a limited number of SNPs reach this stringent significance level, there is signal in the other association tests. The weighted effects of all the genetic variants involved in aggression could produce a polygenic risk score with a certain predictive value (Beaver, Connolly, Nedelec, & Schwartz, 2018).

Many reviews discussed a whole genome approach to understanding aggression, but only three have done so in a systematic manner (Fernàndez-Castillo & Cormand, 2016; Veroude, Zhang-James, et al., 2016; Waltes et al., 2015). We will summarize findings for genes harbouring, or in close proximity to, variants that reached genome-wide ($P \leq 5.0E^{-08}$) or nominal ($P \leq 1.0E^{-05}$) significance levels in all GWAS of aggression phenotypes to date. These include aggression-related phenotypes, i.e., anger, hostility dimensions, aggressive behaviour, physical aggression, ASB, violent offending, CD, ODD, and ASPD.

To provide a complete picture of the GWAS literature available, we chose to include phenotypes which clearly include aggression, but are sometimes conflated with other antisocial behaviours (e.g., rule breaking) or personality characteristics (e.g., being suspiciousness, being loud). These phenotypes can be found in Supplement S4. Most GWASs on aggression were performed in child and adolescent samples that were assessed using rating scales and were done in samples of European ancestry (see Table 3).

GWAS studies have mainly resulted in nominal associations between genetic variants and aggression-related traits and disorders. Collectively these studies reported 10 genome-wide significant findings (Dick et al., 2011; Montalvo-Ortiz et al., 2018; Rautiainen et al., 2016; Tielbeek et al., 2017). Five of these variants are located inside or close to four genes: *LINC00951* (long intergenic non-protein coding RNA 951) (Rautiainen et al., 2016), *C1QTNF7* (C1q tumor necrosis factor-related protein 7) (Dick et al., 2011), *PSMD1* (proteasome 26S subunit, non-ATPase 1) and *HTR2B* (5-hydroxytryptamine receptor 2B) (Montalvo-Ortiz et al., 2018). Lastly, the five remaining significant SNPs are located on chromosomes 11 (Dick et al., 2011; Tielbeek et al., 2017), 13 (Dick et al., 2011), 1 and X (Tielbeek et al., 2017).

In a mixed sample of subjects from European and African-American ancestry, three SNPs inside *C1QTNF7* were significantly associated with Conduct Disorder (CD) symptoms in adults with substance dependence (Dick et al., 2011). When the sample was split on the basis of ancestry, no SNPs reached suggestive levels in the European-American sample. In the African-American sample one out of the three SNPs reached suggestive levels (minimum $p = 4.35E^{-06}$), along with two additional suggestive findings (minimum $p = 2.67E^{-07}$). *C1QTNF7* is less expressed in the brain, compared to such tissues as endometrium, gall bladder, lungs, ovaries and 18 other tissues, and has a potential role in maintaining energy balance (Kaye et al., 2017).

In a study focusing on antisocial personality disorder in Finnish criminal offenders, Rautiainen and colleagues (2016) found one hit (rs4714329, $p = 1.6E^{-09}$) in the cross-sex meta-analysis. This variant is in close proximity to *LINC00951* (long intergenic non-protein coding RNA 951). The same SNPs returned suggestive associations in the male-specific GWAMA of ASPD ($p = 1.38E^{-07}$). The signal from these variants was specific for ASPD, and did not cover a broader range of criminal behaviour. Montalvo-Ortiz and colleagues (2018) found that SNPs located in the *HTR2B* ($p = 2.16E^{-08}$) and *PSMD1* ($p = 1.79E^{-08}$) genes were significantly associated with cannabis-related physical aggression in African-Americans, but these SNPs did not reach even suggestive significance in European-Americans. Cannabis use has been associated with greater impulsive decision-making and increased aggressive behaviour. Notably this is the only GWAS study which focused purely on physical aggression.

Table 3 Overview of genome-wide suggestive and significant associations with aggression-related traits at P≤1E-05 per GWASs

Study	Sample	Phenotype	$N_{variants}$	N_{genes}
Sonuga-Barke et al. (2008)	N=909 probands in trios (~87% males)z ~99% had ADHD diagnosis Age range: 5-17 years European Caucasian ancestry	CD using PACS	18	7
Anney et al. (2008)	N=938 probands in trios (~87% males) ~99% had ADHD diagnosis Age range: 5-17 years European Caucasians ancestry	CD using DSM-IV criteria for CD, PACS and CPRS-R:L, gathered the symptom on a less severe behavioural characteristic of an oppositional defiant individual.	54	41
Viding et al. (2010)	N=600 (69% males) from twin cohort (high- and low-scoring of AB) Replication N=586 (71%males) Age=7 years Caucasian ancestry	ASB/CU: Teacher-rated conduct problems and CU traits using SDQ; 3-point scale	0	0

Genes	Summary of main findings
GxE interaction with "mother's criticism" *PPM1K, ZBTB16*	Suggestive GxE interactions were reported for 18 SNPs, of which 3 SNPs also showed a suggestive main effect. For both the main and interaction effects, no SNP reached genome-wide significance.
GxE interaction with "mother's warmth" *RBFOX1(A2BP1), ADH1C (proximal), MFHAS1, SLC6A1, RIT1 (proximal)*	
LIG4 (proximal), ABHD13 (proximal); AMOLT1 (proximal), CWD15 (proximal), KDM4D (JMJD2D) (proximal); FLJ16077; RXFP1 (proximal); PAWR; LOC729257; SPATA8 (proximal); YWHAZ (proximal); FLJ31818, GPR85 (proximal); KIRREL3; PRPRD (proximal); ATP8B1 (proximal); MYRFL (c12orf28); LIG4 (proximal), ABHD13 (proximal); PKD1L2; c16orf46 (proximal); PKD1L3; KIAA0174 (proximal),; DHODH (proximal); c5orf16 (proximal); c5orf15 (proximal); FLJ39064; FZD10 (proximal); FLJ39063; FZD9 (proximal); FLJ39062; FZD8 (proximal); ILVBL(FLJ39061); FZD7 (proximal); ETV3L (proximal), ETV3 (proximal); FLJ17340; GSX1 (proximal), PDX1 (proximal); PITRM1 (proximal); RBFOX1(A2BP1); GLT25D2 (proximal); RGL1	Suggestive associations were reported for 54 SNPs. These SNPs were located in 11 genes and/or were within a 200kb window of 23 additional genes. The top five association signals were observed on Chr 13, 21, 11, 4, and 12.
Suggestive in replication (p = 4,77E^{-05}) *KCNMA1*	In both the discovery and replication study, no SNP reached genome-wide significance. Several top SNPs were located near neurodevelopmental genes such as ROBO2 (p = 4.61E^{-03})

Table 3 Continued.

Study	Sample	Phenotype	$N_{variants}$	N_{genes}
Dick et al. (2011)	N=3,963 (N_{cases}=872, $N_{controls}$=3091) Age range: 18-77 years European Americans, African Americans	CD: retrospective report of DSM-IV CD symptoms, natural log as primary CD measure.	29	10
Merjonen et al. (2011)	N=2443 (46% males) Age range: 15-30 years Followed up for 15 years European Caucasians ancestry (Finnish population)	Anger in hostility dimensions measured by the Irritability Scale of the Buss-Durkee Hostility Inventory in four time points over a 15-year interval	20	2
Mick et al. (2011)	N = 341 (64% males) ADHD offspring from 339 ADHD affected trio families Age range: 6-17 years Ancestry: NA	CBCL dysregulation subscale (anxiety/depression, aggression, attention problems subscale)	9	5
Tielbeek et al. (2012)	Combined sample N=4816 (41% males) 298 cases, 4518 controls Age range cases: 18-74 years Age range controls: 18-77 years Australians	ASB according to DSM-IV for CD Cohort 1: non-diagnostic measure covering seven items related to antisocial behaviour, case status was 3 symptoms or more Cohort 2: Diagnostic measure of ASPD, cases had a diagnoses of ASPD except for criterion D (the occurrence of antisocial behaviour is not exclusively during the course of schizophrenia or a manic episode)	22	12

Genes	Summary of main findings
In a sample with mixed ancestry *C1QTNF7*; PDE10A; SELPLG; TOX2; LOC343052; ERCC4*	European sample: were only reported for the top 20 SNPs that came out as suggestive/significant for the mixed analysis. None of the SNPs were suggestively associated with either phenotype within the European sample. Mixed sample with European and African ancestry: 4 SNPs reached genome-wide significance level for CD_{symp} – but not for CD_{cc} – two of which were located inside *C1QTNF7*. The other two significant SNPs were not located near any gene.
SHISA6; PURG	One SNP reached significance $p < 9E^{-8}$: Chr 17: rs11656526, closest gene *SHISA6*. Many associations with anger approached significance, among them SNPs located close to genes *PURG*.
FERMT3; LRRC7; STIP1; TRPT1; SEMA3A	Only results for top 50 SNPs were reported. No SNP reached genome-wide significance, but 9 were suggestively associated with DP. Out of these 9, 7 were located within 4 genes. Suggestive evidence for developmentally expressed genes operant in hippocampal dependent memory and learning associated with CBCL-DP is found.
DYRK1A; AL590874.1; CIB1; SEMA4B; TTC7B; IMMT; CSMD1; REEP1; RP11; BAZ2B; STK32A; VRK1	Sample was pooled together from two studies. Suggestive levels of significance were reached by 22 SNPs, located inside 12 genes. The gene with the strongest association was *DYRK1A*, previously related to abnormal brain development and mental retardation.

Table 3 Continued.

Study	Sample	Phenotype	$N_{variants}$	N_{genes}
McGue et al. (2013)	N=7,188 (46% males) Age: adults Caucasian ancestry	Behavioral Disinhibition; composite score consisting of five symptom counts for CD, ASB, Dissocial behavior, Delinquent Behavior Inventory, Aggressive underscore	4	1
Tiihonen et al. (2015)	Violent offending N_{cases}= 360 (94% males) Extreme violent offending N_{cases}= 56 (97% males) $N_{controls}$=5983 (57% males) Age (mean±s.d.) = 29.4±8.2 Finnish population	Violent offending; at least one sentence for violent offence. Extreme violent offending; 10 or more sever violent crimes	14	9
Mick et al. (2014)	N = 8,747 (47% males) From Atherosclerosis Risk in Communities Study. Age range: 45-64 years European ancestry	Angry temperament and angry reaction measured by SSTAS.	8	5
Salvatore et al. (2015)	Discovery N=1,379 (54% males) with alcohol dependency Age range: 18-79 years Replication N=1796 (46% males) Age range: 18-88 years European ancestry	ASB. Symptoms of DSM-IV ASPD. SSTAS	75	NA

Genes	Summary of main findings
GLIS1	Genome-wide suggestive levels were reached by 4 SNPs, tagging 1 gene.
Violent behaviour *SPIN1; NTM; ATP10B (proximal); PRMD2 (proximal); PLCB1; NXPH1 (proximal)* Extremely violent behaviour *CDH13; PRUNE2; LOC101928923*	Genome-wide suggestive levels for violent behavior were reached by 10 SNPs, mapping to 6 genes. Additionally, 4 suggestive SNPs (3 genes) were reported for extreme violent behavior.
Angry temperament *FYN (proximal), IYD (proximal), ZNFX1 (proximal), STAU1 (proximal), DDX27 (proximal)* Angry reaction ($p < 6E^{-03}$) *PHEX (proximal), SLC39A8 (proximal), MBOAT1(proximal), PLEK (proximal)*	p-values results from phenotypes adjusted for principal components representing genetic structure were used. Four SNPs reached suggestive levels of significance for angry temperament. Five SNPs reached suggestive levels for angry reaction $p < 6E^{-03}$, tagging four genes. Both scales were also dichotomized and treated as case-control phenotype, for which no SNP returned suggestive results. Results were only reported for SNPs with $P \leq 5E^{-06}$. 75 SNPs reached genome-wide suggestive levels. The top suggestive SNP on Chr 7, rs4728702, was in the *ABCB1* gene, which encodes a transporter protein. This suggestive association did not replicate in the replication sample. Found enrichment of several immune-related canonical pathways and gene ontologies, suggesting that immune and inflammatory pathways are associated with externalizing spectrum behaviours.

Table 3 Continued.

Study	Sample	Phenotype	$N_{variants}$	N_{genes}
Pappa et al. (2016)	N=18,988 9 cohorts Age range: 3-15 years North European ancestry	Predominantly parent-reported child aggressive behaviour. SDQ, CBCL, and other (parent rated questionnaires) in different cohort	76	16
Rautiainen et al. (2016)	Discovery N=6,220 (59% males) 370 ASPD, 5850 controls Age (mean±s.d.)$_{ASPD}$ = 34.5±8.0 Age $_{Controls}$ = 55.0±13.2 Replication N = 3939 (43% males) 173 ASPD, 3766 controls Age (mean±s.d.)$_{ASPD}$ = 34.2±9.2 Age $_{controls}$ = 55.0±17.0 Finnish population	ASPD (violent criminals, substance abuse, maltreatment). ASPD diagnoses, SCID-II items for DSM-IV	6	1
Aebi et al. (2016)	N=750 (87.8% males) with available ODD Age range: 5-18 years European Caucasian ancestry	ODD. CPRS-R: L. Continuous: defiant/ vindicative; irritable Case-control: low/moderate OPP vs irritable /severe OPP	53	14
Brevik et al. (2016)	N adults=1060 patients with ADHD N children= 750 with ADHD European Caucasian ancestry	Childhood aggressiveness in adult ADHD Adult sample: retroactive measure of childhood symptoms of ADHD. Child sample: CPRS-R:L, subdivided in defiant/ vindictive and irritable dimension	65	20

Genes	Summary of main findings
Overall *LRRTM4 (proximal)*;PDSS2; TRIM27 (proximal); MRC1; MECOM; CASC17 (proximal)* Early childhood *COL13A1; SDK1 (proximal); LOC101928923; TSG1 (proximal); LOC727982 (proximal)* Middle childhood/early adolescence *LRRTM4 (proximal); LOC101927797 (proximal); OPCML; COL13A1; GRIA1; ASBA; CNTN4*	Meta-analysis of nine cohorts reported one genome-wide significant hit. N35 SNPs reached suggestive levels for the overall GWAMA. These SNPs are located inside three genes and near three others.10 and 31 SNPs reached suggestive levels for GWAMA on early and middle childhood/ early adolescence AGG, respectively. Some of these SNPs overlap with the top hits reported in the overall GWAMA. In total suggestive associations were reported for 76 SNPs (66 unique) located in or around 16 genes.
Cross-sex *LINC00951 (proximal)** Males only *LINC00951 (proximal)*	Results based on meta-analysis across discovery and replication reported that for the cross-sex GWAMA, 1 SNP reached genome-wide significance while another SNP ~10Kbp away reached suggestive levels. The closest gene to these SNPs is *LINC00951*. In the male-specific GWAMA, four SNPs reached suggestive levels, two of which are the same ones as the SNPs reported in the overall GWAMA. The other two SNPs are within ~50Kbp
ADAM12; MYLK2 (proximal); OR2AG1 (proximal), OR2AG2 (proximal); BCL2L1; TPX2; DDX24 (proximal), ASB2 (proximal); RARB; RUNX1T1; FOXS1 (proximal); TTLL9 (proximal); COX4I2; SOX5; MYLK2	Results based on multivariate GWAS only reported that 53 SNPs reached genome-wide suggestive levels, which are located inside and/or near 14 unique genes.
NTM; CSMD1; KRT18P42 (proximal); TEPP; CPNE4; MICAL2 (proximal); LOC101929236; LOC101927464; NR_110053.1; H3F3A; LOC105370057; ACBD3 (proximal); LOC101929156; LOC105376469 (proximal); LOC105373223 (proximal); SPINK2; PHLPP1; UFM1	Results based on meta-analysis across adult and children samples reported that 65 SNPs – located in or near 20 genes – reached suggestive levels of associations. The strongest signal was observed at rs10826548 on Chr 10 located within the transcript of a long noncoding RNA (p = 1.07E^{-06}), closely followed by rs35974940 in *NTM* (p = 1.26E^{-06}).

Table 3 Continued.

Study	Sample	Phenotype	$N_{variants}$	N_{genes}
Tielbeek et al. (2017)	N=16,400 (47% males) Replication N=9,381 Mean age range across cohorts=6.7-56.1 years Ancestry: Mixed	Broad-spectrum ASB. Development and well-being assessment, conduct disorder scale, count of the number of APD criteria, rule-breaking behaviour, Teacher report Form, Antisocial Process Screening Device, Retrospective CD, SCID-II for DSM-IV disorders, CBCL: conduct problems (reported by mother), DSM-IV CD criteria	80	NA
Montalvo-Ortiz et al. (2018)	N=2,185 African Americans (~61% males) N=1,362 European Americans (~64% males) Replication N=89 African Americans (49% males) Exposed to cannabis use Age mean ~ 37-45 (in different cohorts European Americans, African Americans	Cannabis related physical aggression assessed with the question, "Did you ever get into physical fights while using marijuana?"	280**	43

From left to right, columns indicate (1) study, (2) sample description, (3) phenotype description, (4) number of (unique) associated SNPs/variants, (5) number of (unique) genes, (6) gene names, and (7) summary of main findings
Selection of associated with aggressive behaviour genes presented in the table is done on the base of associated SNP at $p < 1E^{-05}$ (nominally significant). Genes are sorted by ascending p in SNPs (the lowest level if gene is associated with several SNPs). When gene name has a new name in HUGO, the old name used in the study is given in brackets. The nearby location of nominally significant SNP is given in brackets (proximal), in other cases the location is intragenic.
Genes for SNPs with genome-wide significance ($p < 5.0E^{-08}$) are indicated with *
ASB=antisocial behaviour, CD=conduct disorder, CU=callous-unemotional, ASPD=antisocial personality disorder, ODD = oppositional defiant disorder, DP=dysregulation profile

	Genes	Summary of main findings
		GWAMA across five cohorts. Only independent signals are reported. The cross-sex GWAMA reports 20 suggestive associations, of which 2 are InDels. Two significant associations were found for the female-specific GWAMA. These two SNPs are located on Chr 1 and 11, respectively. The male-specific GWAMA returned one significant association on the X-chromosome. The female- and male-specific GWAMAs returned 37 and 20 suggestive associations, respectively. In total 80 unique variants (64 SNPs) were associated with ASB. ASB has potential heterogeneous genetic effects across sex.
	European ancestry *LPPR1; ARHGEF3; RARB; TMEM92; ERBB4; CCDC171; ATP10A; UST; GPRC5B; CDH13; GRIN2B* African ancestry *PSMD1*; HTR2B*; CCDC157; TBC1D10A; GSG1L; THSD7B; BRINP1; CNTN3; NSG2; SF3A1; SOD3; ADGRV1 (GPR98); KLHL3; SEC31A; ABR; TSPEAR; TMEM53; CCDC141; STAB2; RTN1; CDYL; UBE2H; LRMDA (C10orf11); ANO4; STRC; TASOR2 (FAM208B); SERTAD1; ARMH1 (C1orf228); CEP126 (KIAA1377); ABCA13; SLC17A6; LRRC4C*	European-American sample: suggestive associations were found for 76 variants, of which 7 were structural variants. The 76 variants implicate 11 genes African-American sample: the top SNPs included rs35750632 in *PSMD1* and rs17440378 in *HTR2B*. Based both on its demonstrated contribution to aggressive behaviour and functional annotation analysis, *HTR2B* is suggested to be the relevant gene.

Chr=chromosome, GWS = genome-wide significant, NA= not available, GWAMA=genome-wide association meta-analysis
PACS=Parental Account of Childhood Symptoms; CPRS-R: L=long version of the Conners Parent Rating Scale; CBCL=Child Behavioural Checklist; SCID-II=Structured Clinical Interview Axis II; DSM-IV=Diagnostic and Statistical Manual of Mental Disorders; SSTAS=Spielberger State-Trait Anger Scale; BDHI= Buss-Durkee Hostility Inventory; SDQ=Strengths and Difficulties Questionnaire; SCID-II=Structured Clinical Interview Axis II
**for Montalvo-Ortiz et al. (2018) SNPs, variants and genes are included at $p < 1E^{-06}$

Anney and colleagues (2008) listed 54 SNPs nominally associated with conduct problems. These SNPs tagged 41 genes 3 of which are with known functions and are involved in the regulation of dopamine receptor D2 signalling (*PAWR* (pro-apoptotic WT1 regulator)), synaptic plasticity (*KIRREL3* (kirre like nephrin family adhesion molecule 3)) and neuronal development (*RBFOX1* (ral guanine nucleotide dissociation stimulator like 1)). Sonuga-Barke and colleagues (2008), analysed interactions between CD symptoms and maternal warmth. Nominal effects were found for SNPs located in genes involved in brain maturation, neurotransmission, neuronal development and regeneration. Viding and colleagues (Viding et al., 2010) examined teacher-reported conduct problems in children and found no suggestive SNPs (minimum $p = 4.6E^{-05}$).

For adult ASB (Tielbeek et al., 2012) the strongest signal was for a SNP (rs346425; $p = 2.51E^{-07}$) located on chromosome 5. Salvatore and colleagues (2015) in an adult ASB sample observed the strongest association for rs4728702 ($p = 5.77E^{-07}$), located in *ABCB1* (ATP binding cassette subfamily B member 1) on chromosome 7 that may confer general risk across a wide range of externalizing behaviours. Enrichment analyses further indicated involvement of immune-related pathways. Two genome-wide association studies compared cohorts of Finnish violent offenders to the general population (Rautiainen et al., 2016; Tiihonen et al., 2015), and obtained association signals at genes involved in neuronal development (Tiihonen et al., 2015) and adaptive immunity (Rautiainen et al., 2016).

Aebi and colleagues (2016) hypothesized that *BCL2L1* (BCL2 like 1) is likely associated with oppositional behaviour, because of its influence on presynaptic plasticity through regulation of neurotransmitter release and retrieval of vesicles in neurons. Brevik and colleagues (2016) applying gene-based tests observed *NTM* (neurotrimin) as the top gene, that is differentially expressed in aggression-related structures of the amygdala and the prefrontal cortex in early stages of brain development.

Merjonen and colleagues (2011) saw suggestive associations for SNPs that lie inside genes involved in the maintenance of high frequency synaptic transmission at hippocampal synapses, and regulating synaptic activation [*SHISA6* (shisa family member 6) in a Finnish population sample]. Mick and colleagues (2011) found associations for SNPs that lie inside or close to multiple genes, including *LRRC7* (leucine rich repeat containing 7), involved in neuronal excitability and used as postsynaptic marker of hippocampal glutamatergic synapse integrity, and

STIP1 (stress induced phosphoprotein 1), involved in astrocyte differentiation and highly expressed in the brain. A second GWAS by Mick and colleagues (2014) observed a nominal association of proneness to anger with the gene, involved in calcium influx and release in the post-synaptic density, and in long-term potentiation (*FYN* (FYN proto-oncogene, Src family tyrosine kinase)). McGue et al. (2013) reported four SNPs associated with behavioural disinhibition including symptoms of CD and aggression, one of which (rs1368882; $p = 1.90E^{-06}$) was located inside the *GLIS1* (GLIS family zinc finger 1) gene responsible for a transcription factor that is involved in regulating the expression of numerous genes.

Recently, two larger studies attempted to identify genes associated with aggression or antisocial behaviour by increasing power through the inclusion of multiple cohorts. Pappa and colleagues (2016) collected a sample of 18,988 children 3 – 15 years for meta-analysis and reported a near genome-wide significant locus on chromosome 2p12 ($p = 5.3E^{-08}$). This locus is in close proximity to two genes: *LRRTM4* (leucine rich repeat transmembrane neuronal 4), which regulates excitatory synapse development, and *SNAR-H [small NF90 (ILF3) associated RNA H]*, which is implicated in the transcription process and is expressed in neurons. They found 19 genes nominally related to aggression from gene-based tests. Among them, *LRRTM4*, *PDSS2* (decaprenyl diphosphate synthase subunit 2), *TRIM27* (tripartite motif containing 27), *MRC1* (mannose receptor C-type 1), *MECOM* (MDS1 and EVI1 complex locus), and *CASC17* (cancer susceptibility 17).

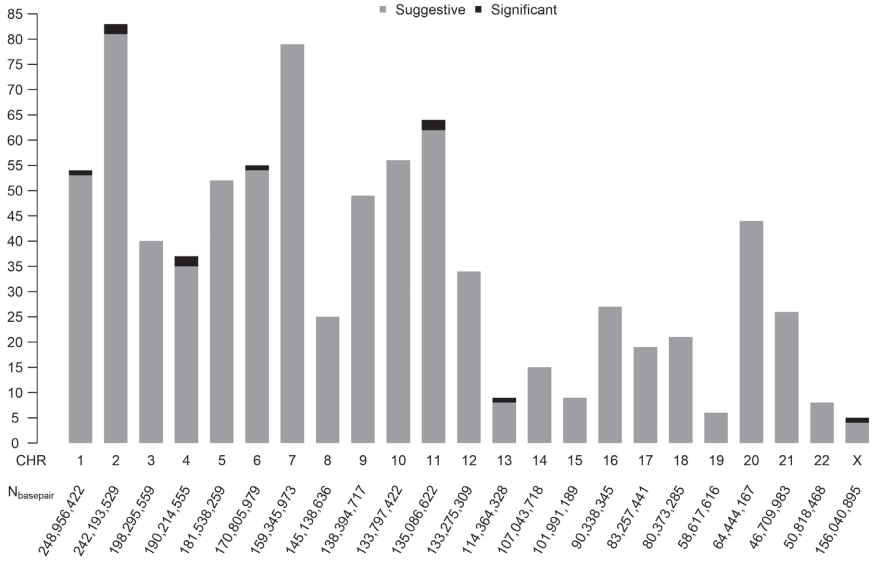
Another larger study by Tielbeek and colleagues (2017) focused on the broader antisocial behaviour phenotype in 16,400 individuals. The overall GWAMA found no hits, but sex-stratified GWAMAs returned three genome-wide significantly associated SNPs (minimum $p = 1.95E^{-08}$), but failed to identify significant genes. This suggested that there might be sex-specific genetic effects on antisocial behaviour and focusing on a more specific phenotype could improve chances of findings significant results.

Thus, nominal genome-wide associations ($p < 1E^{-05}$) have been found in genes involved in a wide variety of biological systems: the immune system, the endocrine system, pathways involved in neuronal development and differentiation and synaptic plasticity. These findings have not been replicated across GWASs, but some studies reported the same genes independently: *NTM* (Brevik et al.,

2016; Tiihonen et al., 2015) and *RBFOX1(A2BP1)* (Anney et al., 2008; Sonuga-Barke et al., 2008).

In summary, the 17 GWASs in our review show that genome-wide significant and/or suggestive associations between aggression-related traits and SNPs are found on all chromosomes (range: 1 - 63; see Supplement S5-6). As shown in Figure 3 near 55% of suggestive associations were found on chromosomes 1, 2, 5, 6, 7, 9, 10, and 11, with the majority of suggestive SNPs on chromosome 7 reported in the sample of African ancestry (Montalvo-Ortiz et al., 2018). The genome-wide significant associations are located on chromosomes 1, 2, 4, 6, 11, 13 and X.

Figure 3 Number of genetic variants associated with aggression-related traits at P≤1E-05 on different chromosomes reported the included GWAS studies



The X-axis shows chromosome number and length (in base pairs)
Nstudies = 17, Nvariants = 817

DISCUSSION

Aggression has a considerable genetic component, as indicated by decades of behaviour genetics research. However, no genomic variants have (yet) been identified. In our review covering GWASs on human aggression, only 4 out of 17 studies reported genome-wide significant hits in primary or replication samples (Dick et al., 2011; Rautiainen et al., 2016; Tielbeek et al., 2017; Montalvo-Ortiz et al., 2018). In the reviews on aggression and GWASs, several explanations are offered for the discrepancy between heritability estimates in behavioural and molecular genetic studies, for example the heterogeneous, context-dependent, and developmental nature of aggression, but foremost, small sample sizes. Fortunately, these limitations can be remedied, and provide future directions for research.

Most of the reviews covered, mention the often cited heritability estimates of 50% for aggression by Miles and Carey (1997), and 41% for antisocial behaviour by Rhee and Waldman (2002) and these estimates are confirmed in more recent empirical studies. Moderation, or any genotype x environment effects seem small, and most pronounced for non-aggressive antisocial behaviour (Burt et al., 2016).

How to address non-significant findings in GWAS studies on psychiatric problems is a pressing issue. Opinions are divided on what approach is most optimal to define phenotypes for GWAS analyses. Some believe that reduction of phenotypic heterogeneity could lead to more genome-wide significant findings (Anholt & Mackay, 2012; CONVERGE Consortium, 2015; Runions et al., 2019). This view is supported by the GWASs covered in this review that did find genome-wide significant hits. These relatively underpowered studies (Nrange = 2,185-6,220 participants) focus on individuals with severe antisocial behaviour and specific types of aggression: individuals with DSM-defined CD symptoms (Dick et al., 2011), cannabis-induced physical aggression (Montalvo-Ortiz et al., 2018), and criminal offenders with antisocial personality disorder (Rautiainen et al., 2016). Two studies were conducted in specific samples; exclusively male, with associations only in African-American subgroup (Montalvo-Ortiz et al., 2018), and predominantly male (89% of cases) and ethnically homogeneous (Rautiainen et al., 2016).

In contrast, other researchers propose a broader approach which includes more lenient phenotypes (Ormel, Hartman, & Snieder, 2019; Vassos, Collier, & Fazel, 2014). This lenient phenotyping approach has already achieved success in depression research, for example, although here the value of minimal versus

broader phenotyping is debated as well (Cai et al., 2019). The two largest GWASs on aggression that were covered by this review used broad, lenient measures of childhood aggression (Pappa et al., 2016) and antisocial behaviour (Tielbeek et al., 2017). Pappa and colleagues (2016) found no significant hits, but several promising loci on chromosomes 2, 3, 6 and 17 (minimum $p = 5.3E^{-08}$). Tielbeek and colleagues (2017) reported three significant hits for the sex-stratified GWAMAs.

Early linkage studies on aggression indicated chromosomes 1 (Criado et al., 2012), 2 and 19 (Dick et al., 2004) as potential loci. GWAS findings in our review confirm loci on chromosomes 1 and 2 which gave more associated variants and significant results. The X- and Y-chromosomes did not give evident results, even if one significant sign was reported in X-chromosome (Tielbeek et al., 2017).

In order to identify 80% of all causal SNPs, depending on the extent of SNP heritability, between 10^5 and 10^7 (100,000 – 10,000,000) independent subjects would be required (Holland et al., 2019). This means that, with sample sizes l0 time less than the lower bound, current GWASs were clearly underpowered. At present, several initiatives are under way to collaborate in achieving larger sample sizes. One example of a large collaborative project is the ACTION consortium (Aggression in Children: unraveling gene-environment interplay to inform Treatment and InterventiON strategies: http://www.action-euproject.eu/) which has brought together over 30 cohorts with childhood data on aggression for GWAS, EWAS and biomarker studies.

As mentioned, multiple reviews suggest that heterogeneity of aggression is a problem in research, with several reviews suggesting some kind of distinction between subtypes, subgroups, or developmental stages. Standardized phenotypic and environmental assessments are proposed as a solution (Craig & Halton, 2009). Although this standardization of assessment could be an option, recent advances in multivariate modelling allow for exploration of other potential avenues (e.g., Baselmans et al. 2019). This approach is also discussed in the meta-analyses of Zhang-James and Faraone (2016), in which aggression might be considered a multi-dimensional trait consisting of distinct, but related, constructs with shared aetiologies (Zhang-James and Faraone, 2016). In other words, although some individuals show different problem behaviours, including aggression, they all share a common genetic vulnerability. Taking a multivariate, approach would allow the inclusion of large cohorts with existing phenotypic (Bartels et al., 2018) and SNP data. However, the focus on ever broader phenotypes and bigger

samples, raises the question how to translate results into practice, to alleviate problems of individuals.

Future directions

We should recognize that the nature-nurture debate has moved on from the question whether aggressive behaviour is heritable to the discovery of the biological bases of aggression. This is currently achieved by investigating aggression's relation to genes, SNPs, and relevant biological pathways. It is expected that GWASs with larger or combined datasets will improve our understanding of the mechanisms of gene regulation of aggression. Individual GWASs on aggression and aggression-like traits are still limited in terms of explaining variation in the population, but ongoing GWASs and other efforts, e.g., in epigenetics and biomarker studies are likely provide insight into the aetiology of aggressive behaviour. Expansion of disease gene maps (Goh et al., 2007) by including aggression-related traits into, for example, OMIM datasets can help in future analyses of underlying cellular network-based relationships between genes and functional modules of aggressive behaviour, and future work should determine if genes mediating aggression pathways are enriched in the polygenic background of disorders associated with aggression.

Also, leveraging on Genotype-Tissue Expression (GTEx; (eGTAxProject, 2017)) GWAS findings can be annotated with additional information and thereby identify biologically relevant systems. One particularly interesting source of biological annotation revolves expression quantitative trait loci (eQTL), i.e., SNPs that have been associated with gene expression levels. Once genome-wide hits are found, overlapping these with known eQTLs could identify genes that are of biological interest (Gusev et al., 2016; Lowe & Reddy, 2015; Zhu et al., 2016).

Systematic reviews with automated functions

The workload on selection process of researchers in our systematic review was around 60 hours (screening and selecting relevant papers from list of 2,069 records). By using automated procedures to screen for relevant literature for inclusion in systematic reviews, it was possible to save 39.1% (23.5 hours) of reading/scanning time. The downside of automated methods is that relevant literature can be missed. On the other hand, even an expert reviewer might omit studies that the automated procedures include. Optimization of the expert reviewer is covered by education and training, whereas optimization of automated

selection is under active development (Borah, Brown, Capers, & Kaiser, 2017; Cohen, Hersh, Peterson, & Yen, 2006; Khabsa, Elmagarmid, Ilyas, Hammady, & Ouzzani, 2016). We opted for a recent approach that utilizes a machine learning algorithm to obtain a selection of papers that could be relevant for this systematic review.

Although the automated systematic review tool we applied is quite new and is still under active development, we found that applying the machine learning approach as implemented in the software hosted at https://github.com (*Automated systematic reviews by using Deep Learning and Active Learning*, 2019) could be indeed of considerable aid to the researcher performing a systematic review solving problems of missed literature in screening phase due to human errors or excluded by searching algorithms.

For the benefit of further developments in automated selection approaches aiding the review process, we advise review authors to supply their search results as additional information to their work. These results can then serve for further refinement of literature search models. This would avoid double work across research groups, create a comprehensive overview of aggression literature, and increase our understanding of the genetic nature of human aggression.

CONCLUSIONS

Aggression in humans is a heritable trait, whose genetic basis largely remains to be uncovered. No sufficiently large genome-wide association studies have been carried out yet. With increases in sample size, we expect aggression to behave like other complex human traits for which GWAS has been successful. There are several ongoing efforts to achieve genome-significant GWAS findings – merging samples in consortia, replication strategies, searching for close phenotypes from other domains associated with aggression for sample extension, developing new approaches of partitioning genetic heterogeneity and sample stratification. Automated tools for systematic review, which are based on machine learning, could be used to optimize the integration of research findings from different studies.

CHAPTER 6

URINARY AMINE AND ORGANIC ACID METABOLITES EVALUATED AS MARKERS FOR CHILDHOOD AGGRESSION: THE ACTION BIOMARKER STUDY

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ABSTRACT

Objective: Biomarkers are of interest as potential diagnostic and predictive instruments in personalized medicine. We present the first urinary metabolomics biomarker study of childhood aggression.

Methods: We aim to examine the association of urinary metabolites and neurotransmitter ratios involved in key metabolic and neurotransmitter pathways in a large cohort of twins (N = 1,347) and clinic-referred children (N = 183) with an average age of 9.7 years. This study is part of ACTION (Aggression in Children: unraveling gene-environment interplay to inform Treatment and InterventiON strategies), in which we developed a standardized protocol for large-scale collection of urine samples in children. Our analytical design consisted of three phases: a discovery phase in twins scoring low or high on aggression (N = 783); a replication phase in twin pairs discordant for aggression (N = 378); and a validation phase in clinical cases and matched twin controls (N = 367).

Results: In the discovery phase, 6 biomarkers were significantly associated with childhood aggression, of which the association of O-phosphoserine ($\beta = 0.36$; SE = 0.09; $p = 0.004$), and gamma-L-glutamyl-L-alanine ($\beta = 0.32$; SE = 0.09; $p = 0.01$) remained significant after multiple testing. Although non-significant, the directions of effect were congruent between the discovery and replication analyses for six biomarkers and two neurotransmitter ratios and the concentrations of 6 amines differed between low and high aggressive twins. In the validation analyses, the top biomarkers and neurotransmitter ratios, with congruent directions of effect, showed no significant associations with childhood aggression.

Conclusion: We find suggestive evidence for associations of childhood aggression with metabolic dysregulation of neurotransmission, oxidative stress, and energy metabolism. Although replication is required, our findings provide starting points to investigate causal and pleiotropic effects of these dysregulations on childhood aggression.

INTRODUCTION

Biomarkers are of interest in etiological research, or as applications in clinical practice as either diagnostic or predictive instruments in personalized medicine (Amur, LaVange, Zineh, Buckman-Garner, & Woodcock, 2015). In general, a biomarker is a measurable characteristic that can serve as an indicator of the presence or absence of a trait or disorder, as an indicator of severity, or to distinguish subgroups (Strimbu & Tavel, 2010). Biomarkers can be molecules, genes, or characteristics from invasively or non-invasively collected biomaterials, for example blood or urine, and may also include measures of some biological state like neuroimaging or resting heart rate (Strimbu & Tavel, 2010). This paper focuses on childhood aggressive behavior and addresses the question to what extent variation in aggressive behavior is associated with biomarkers assessed in urine, which is a tissue that can be obtained non-invasively. Aggressive behavior is common in children and shows considerable individual variation, with more pathological levels of aggression thought to be at the extreme end of a continuous phenotype (Veroude, Zhang-James, et al., 2016). Because of the large impact of aggression problems on children, their families, teachers, and their broader environment, there is a substantial interest in studying aggression from a wide range of disciplines, including genome, biomarker, and exposome research (Boomsma, 2015a).

Aggression can be defined as a behavior that intends to cause physical or emotional harm to others (Anderson & Bushman, 2002; Siever, 2008). Odintsova et al. (2019) summarized all reviews of genetic studies in human aggression, including an overview of "What is considered to be aggression?" They indicated that the definitions of aggression vary considerably, ranging from broadly-defined externalizing and antisocial behaviors, including rule-breaking behavior, to narrow definitions of chronic physical aggression. The broader definitions entail a range of behaviors, which are expressed differently with age (Bolhuis et al., 2017; Lubke, McArtor, Boomsma, & Bartels, 2018). For example, physical aggression peaks in early childhood around 42 months (Loeber & Hay, 1997; Loeber & Stouthamer-Loeber, 1998), while relational aggression increases during adolescence (Björkqvist & Österman, 2018). Decreases in specific types of aggression can reflect actual cessation from aggression, while sometimes a transition is made to types of aggression which are more cognitively demanding, for example, from physical aggression into relational aggression (Voulgaridou &

Kokkinos, 2015). Aggression rarely occurs in isolation, and aggressive children often experience co-occurring behavioral and social problems (Bartels, Hendriks, Mauri, Krapohl, Whipp, Bolhuis, Conde, Luningham, Fung Ip, et al., 2018; Whipp et al., 2019).

A review of the biochemical biomarker literature on aggressive behavior indicated a possible role of inflammation, neurotransmitters, lipoproteins, and several classes of hormones (Hagenbeek et al., 2016). Particularly, research has focused on the role of neurotransmitter pathways in aggressive behavior.

In general, it has been hypothesized that the dopaminergic system is involved in the initiation of aggressive behavior, the serotonergic system regulates the inhibition of aggression, while the appraisal of aggression-related cues is controlled by the gamma-aminobutyric acid (GABA) system (Willner, 2015). Most biomarker studies of aggression have been done in adults, and much of the biochemical biomarker research is on a limited range of biomarkers (Hagenbeek et al., 2016). As a consequence, it is often unknown whether changes in selected biomarkers reflect accurate representations of their putatively associated biological pathways or systems.

Recent advances in high-throughput technologies have enabled the transition to more holistic approaches in biomarker discovery in the form of metabolomics (Koulman, Lane, Harrison, & Volmer, 2009). Metabolomics allow for the measurement of a large number of metabolites, which are small molecular intermediates and products of metabolism, such as amino acids, lipids, sugars, and nucleic acids (Dunn & Ellis, 2005). Metabolomics profiles represent a functional read-out of the physiological state of the human body (Gieger et al., 2008; Suhre & Gieger, 2012). With the complex and heterogeneous nature of aggression, the combination of multiple biomarkers through metabolomics, as compared with single biomarkers, may reflect its etiology more comprehensively, and provide further insight into underlying biological processes (Boksa, 2013; Glenn, 2009). Metabolomics approaches may identify more informative markers, while knowledge from single biomarker studies can guide the selection of pathways most relevant to aggression (Hagenbeek et al., 2016). Two classes of compounds that are likely to be important in the study of aggression are organic acids, which play vital roles in critical metabolic pathways and neurotransmitter turnover (Tsoukalas et al., 2017), and biogenic amines. Neurotransmitters like serotonin, dopamine, norepinephrine, epinephrine, and histamine are all biogenic amines (Plenis, Olędzka, Kowalski, Miękus, & Bączek, 2019).

Here we present the first results from a large study on the association of childhood aggression with urinary amines, and organic acids in school-aged children (average age 9.7 years). The study is part the ACTION project [Aggression in Children: unraveling gene-environment interplay to inform Treatment and InterventiON strategies; (Bartels, Hendriks, Mauri, Krapohl, Whipp, Bolhuis, Conde, Luningham, Fung Ip, et al., 2018; Boomsma, 2015a)]. ACTION is a large collaborative endeavor which includes genome-wide genetic and epigenetic association studies, biomarker discovery, and epidemiological projects into the antecedents, characteristics, and consequences of childhood aggression. We describe the biomarker component of the ACTION project with a focus on metabolomics. ACTION has collected data for two metabolomics platforms, targeting amines and organic acids, as well as some other biomarkers of larger molecular weight: creatinine (indicator of renal health), neopterin (infection marker), oxidized DNA/RNA (oxidative stress marker), the neuropeptide Substance P, and C-peptide (indicator of insulin production). Participants were recruited from the Netherlands Twin Register (NTR; $N = 1{,}349$) and from an academic center for child and youth psychiatry in the Netherlands (Curium-LUMC, Oegstgeest; $N = 183$). We developed a standardized protocol for the large-scale collection of urine samples in children, which has been made available to the scientific community (http://www.action-euproject.eu/content/data-protocols).

The two aims of this paper were to examine whether concentrations of urinary metabolites and some larger, selected, biomarker differed between children scoring low and high on aggressive behavior and to see if we could validate the role of neurotransmitter pathways in childhood aggression. Therefore, we applied an analytical design consisting of three phases, each conducted in independent samples. First, the discovery phase assessed if aggression status was associated with urinary biomarkers levels in a sample of twins concordant for high or low aggression. Second, in the replication phase, the levels of the top 25% most strongly associated biomarkers were compared within twin pairs discordant for aggression, i.e., pairs selected in which one twin scored high and the co-twin scored low. Third, in the validation phase we assessed the top biomarkers for childhood aggression in a sample of aggressive clinical cases and low scoring twins (controls). The second aim of this paper was to examine whether we could validate the role of serotonergic, dopaminergic, and GABAergic neurotransmitter pathways in aggressive behavior for children.

To do so, we used ratios of metabolites involved in neurotransmitter anabolism (synthesis) and catabolism (degradation) in the same analytical design as described above. A series of follow-up analyses was done in which case-control status was defined at the level of the individual items. We used the same analytical design, with a discovery, replication, and validation step.

MATERIALS AND METHODS

Study population and procedures

Twin cohort

Twins from the longitudinal Netherlands Twin Register [NTR; (Boomsma et al., 2006; Ligthart et al., 2019)] were invited for participation in the biomarker study based on their longitudinal data on aggressive behavior at ages 3, 7, and/or 9/10 years. At, or around these ages, parents of twins received surveys that included the Dutch version of the Achenbach System of Empirically Based Assessment (ASEBA) Child Behavior Checklist (CBCL) for pre-school children (1.5– 5 years) or school-aged children [6–18 years; (Achenbach et al., 2017)]. Maternal data were always collected, paternal ratings are missing for some birth cohorts due to financial constraints. At ages 7 and 9/10, teachers of twins also received surveys that included the Dutch version of the ASEBA Teacher Rating Form [TRF; (Achenbach et al., 2017)] after parents consented to approach the teachers and provided contact information. Twin pairs were invited for participation in the biomarker study based on concordance or discordance for aggressive behavior rated by either the mother (93%) or teacher(s; 7%) on the Aggressive Behavior subscale of the CBCL/TRF, with an intentional oversampling of monozygotic (McLaughlin et al.) pairs. The design included twins from high-high and low-low scoring concordant pairs, and twins from discordant high-low pairs (81% MZ pairs). NTR defined age- and sex-specific Aggressive Behavior T-scores by multiplying a z-score by 10 and adding 50. High-scoring children had T-scores ≥ 65. Low-scoring children had sum scores lower than five. We selected high-high, low-low, and high-low pairs based on these criteria and additionally matched low-low pairs to the other pairs based on postal code. In the last phase of recruitment, an age-specific sum score defined high-scoring children based on mother ratings as: age 3 ≥ 13, age 7 ≥ 5, and age 10 ≥ 4.

Prior to biological sample collection in the twin cohort, a feasibility study established achievability of urine collection and storage in the home context. Parents collected first-morning urine samples (see Supplement 1 for description of buccal cell collection). Urine samples were stored at home and transported by researchers to the lab at −18 degrees Celsius. In the lab, urine samples were stored at −80°C until further processing. All parents provided written informed consent for their children's participation. At the time of sample collection, they answered a set of questions about the precise dates and times of urine collection, their children's general health, and current medication use. Parents also completed the CBCL, of which the Aggressive Behavior subscale was used to measure the twins' aggressive behavior at the time of urine collection.

From December 2014 to May 2017, 3,304 twins were invited with 1,367 twins (41.4%) agreeing to take part. The invited group comes from the larger Netherlands Twin Register. Heritability estimates of aggression were calculated from CBCL Aggression scores of the entire twin sample from which the twins who were invited into the biomarker study were drawn. The ACTION biomarker project included 1,362 twins with first-morning urine (Table S1). Twins were excluded if the collected urine was not the first-morning urine (e.g., parent-reported time of urine collection was after 12:00 in the afternoon; $N = 13$) or if the urine sample was too small to analyze both metabolomics platforms and all biomarkers ($N = 2$). This resulted in a total of 1,347 urine samples (673 complete twin pairs) in which analyses were performed. Study approval was obtained from the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center, Amsterdam (NTR 25th of May 2007 and ACTION 2014.252), an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance- FWA00017598; IRB/institute codes).

Clinical cohort

Six- to 13-years-old children were recruited who were referred to an academic center for child and youth psychiatry in the Netherlands (Curium-LUMC) between February 2016 and June 2018. This center provides inpatient and outpatient treatment programs and treats children with severe and complex mental health problems who are in need of intensive care. As part of a standardized clinical assessment, parents completed the Dutch version of the CBCL (Achenbach et al., 2017), of which the Aggressive Behavior subscale was used as an index of aggression. These data were made available to the authors

for the purpose of the present study. Specifically, parents were approached in the context of an ongoing biobank protocol approved by the ethics board of Leiden University Medical Center. For children for whom parents agreed to participate, biomaterials (buccal cells and urine) and physical measures (height, weight, resting heart rate) were also collected. Collection of biomaterials was identical to the twin sample's procedure. In total, 809 parents and children were invited to participate in the study, of which 189 (23.4%) agreed to participate (including eight sibling pairs and sibling trio). Several children refused to participate during urine collection ($N = 3$) or donated urine in the afternoon ($N = 2$). One child was excluded as this child and its co-twin were also included as part of the twin cohort. This resulted in a total of 183 clinical cases with urine samples available. Information on psychiatric disorders in the clinical sample is available in Table S2.

For the 183 clinic-referred children who donated morning urine (mean age = 10.2 years, *SD* age = 1.8; 25.7% female), 180 children had CBCL parent reports available and 164 children also had TRF teacher reports. ASEBA questionnaires were completed a maximum of 6 months before or after urine collection. All clinic-referred children were considered aggressive cases in our design, which was confirmed by the ASEBA sex-specific norm scores. Specifically, the clinical sample displayed subclinical levels of parent-rated CBCL aggression with average T-scores of $M = 66.08$ ($SD = 11.13$), with $T \geq 65$ conferring to subclinical levels of aggression, and $T \geq 70$ to clinical levels of aggression. Teacher-reported aggression was substantially elevated in the clinical sample with an average T-score of $M = 60.45$ ($SD = 8.19$), with a score of $T = 60$ referring to one standard deviation elevation above the sample mean.

Biomarker measurement

Biomarker quantification

Dipstick. A dipstick (Siemens, Marburg, Germany) was used to screen for infections in urine and to measure leukocytes, nitrite, proteins, glucose, and blood presence in the urine. The dipstick was applied to the first thaw of the urine samples either by dipping in the residual urine volume after aliquoting or by dropping urine on the dipstick. No children had to be excluded.

Density. Density of urine was measured using the Atago ^{R} refractometer PAL-10S BLT/A+W (Atago, Tokyo, Japan). The refractive index is a ratio of

the velocity of light in air to the velocity of light in solution, which is directly proportional to the number of dissolved solids in urine.

Creatinine. Creatinine was measured using a colorimetric assay kit according to manufacturer's instructions (Cayman, Ann Harbor, MI, USA). Creatinine values are reported in μmol/L.

Neopterin. Neopterin is a peptide which responds to damage and infection, especially to tissue damage and viral infection. Neopterin was measured using a competitive ELISA according to manufacturer's instructions (IBL International GmbH, Munich, Germany) Neopterin levels are reported in nmol/L.

Oxidized DNA/RNA. DNA and RNA are damaged by oxidation, with guanine as most prone to oxidation. Using a competitive ELISA (Cayman, Ann Harbor, MI, USA), different oxidized guanine species were measured in urine including 8-hydroxyguanosine, 8-hydroxy-2'-deoxyguanosine, and 8-hydroxyguanine. We used these oxidized guanine species as marker for oxidized DNA and RNA. Oxidized DNA/RNA levels are reported in pg/ml.

C-peptide. Insulin is synthesized in the pancreatic beta cells as proinsulin. Proinsulin is cleaved enzymatically, releasing insulin and its byproduct C-peptide. C-peptide was measured using an ELISA according to manufacturer's instructions (IBL International GmbH, Munich, Germany) and was used as a marker of insulin in urine. C-peptide levels are reported in ng/ml.

Substance P. The peptide neurotransmitter substance P was measured in urine using competitive ELISA according to manufacturer's instructions (Cayman, Ann Harbor, MI, USA). Substance *P* levels are reported in pg/ml.

Metabolite quantification

LC-MS amines platform. The amine metabolites were measured using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) employing an Accq-Tag derivatization strategy adapted from the protocol supplied by Waters. Sample preparation consisted of protein precipitation by the addition of methanol to 5 μL of urine spiked with internal standards. The centrifuged supernatant was then evaporated using a speedvac prior to reconstitution in borate buffer (pH 8.5) with AQC reagent. Chromatic separation was done on an Accq-Tag Ultra column (Waters Chromatography B.V., Etten – Leur, The Netherlands) using a UPLC Agilent Infinity II (1290 Multisampler, 1290 Multicolumn Thermostat and 1290 High Speed Pump; Agilent Technologies, Waldbronn, Germany) coupled to an AB SCIEX quadrupole-ion trap (QTRAP; AB Sciex, Massachusetts, USA). Analytes

were detected in the positive ion mode and monitored in Multiple Reaction Monitoring (MRM) using nominal mass resolution. The amine method has been described in detail elsewhere (Noga et al., 2012). Metabolites are reported as 'relative response ratios' (target area/area of internal standard) after quality control (QC) correction.

GC-MS organic acids platform. The organic acid metabolites were measured using gas chromatography mass spectrometry (GC-MS). Sample preparation of 50 μL of urine spiked with internal standards consisted of liquid-liquid extraction with ethyl acetate to extract the organic acids and remove urea present in the urine. After collecting the organic phase, the samples were evaporated to dryness using a speedvac. Then, two-step derivatization procedures were performed on-line: oximation using methoxyamine hydrochloride (MeOX, 15 mg/mL in pyridine) as first reaction and silylation using N-Methyl-N-(trimethylsilyl)- trifluoroacetamide (MSTFA) as second reaction. Chromatic separation using helium as carrier gas (1,7 mL/min) was performed on a 30 × 0.25 m ID column with a film thickness of 25 m (HP-5MS UI). The mass spectrometer (Agilent Technologies, Waldbronn, Germany) with a single quadrupole using electron impact ionization (70 eV) was operated in SCAN mode (mass range 50–500). Metabolites are reported as "relative response ratios" (target area/area of internal standard) after QC corrections. The acceptance criteria for metabolite reporting was a relative standard deviation (RSD) of the QCs (RSDqC) of <15% and background signal <20%, metabolites with RSDqc values of 15–30% should be interpreted with caution.

Metabolomics measurement protocol. In order to minimize the analytical error in the data, a number of measures were taken. A QC sample was created by pooling aliquots from all urine samples. Randomization of the subjects was done in such a manner that low and high aggression subjects, and therefore twin and clinical samples were randomly distributed across batches. Twin pairs were included in the same batch. Samples were run in 20 batches which included a calibration line, QC samples, sample replicates and blanks. QC samples were analyzed every 10 samples, and used to assess data quality and to correct for instrument response. Blank samples were used to determine if there was any interference from background signal. In-house developed algorithms were applied using the pooled QC samples to compensate for shifts in the sensitivity of the mass spectrometer over the batches. The performance and reproducibility of individual metabolites were evaluated with the RSDqc. The

acceptance criteria for metabolite reporting was RSDqc <15% and background signal <20%, metabolites with RSDqc of 15–30% should be interpreted with caution.

Data pre-processing for analysis. Preprocessing of the metabolomics data was done for each platform. To avoid the exclusion of potentially relevant metabolites and to avoid including metabolites with very poor RSDqc values, metabolites with a RSDqc value of >20% were removed (RSDqc values are given in Table S3). Metabolite measurements that fell below the limit of detection/quantification were imputed with half of the value of this limit, or when this limit was unknown with half of the lowest observed level for this metabolite (the number of imputed values per metabolite have been included in Table S3). Urine volume fluctuates among individuals and throughout the day; therefore, correction for dilution in urinary metabolite concentrations is essential. It is common practice to normalize to urinary creatinine output to correct for dilution differences (Warrack et al., 2009). However, creatinine was associated with childhood aggression (unpublished pilot study), therefore, normalization to creatinine levels would bias our results. Instead we applied an adjusted variant of density normalization. The density reflects the dilution of the urine sample and thus can be used to account for hydration state of the subject. In a healthy representative population, one can account for hydration state by dividing the metabolite concentrations by $(d_i - d_w)$, where d_i is the density of sample *i and* $d_w = 1$ the density of pure water. In this study, we took the data from the control group to construct the linear models that predict the concentration of each metabolite from the density measure. The density effect size β_m for each metabolite *m* is then used as a scaling factor in the density normalization for the entire population as follows:

$$[m_i]' = [m_i]/(\beta_m{}^*(d_i - d_w)),$$

where $[m_i]$ denotes the measured concentration of metabolite m in sample *i* and $[m_i]$' the corrected concentration. For convenience, densities and concentrations are expressed as a percentage of their median. The regression parameters are all listed in Table S3. In generating the models, we imputed data points that deviated more than 2.5 *SDs* from the mean by the mean metabolite or biomarker concentration. After normalization we verified if the effect of density on $[m_i]$' disappeared as one would expect. This was indeed the case by considering data

points within 3 *SDs* from the mean for each metabolite, c.f. Table S3. Finally, the metabolites and biomarkers were transformed by inverse normal rank transformation (Demirkan et al., 2015; Kettunen et al., 2016).

To get an indication of the metabolic functioning of serotonergic, dopaminergic, and GABAergic neurotransmitter pathways, ratios were calculated between metabolites which have been associated with these pathways. Specifically, we targeted serotonergic, dopaminergic, and GABAergic anabolism (synthesis) and catabolism (degradation). Serotonergic anabolism was represented by the ratios of L- tryptophan to 5-hydroxy-L-tryptophan (5HTP) and 5HTP to serotonin. Dopaminergic anabolism was assessed with the ratio of L-phenylalanine to L-tyrosine, while the ratio of 3-methoxytyramine (3MT) to homovanillic acid (HVA) represented dopamine catabolism. The ratios of L-glutamine to L-glutamic acid and L-glutamic acid to GABA represented GABA synthesis and GABA to succinic acid GABA degradation.

Statistical analyses

Because twins were selected for the biomarker study on the basis of prior longitudinal data, it was important to assess whether these group differences in aggression were still present at the time of urine collection. Generalized estimation equation (GEE) models tested whether twins selected for high or low aggression and clinical cases and twin controls differed in aggressive behavior at the time of urine collection (see Main analyses for details on GEE analyses). Similarly, a paired sample *t*-test was used to assess differences within twin pairs discordant for aggression (i.e., high co-twin vs. low co-twin). All analyses were carried out in the R programming language [version 3.6.0; (Team, 2019)]. For the entire NTR group (1,502 MZ twins and 2,298 DZ twins), from which the ACTION biomarker subsample was drawn, we analyzed the CBCL aggression scores with genetic structural equation modeling (Neale, Boker, Xie, & Maes, 2006; Posthuma et al., 2003) to obtain estimates of heritability, influences of shared (common), and unshared (unique) environmental factors.

Analytical design

We employed a three-step analytical strategy, with independent samples included in each step: (1) discovery in between-family analyses; (2) replication in within-family analyses; and (3) validation in clinically referred aggression cases and twin controls. In the discovery phase we explored the differences between high

and low concordant twin pairs in biomarkers levels and neurotransmitter ratios in between-family analyses with GEE models. The within-family replication analyses were performed for the top 25% most strongly associated biomarkers or ratios from the discovery phase. In the within-family analyses we compared the biomarker levels and neurotransmitter ratios of the low and high scoring twin of discordant twin pairs. Finally, for the biomarkers or ratios that differed consistently for aggression status in the discovery and replication phase we performed validation analyses to compare the levels of these biomarkers or the neurotransmitter ratios between clinical cases and controls.

Sensitivity analyses were performed to assess the impact of confounders (preexisting chronic conditions, medication use, and vitamin supplementation) on the results. After removal of individuals scoring positive on these potential confounders, we repeated the within-family analyses, for the biomarkers and ratios included in the replication phase of the analytical strategy.

Main analyses

The between-family discovery analyses included the twins scoring high or low on aggression. To investigate the first aim of the study, the relation of amines, organic acids, and biomarkers with childhood aggression, GEE analyses were performed to model the relationship between biomarkers (outcomes) and aggression status (predictors), with sex and age at urine collection as covariates. The second aim of this study, to investigate the contribution of neurotransmitter pathways (i.e., serotonergic, dopaminergic, and GABAergic) to aggression, was explored through identical GEE models, except with neurotransmitter ratios as outcomes. Aggression case-control status was the predictor in all analyses. GEE uses a sandwich or robust variance estimator that adjusts the standard errors to correct for clustering in the data (Rogers & Stoner, 2018). In our analyses the clustering in the data is due to relatedness of participants (i.e., twins within families), to correct for this we used the "exchangeable" correlation structure option in GEE. To correct for multiple testing (p.adjust function in R) we used the False Discovery Rate [FDR; (Benjamini & Hochberg, 1995)] of 5% for 89 (biomarkers) or 7 (ratios) tests, the significance threshold was set at $p \leq 0.05$.

The within-family replication analyses was done in twin pairs that were discordant for aggression status (high-low) and tested the top 25% most strongly associated biomarkers or ratios from the between-family analyses. Biomarker concentrations or ratios were corrected for the effects of sex and age at urine

collection by regressing out their effects. We then employed paired *t*-tests to analyze the residuals of the regression analysis. The FDR of 5% for 23 (biomarkers) or 3 (ratios) tests was used to correct for multiple testing, with the significance threshold at $p \leq 0.05$.

The top five most strongly associated biomarkers and top ratio were included in the validation analyses; these were required to have the same direction of effect in both the discovery and validation analyses. To assess if levels of the biomarkers and ratio selected by the discovery and validation analyses can differentiate between low and high aggressive children, we performed replication analyses in clinical cases and twin controls (92 twin pairs not previously included in the discovery between-family analyses). As for the discovery analysis, we performed GEE analyses to model the relationship of the biomarkers and ratio with aggression status. Sex and age at urine collection were included as covariates and we used to "exchangeable" correlation structure to correct for relatedness in our sample and obtain robust standard errors. For the biomarkers we used the FDR of 5% for 5 tests to account for multiple testing, $p \leq 0.05$ was considered significant.

Sensitivity analyses

Sensitivity analyses were done in the discordant monozygotic twin pairs, and comprised of the biomarkers and the neurotransmitter ratio included in the validation phase. These analyses only included data from twins without a preexisting chronic condition ($N = 24$ excluded), who were medication ($N = 48$ excluded) or vitamin supplement ($N = 67$ excluded) naive (see Supplementary Text 2 for more information). After exclusions, we performed paired *t*-tests to re-evaluate the differences in biomarker levels and the neurotransmitter ratio between the aggressive and non-aggressive twins. The FDR of 5% for 15 (biomarkers) or 3 (ratios) tests was used to correct for multiple testing, with the significance threshold at $p \leq 0.05$.

Finally, we carried out sensitivity analyses on item level data (see Table S4). These sensitivity analyses entailed association analyses of each metabolite, other biomarker of neurotransmitter ratio with each item from the CBCL Aggressive Behavior subscale (see Supplement 3).

RESULTS

Participant and aggression description

The present study contains data from 1,530 children, including twins and clinical cases, aged 9.7 years on average (range 5.6 to 13.4 years; *SD* = 1.8) of which 693 (45.3%) were females. In total, we included 794 (51.9%) children scoring low on aggression and 736 (48.1%) children with a high aggression score (Table 1). Twin pairs were invited for participation based on longitudinal data on childhood aggressive behavior (Table S5 and Supplement 4). We compared the CBCL aggression scores, obtained at time of urine collection, to assess whether differences in aggression between the high and low scoring twins were still present at the time of urine collection. At the time of urine collection, twins selected for high aggression indeed had significantly higher CBCL aggression scores as compared to twins selected for low aggression ($\beta = 5.09$; $SE = 0.50$; $p = 1.83 \times 10^{-24}$). Similarly, when comparing the discordant twin pairs, the high aggressive twins ($M = 6.2$, $SD = 5.8$) had significantly higher aggression scores at the time of urine collection than their low aggressive co-twins ($M = 4.4$, $SD = 4.4$; $t(185) = 5.73$, $p = 4.08 \times 10^{-08}$). Finally, the clinical cases and low aggressive twin controls, differed greatly in their levels of aggression ($\beta = 10.19$; $SE = 0.74$; $p = 8.25 \times 10^{-43}$). The heritability of the CBCL aggression scores as analyzed in our project was 0.63 (90% CI: 0.53–0.74). The proportion of variation explained by common environment shared by twins growing up in the same family was 0.14 (90% CI: 0.03–0.24) and the proportion of variation explained by unique environment was 0.23 (90% CI: 0.21–0.25).

Table 1 Participant characteristics of the twins (N = 1,347) and clinical cases (N = 183)

	Twins				Clinical cases
	Concordant low	Discordant		Concordant High	
	n = 605			*n* = 364	*n* = 183
		Low (*n* = 189)	High (*n* = 189)		
N complete twin pairs	302	189		182	
Mean (SD) age sample collection	9.4 (1.9)	10.1 (1.7)		9.5 (1.8)	10.2 (1.8)
Range age sample collection	5.6–12.6	6.1–12.7		5.8–12.9	6.3–13.4
N (%) MZ twins	469 (77.5%)	306 (81.0%)		330 (90.7%)	
N (%) females	323 (53.4%)	85 (45.0%)	79 (41.8%)	159 (43.7%)	47 (25.7%)
CBCL mother (SD) aggression score	2.7 (3.8)	4.4 (4.4)	6.2 (5.8)	7.6 (6.0)	13.0 (7.6)
Current psychotropic medication use					
Stimulants	10 (1.7%)	7 (3.7%)	13 (6.9%)	25 (7.0%)	46 (24.6%)
Analgesics	1 (0.2%)	1 (0.5%)	3 (1.6%)	1 (0.3%)	0 (0.0%)
Antipsychotics	1 (0.2%)	0 (0.0%)	1 (0.5%)	3 (0.8%)	36 (19.7%)
Hypnotics/sedatives	7 (1.2%)	1 (0.5%)	2 (1.1%)	6 (1.7%)	6 (3.3%)

CBCL, Child Behavior Checklist; MZ, monozygotic. The clinical cases CBCL scores include either mother of father-report (90% mother report).

Association of urinary metabolites and other biomarkers with childhood aggression

Discovery analyses

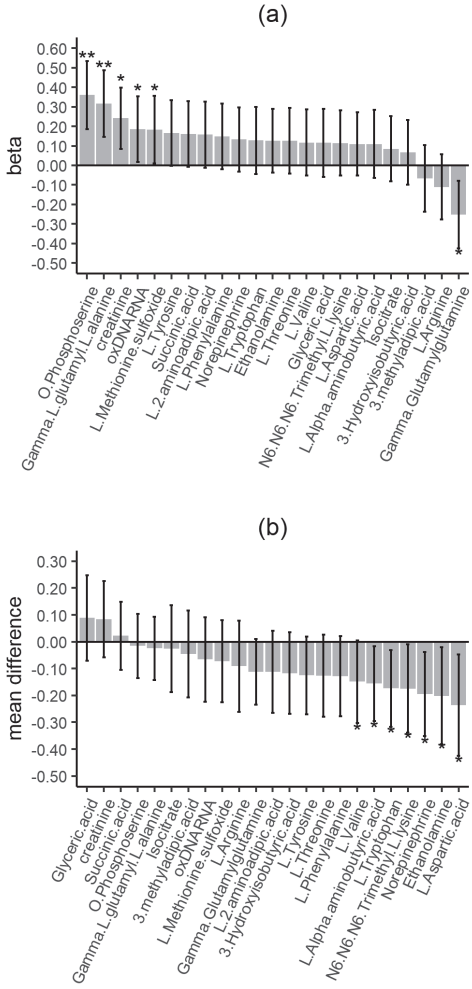
To determine the association of urinary amine, organic acid, and biomarker levels with childhood aggression, we first performed discovery analyses using a between-family design. The discovery analyses were conducted using 421 low scoring and 364 high scoring twins (average age = 9.4; *SD* = 1.8) and included 48.8% females and 84% MZ twins (Table 1). The discovery analyses showed significant associations for 4 amines and two other biomarkers with childhood aggression. We observed positive associations of childhood aggression with creatinine ($\beta = 0.24$; $SE = 0.08$; $p = 0.003$; FDR $p = 0.08$), oxidized DNA/

RNA (β = 0.19; *SE* = 0.09; *p* = 0.03; FDR *p* = 0.54), and L-methionine sulfoxide (β = 0.18; *SE* = 0.09; *p* = 0.04; FDR *p* = 0.57) and negative associations with gamma-glutamylglutamine (β = −0.25; *SE* = 0.09; *p* = 0.004; FDR *p* = 0.09; Table S7). After correction for multiple testing, the positive associations of O-phosphoserine (β = 0.36; *SE* = 0.09; FDR *p* = 0.004), and gamma-L-glutamyl-L-alanine (β = 0.32; *SE* = 0.09; FDR *p* = 0.01) remained significant (Table S6).

Replication analyses

The top 25% most strongly associated amines (Hagenbeek et al., 2016), organic acids (Siever, 2008), and biomarkers (Strimbu & Tavel, 2010), from the discovery analysis were examined in within-family analyses, conducted in 189 twin pairs discordant for childhood aggression status (Table 1). There was no replication of associations with childhood aggression of the discovery phase, where 2 amines were significantly associated after correction for multiple testing (Table S7). As compared to their low aggression co-twin, twins with high aggression had significantly lower concentrations of L-aspartic acid (mean difference = −0.24; $t(188) = -2.46$; $p = 0.01$), norepinephrine (mean difference = −0.19; $t(188) = -2.44$; $p = 0.02$), L-tryptophan (mean difference = −0.17; $t(188) = -2.40$; $p = 0.02$), ethanolamine (mean difference = −0.20; $t(188) = -2.20$; $p = 0.03$), L-alpha-aminobutyric acid (mean difference = −0.16; $t(188) = -2.20$; $p = 0.03$), and N6-N6-N6-trimethyl-L-lysine (mean difference = −0.17; $t(188) = -2.09$; $p = 0.04$; Table S7). However, none of these associations survived multiple testing (Table S7). Overall, we observed congruent directions of effect in the discovery and validation analyses for 6 out of 23 (26.1%) top 25% amines, organic acids and biomarkers (Figure 1).

Figure 1 Association of the top 25% amines, organic acids and other biomarkers with childhood aggression in the discovery and validation phases. The between-family analyses in the discovery phase are based on gee models for the 783 twins scoring low or high aggression. The within-family analyses in the validation phase are based on paired t-tests among the 189 twin pairs discordant for aggression. The whiskers denote the 95% confidence intervals for the GEE betas or the mean differences. Single asterisk represents a significant finding before correction for multiple testing at $p \leq 0.05$, double asterisks represent a significant finding after correction for multiple testing.



(A) The top 25% between-family results for the amines, organic acids and other biomarkers in the discovery phase. Correction for multiple testing was done with the False Discovery Rate (FDR) of 5% for 89 tests. (B) The top 25% within-family results for the amines, organic acids and other biomarkers. Correction for multiple testing was done with the FDR of 5% for 23 tests.

Validation analyses

To assess if biomarkers selected in the discovery and replication analyses could differentiate between low and high aggressive children from an independent sample, we analyzed data from 183 clinical cases and 184 controls (92 twin pairs concordant low for childhood aggression). This validation sample included children with an average age of 9.8 years (*SD* = 1.9), 39.8% females and 38.2% MZ twins. The analyses included the top 5 biomarkers with congruent direction of effect in the discovery and validation analyses: gamma-glutamylglutamine, L-arginine, glyceric acid, creatinine, and succinic acid. None of the biomarkers were significantly associated with childhood aggression in the validation analyses (Table 2 and Table S8). We observed the same direction of effect in the validation analysis for 3 (60%) biomarkers (Table 2).

Sensitivity analyses

For the five biomarkers included in the validation analyses we performed sensitivity analyses to assess if the mean difference between high and low aggressive children changed after excluding twins with potentially confounding characteristics (preexisting chronic condition, currently on medication, or on vitamin supplements). As compared to the within-family analyses (Table 2), we observed no differences after exclusions for preexisting chronic disorder, medication or vitamin use for any of the biomarkers (Table S9). Item-based analyses found no significantly associated metabolites or other biomarkers after correction for multiple testing. Replication and validation analyses also found no significant metabolites or other biomarkers per item after correction for multiple testing (Tables S13 – S16). The complete results have been included in Supplement 3.

Association of urinary neurotransmitter pathways in childhood aggression

Discovery analyses

To elucidate the role of serotonergic, dopaminergic, and GABAergic neurotransmitter pathways in childhood aggression we analyzed neurotransmitter ratios representing anabolism (synthesis) and catabolism (degradation) of the key neurotransmitters in these pathways. The discovery analyses using a between-family design to assess the association of urinary neurotransmitter ratios with childhood aggression found no neurotransmitter ratios involved in the anabolism or catabolism of serotonin, dopamine or GABA significantly associated with childhood aggression (Table S10).

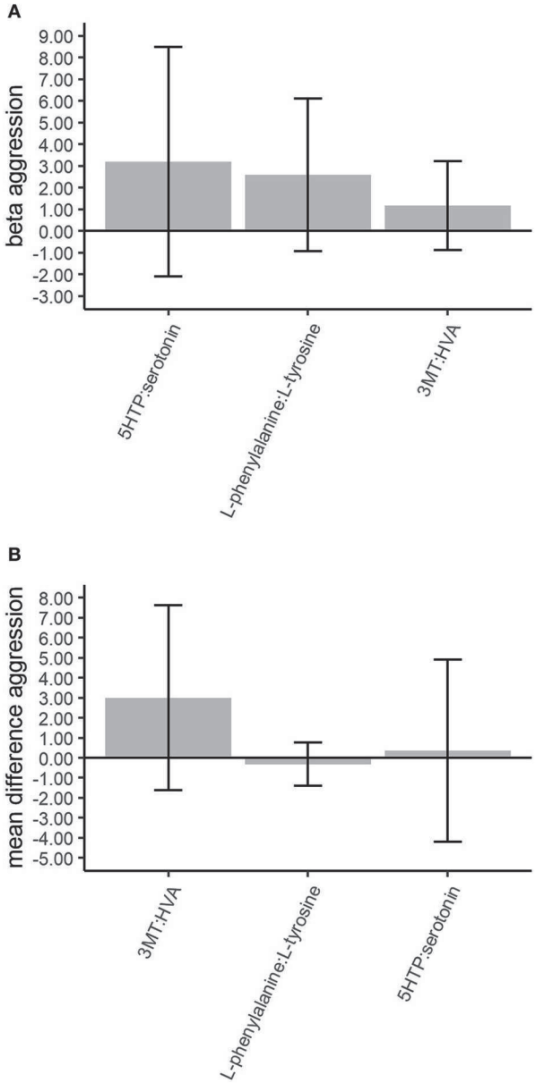
Replication analyses

Replication in the top 25% most strongly associated neurotransmitter ratios (3) from the discovery analysis were done in within-family analyses. The 3 top 25% neurotransmitter ratios included the dopamine ratios 3MT:HVA and L-phenylalanine:L-tyrosine and the serotonergic ratio 5HTP:serotonin. None of the neurotransmitter ratios showed significant differences between high and low aggressive twins (Table S11). We observed congruent directions of effect in the discovery and replication analyses for 2 of the 3 (66.6%) top 25% neurotransmitter ratios (Figure 2).

Validation analyses

To assess if neurotransmitter ratios selected in the discovery and replication analyses could differentiate between low and high aggressive children we analyzed data from 183 clinical cases and 184 twin controls. The top neurotransmitter ratio with the same direction of effect in the discovery and replication analyses was 3MT:HVA. The catabolic dopaminergic ratio 3MT:HVA was not significantly associated with childhood aggression in a sample of clinical cases and twin controls ($B = 2.12$; $SE = 1.57$; $p = 0.18$).

Figure 2 Association of the top 25% neurotransmitter ratios with childhood aggression in the discovery and validation phases. The between-family analyses in the discovery phase are based on gee models for the 783 twins scoring low or high aggression. The within-family analyses in the validation phase are based on paired t-tests among the 189 twin pairs discordant for aggression. The whiskers denote the 95% confidence intervals for the GEE betas or the mean differences. The neurotransmitter ratios denote the following: 3MT, 3-methoxytyramine; 5HTP, 5-hydroxy-L-tryptophan; HVA, homovanillic acid. **(A)** The top 25% between-family results for the neurotransmitter ratios in the discovery phase. Correction for multiple testing was done with the False Discovery Rate (FDR) of 5% for 7 tests. **(B)** The top 25% within-family results for the neurotransmitter. Correction for multiple testing was done with the FDR of 5% for 3 tests.



Sensitivity analyses

We performed sensitivity analyses to assess if the mean difference in the top neurotransmitter ratio between high and low aggressive children changed after excluding participants with potentially confounding characteristics. Excluding twins with a preexisting chronic condition or who were on medication or vitamin supplements, did not result in significant differences between aggressive and non-aggressive twins for the 3MT:HVA ratio (Table S12).

This table includes the results from the between-family discovery, the within-family validation and replication analyses for all 5 biomarkers. Discovery analyses were performed with GEE for 783 twins with low or high aggression. The p-values in the discovery analysis have been adjusted for multiple testing using the FDR of 5% for 89 tests. Validation analyses were performed with paired t-tests for 189 twin pairs discordant (high-low) on aggression status. The p-values in the validation analysis have been adjusted for multiple testing using the FDR 5% for 23 tests. Replication analyses were performed with GEE for 183 clinical cases and 184 twin controls. The p-values in the replication analysis have been adjusted for multiple testing using the FDR of 5% for 5 tests. All $p \leq 0.05$ have been given in bold. Full model information of the discovery, validation and replication analyses have been included in Tables S6–S8, respectively.

After correction for multiple testing none of the neurotransmitter ratios were significantly associated to any of the Aggressive Behavior items in the discovery analyses. Similarly, replication and validation analyses also found that associations of Aggressive Behavior items with neurotransmitter ratios did not survive multiple testing (Tables S17–S20). Supplement 3 contains a complete description of the results.

Table 2 Results of the top five associated biomarkers as included in the replication analysis

	Platform	Amines	Amines	Other	Organic acids	Organic acids
	Metabolite	Gamma-glutamylglutamine	L-arginine	Creatinine	Glyceric acid	Succinic acid
Discovery analysis	N	783	783	785	783	783
	B	−0.25	−0.11	0.24	0.12	0.16
	SE	0.09	0.08	0.08	0.09	0.09
	p-value	**0.004**	0.193	**0.003**	0.193	0.060
	FDR p-value	0.09	0.79	0.08	0.79	0.66
Replication analysis	Mean difference	−0.11	−0.09	0.08	0.09	0.02
	df	188	188	188	188	188
	T	−1.80	−1.05	1.17	1.09	0.34
	p-value	0.07	0.29	0.24	0.28	0.73
	FDR p-value	0.21	0.42	0.40	0.42	0.79
Validation analysis	N	367	367	367	367	367
	B	−0.17	0.12	0.16	0.09	−0.23
	SE	0.14	0.11	0.12	0.12	0.13
	p-value	0.25	0.27	0.16	0.42	0.07
	FDR p-value	0.34	0.34	0.34	0.42	0.34

DISCUSSION

Discovery of biomarkers that would aid in the diagnostics and treatment of childhood aggression could be of great benefit. To illustrate, poorer adult outcomes have been reported for later diagnosis, and thus treatment, of aggression (Campbell, Lundstrom, Larsson, Lichtenstein, & Lubke, 2019). Here, we describe the first urinary metabolomics study for childhood aggression, conducted in a sample of 1,347 twins selected for high or low aggression and a sample of 183 clinically- referred children with high aggression. Our first aim was to identify metabotypes for childhood aggression based on a total of 89 amines, organic acids, and other biomarkers of larger molecular weight. The second aim was to validate the role of serotonergic, dopaminergic, and GABAergic neurotransmitter pathways in childhood aggression. Thus, we compared seven ratios of metabolites reflecting neurotransmitter anabolism (synthesis) and catabolism (degradation) between aggressive and non-aggressive children.

Out of the 89 tested amines, organic acids, and other biomarkers of larger molecular weight, we observed significant associations for 4 amines (O-pshosposerine, gamma-L-glutamyl-L-alanine, gamma-glutamylglutamine, and methionine-sulfoxide) and 2 biomarkers (creatinine and oxidized DNA/RNA) in the discovery stage before correction for multiple testing. After correction for multiple testing, only O-phosphoserine, and gamma-L-glutamyl-L-alanine remained significantly associated. None of the organic acids or neurotransmitter ratios were significantly associated with childhood aggression. The replication phase included the top 25% most strongly associated amines, organic acids, other biomarkers, and neurotransmitter ratios from the discovery phase. The replication analyses revealed significant differences between low and high aggressive twins for the levels of 6 amines (L-aspartic acid, norepinephrine, L-tryptophan, ethanolamine, L-alpha-aminobutyric acid, and N6-N6-N6-trimethyl-L-lysine). These 6 amines were among the top 25% most strongly associated traits in the discovery phase, but did not reach a significance of FDR 5%. Consequently, validation was performed on the top biomarkers (gamma-glutamylglutamine, L-arginine, glyceric acid, creatinine, and succinic acid) and neurotransmitter ratio (3MT:HVA), which had congruent directions of effect in both the discovery and replication samples. The validation analyses were conducted in an independent sample of aggressive clinical cases and non-aggressive twin controls and did not show any significant differences between

groups. We compared these results to those obtained when applying sample median normalization of the metabolomics measurements and found results to be highly similar (for correlations between the beta's obtained by both normalizations: Pearson's r correlation 0.87, $p = 1.89 \times 10^{-27}$).

To assess if the heterogeneous nature of aggression prevented us from finding robust biomarkers or neurotransmitters associated with childhood aggression, all biomarkers and neurotransmitter ratios were reanalyzed for their association with endorsement of individual aggressive behavior questionnaire items. While we found some evidence for biomarkers and neurotransmitter ratios being differentially associated with distinct aggressive behaviors, like threatens or argues, none of the associations survived multiple testing.

Based on our findings for overall aggression and on the current state of the art in the field of human studies on the aetiology of aggression with respect to biomarkers, including genetic factors, hormones, and metabolites, below, we address three biochemical pathways and discuss their roles in aggression.

Serotonergic, dopaminergic, and GABAergic pathways and childhood aggression

It has been suggested that serotonergic, dopaminergic, and GABAergic pathways play a role in aggression (Willner, 2015). The role of these neurotransmission systems in aggression in humans is largely based on candidate gene studies. Candidate gene studies have mainly focused on the monoamine oxidase A gene *MAOA*, the catecholamine o-methyltransferase gene *COMT* and transporter and receptor genes for dopamine and serotonin, including *5HTTP*, *DRD2, DRD4*, and *DRD5* (Odintsova et al., 2019; Veroude, Zhang-James, et al., 2016). However, results from candidate gene studies replicated poorly, and well-powered genome-wide association studies are required to determine the value of these candidate genes for aggressive behaviour (Odintsova et al., 2019). Nevertheless, neurotransmission pathways remain interesting candidates for biomarker discovery. Therefore, we compared ratios of urinary metabolites representing anabolism (synthesis) and catabolism (degradation) of serotonin, dopamine, and GABA, between aggressive and non-aggressive children. We found no significant associations of urinary neurotransmitter ratios and childhood aggression in the discovery, replication, or validation phase. However, several of the metabolites included in our top 25% most strongly associated biomarkers indicate that

dysregulation of serotonergic, dopaminergic, and GABAergic neurotransmitter pathways can be involved in childhood aggression.

In the between-family discovery analysis we observed higher, non-significant, levels of L-tryptophan in children with high aggression, though the replication analysis revealed significantly lower L-tryptophan levels in children with high aggression. A previous study reported lower serum levels of tryptophan in aggressive inmates and increases in the ratio of tryptophan to serotonin (Comai et al., 2016). Similarly, lower plasma L-tryptophan levels have been observed in patients with major depressive disorder (MDD) as compared to controls (Ogawa et al., 2018). In the discovery analyses we observed significantly higher levels of O-phosphoserine, an ester of serine and phosphoric acid. High levels of phosphoserine indicate dysregulation of serotonin and dopamine metabolism pathways as it expresses a lack of pyridoxal-5-phosphate (Lui, Lumeng, & Li, 1985). Due to low pyridoxal-5-phosphate levels L-tryptophan cannot be converted to serotonin, nor can the conversion of L-tyrosine to dopamine occur (Lui et al., 1985). While none of the dopamine metabolites have been included in our top 25% most strongly associated biomarkers, norepinephrine, which is synthesized through catabolism of dopamine, was included in this top 25%. The role of norepinephrine in depression and anxiety disorders is well-established (Liu, Zhao, & Guo, 2018), for example, increased plasma norepinephrine levels were observed in new mothers suffering from postpartum depression as compared to control new mothers (Xie, Xie, Krewski, & He, 2018). In children, plasma norepinephrine levels were correlated with inefficient conditioned pain modulation response (Ferland et al., 2019). Furthermore, norepinephrine is increased by S-Adenosyl-Methionine (SAMe), which is the primary methyl group donor for several metabolic compounds and cysteine (Cai et al.) methylation (Sharma et al., 2017). SAMe is believed to have a positive influence on multiple neuropsychiatric disorders and due to its role in increasing catechol-O-methyltransferase (COMT) activity, SAMe has been suggested to reduce aggressive behavior in psychiatric patients (Sharma et al., 2017; Strous et al., 2009).

Of the GABAergic metabolites, only succinic acid was included in the top 25% most strongly associated biomarker results. In the discovery and replication analyses succinic acid showed, non-significant, higher levels in children with high aggression, though the direction of effect flipped in the replication analysis. In contrast to our findings, succinic semialdehyde dehydrogenase (SSADH) deficiency, a rare inherited metabolic disorder, causes lower succinic acid

levels and has been associated with a number of neuropsychiatric symptoms, including aggressive behavior (Gibson et al., 2003). The top 25% did include other metabolites involved in the metabolism of GABAergic metabolites. For example, gamma-glutamylglutamine is a dipeptide obtained from glutamine and L-glutamic acid, low levels of gamma-glutamylglutamine reflect a deficiency in gamma-glutamyltransferase system responsible for glutamate transport across the membrane (Meister, 1974); congruent with a previous study in drug naive patients with schizophrenia, where lower levels of cerebrospinal fluid (CSF) were observed as compared to controls (Do et al., 1995), we reported lower levels in children with high aggression. Furthermore, in the discovery analyses we observed significantly increased levels of gamma-L-glutamyl-L-alanine, after correction for multiple testing. Gamma-L-glutamyl-L-alanine is formed by the condensation of L-glutamic acid and L-alanine. Finally, we also observed dysregulation of metabolites downstream from GABAergic metabolites, such as L-arginine, which is synthesized from glutamine through citrulline. We observed lower levels of L-arginine in children with high aggression. Our results are consistent with results obtained for other psychiatric disorders, so have lower serum L-arginine levels been associated with antisocial personality disorder (APD) and schizophrenia (Cao et al., 2020; Gulsun et al., 2016).

Dysregulation in oxidative stress pathways and childhood aggression

Inflammation has been identified as a potential mechanism underlying aggressive behavior (Hagenbeek et al., 2016; Hagenbeek et al., 2018). One of the mechanisms believed to induce chronic inflammation is oxidative stress, characterized by the disturbed balance between antioxidant defenses and the production of reactive oxygen species (Betteridge, 2000). In the discovery analyses we reported significantly higher levels of the composite measure for oxidized DNA/RNA in children with high aggression, though in the replication analysis we observed non-significant lower oxidized DNA/RNA levels for children with high aggression. A study investigating the role of oxidative stress in adults with intermittent explosive disorder (IED) observed increased plasma levels of the oxidative stress markers 8-hydroxy-2′ -deoxyguanosine and 8-isoprostane (Coccaro, Lee, & Gozal, 2016). Congruent with our results in the discovery analyses, Coccaro et al. (2016) also reported significant positive correlation of oxidative stress markers with aggression.

In addition to dysregulation in oxidative stress markers, we have observed dysregulation in several metabolites involved in oxidative stress pathways. As discussed, we found lower levels of L-arginine in children with high aggression. L-arginine is synthesized from glutamine through citrulline. Both L-arginine and citrulline are precursors for nitric oxide, with low citrulline levels indicating overconsumption of citrulline for nitric oxide synthesis (Cynober, 2013). Through nitric oxide mediation citrulline can play a role in oxidative stress.

Similarly, SAMe has been discussed for its role in norepinephrine metabolism. While SAMe was not measured in the current study, methionine sulfoxide was included in the top 25% most strongly associated biomarkers. Methionine sulfoxide is obtained by oxidation of the sulfur in methionine and high serum methionine levels have been associated with anger and indirect aggression in APD patients (Gulsun et al., 2016). In contrast, lower plasma methionine levels have been reported in MDD patients as compared to controls (Kawamura et al., 2018; Ogawa et al., 2018). Furthermore, after correction for multiple testing higher levels of the methionine precursor, L-alpha-aminobutyric acid, were observed for children with high aggression. SAMe is also a precursor for the cysteine metabolism pathway, which is involved in the synthesis of the antioxidant glutathione (Sekhar et al., 2011). Low glutathione production might cause oxidative stress (Betteridge, 2000). Further suggestive evidence for a role of the cysteine metabolism pathway comes from the significant positive association of gamma-L-glutamyl-L-alanine with childhood aggression. Gamma-L-glutamyl-L-alanine is a gamma-glutamyl peptide and a substrate of a metabolite involved in glutathione metabolism. A study in mice showed that gamma-glutamyl peptides are synthesised through reactions with gamma-glutamylcysteine and glutathione synthetase and that this particularly occurs when glutathione is depleted (Soga et al., 2006). This is evident from the observation that elevated gamma-glutamyl peptide levels coincide with decreased glutathione levels in mice (Soga et al., 2006; Soga et al., 2011). These findings suggest that increased levels of the gamma-glutamyl peptide, gamma-L-glutamyl-L-alanine, may reflect depleted glutathione levels and supports a role for oxidative stress in childhood aggression.

In general, inflammation and oxidative stress have been associated with a great number of neuropsychiatric disorders (Salim, 2014), therefore, it is likely that these mechanisms do not play a role in childhood aggression specifically, but might be more general mechanisms underlying neuropsychiatric disorders.

However, knowledge of the causal mechanisms linking inflammation and oxidative stress with neuropsychiatric disorders is largely lacking.

Energy metabolism and childhood aggression

The results as obtained in the discovery replication and validation analyses also suggest a potential role of energy metabolism dysregulation in childhood aggression. Many of the main metabolic pathways are involved in converting glucose into energy (glycogenesis) and the breakdown of proteins to produce glucose (gluconeogenesis) to maintain blood glucose levels (Chung, Chacko, Sunehag, & Haymond, 2015). We found L-aspartic acid, which is involved in gluconeogenesis to differ significantly between twins scoring high on aggression as compared to their low scoring co-twins in the within-family replication analyses. While we reported lower urinary L-aspartic acid levels in twins with high aggression, a previous study reported increased serum levels in patients with APD (Gulsun et al., 2016). Congruent with our findings, lower plasma levels of L-aspartic acid were reported in MDD patients as compared to controls (Kawamura et al., 2018). While glucose is the main energy source in the human body, in cells and tissues with high-energy demand, such as the skeletal muscles, the phosphorylation of creatine produces phosphocreatine, a major source for adenosine triphosphate [ATP; (Nabuurs et al., 2013; Wyss & Kaddurah-Daouk, 2000)]. During the conversion of creatine to phosphocreatine, creatinine is formed spontaneously (Wyss & Kaddurah-Daouk, 2000). We consistently, but not always significantly, report higher creatinine levels in children with high aggression as compared to children with low aggression across all three phases of the study. Plasma creatinine has been associated with the severity of depression symptoms (Setoyama et al., 2016) and patients with schizophrenia showed decreased blood creatinine levels as compared to controls (Liu et al., 2014). Processes for storing and obtaining energy in and from fatty molecules are related to energy metabolism. In the current study the current study we find associations with childhood aggression for ethanolamine, involved in the synthesis of phospholipids, N6-N6-N6-trimethyl-L-lysine, involved in oxidation of fatty acids, and glyceric acid, involved in glycerolipid metabolism. Previously, glyceric acid was included in a panel capable of discriminating between patients with schizophrenia and controls with an AUC of 0.94 (Yang et al., 2013), lower serum levels of ethanolamine were observed in APD patients as compared to controls (Gulsun et al., 2016), lower CSF levels of ethanolamine were reported

in MDD patients as compared to controls and associated to depression severity and increased somatic anxiety symptoms in MDD patients (Ogawa et al., 2015), in addition, serum levels of N6-N6-N6-trimethyl-L-lysine have been associated with cognitive decline (Low et al., 2019).

Strengths and limitations

This study has several assets. First of all, the large-scale study design, which could be achieved by investigating urinary biomarkers, is a major strength of the current study. Urine is an easily accessible biofluid and may be obtained with minimal invasiveness, making it an ideal measure for large-scale data collection in vulnerable groups, like children. We showed that large scale standardized collection of urine and buccal samples is feasible in epidemiological projects and attained a fairly high response rates, considering that the sample included families who had to cope with difficult children. Families successfully kept samples at home in their freezers, until transport to the laboratory. Obviously, collection of frozen samples from a population-based sample at home is only feasible in a small country like the Netherlands.

The use of a longitudinal twin cohort permitted us to select children that were stable in their aggression status over time (see Table S5). We have shown that the operationalization of high and low aggression in our twin sample on the basis of previously collected data across ages, raters, and instruments did not impact mean aggression differences between concordant and discordant twin pairs at urine collection. By including twin pairs who were concordant (high-high or low-low) in their aggression scores, we further optimized toward more extreme groups. The MZ twin pairs discordant for aggression, enabled the analysis of within-family differences and controlled for genetic differences between individuals as well as potential confounders from the shared home or school environment, as these are largely shared between MZ twins. Finally, the clinical cases as included in the validation sample had aggression scores at the extreme end of the aggression distribution. As such, differences reported in the validation analyses between clinical cases and twin controls, are likely to offer the best indication of dysfunctional aggression. However, it should also be noted that the Aggressive Behavior subscale of the CBCL is derived from data-driven, factor analytic approaches. Consequently, the scale includes several items that wouldn't be considered aggressive based on their content (e.g., Unusually loud,

Sulks). Therefore, approaches with more theory-driven definitions of aggression (e.g., predatory aggression) should also be explored.

Our collection protocol was tested extensively, and it was kept relatively simple to ensure compliance. As a consequence, the collected first-morning urine was not mid-stream, as is sometimes recommended to avoid potential bacterial contamination of the upper urinary tract (Vaillancourt, McGillivray, Zhang, & Kramer, 2007). Fortunately, dipstick results for the urine samples did not indicate serious contaminations (data not shown), indicating that these did not play a major role in our findings. Because urine collection was performed by parents and children in the home-setting, deviations from the collection protocol were poorly monitored. Future studies may consider pairing the urine collection brochures with short videos describing the protocol to make it more accessible. Integrating such videos in an app, together with the phenotypic data collection can allow for the monitoring of the collection protocol and may also increase protocol compliance.

In interpreting our results, the wide age range (5–13 years of age) included in our study should be considered. This is because the onset of puberty likely influences both aggressive behavior and urinary metabolite profiles in older children. A caveat of the analyses targeting neurotransmitter ratios is the inability of targeting the complete neurotransmitter pathways. Our platforms did not target 5-hydroxyindoleacetic acid (5-HIAA), succinate semialdehyde, levodopa, dopamine, or 3,4-dihydroxyphenyl acetic acid (DOPAC). Moreover, the relationship of urine and brain metabolites is poorly understood, as many of our metabolites of interest are also synthesized in peripheral systems, therefore urinary metabolites do not necessarily reflect processes in the brain (An & Gao, 2015). Finally, in addition to all item-specific analyses, the results for 19 metabolites in general must be interpreted with caution because their RSDqc values fell outside of the acceptable range (>15%), this includes gamma-L-glutamyl-L-alanine, which was included in the top 25% most associated metabolites.

CONCLUSIONS AND FUTURE DIRECTIONS

This was the first metabolomics study on childhood aggression. In both the discovery and replication phases of this study we reported metabolites significantly associated with childhood aggression, however, these results were not congruent between the analyses and could not be validated. Our top metabolites play roles in central metabolic processes, specifically energy metabolism, neurotransmission, and oxidative stress. While most of the metabolites have previously been associated with neuropsychiatric disorders, only L-tryptophan and oxidized DNA/RNA are known to be involved in adult aggression. Further work is required to replicate our results and to establish the viability of the suggested urinary biomarkers in the early detection or treatment of childhood aggression, as the translational applicability for the current results are still limited. For a biomarker panel to be of practical utility it needs to exhibit good discrimination among phenotype classes, with high specificity and sensitivity (Strimbu & Tavel, 2010). The metabolite levels analyzed were quantified relative to an internal standard. To develop a biomarker panel with practical utility and recommended threshold values, absolute quantified values are preferred.

Moreover, while this study described the associations for a large number of amines and organic acids, it has not included the contribution of steroid hormones, as well as their interaction with neurotransmitters. This is an active topic in aggression research and in our ACTION project we aim to include the measurement of steroid hormones. Elucidating the role of steroid hormones, particularly in conjunction with metabolomics, may be of benefit to the field. Finally, all current results are correlational, therefore considerably more work needs to be done to determine the causal role of metabolic dysregulation in (childhood) aggression, combining multiple types of 'omics techniques (e.g., genomics, epigenomics, metabolomics) could be of aid here.

CHAPTER 7

GENERAL DISCUSSION

AIMS

This thesis aimed to provide insight in the etiology, predictors, and outcomes of aggression and antisocial behavior. The first part of this thesis focused on more conventional prediction of outcomes and continuation of aggression and antisocial behavior on the basis of the following constructs: parental psychopathology (**Chapter 2**), anxiety and depression (**Chapter 3**), and Oppositional Defiant Disorder symptoms (**Chapter 4**). Next, the second part of this thesis focused on novel biological markers of aggression, consisting of a review on the genetics of aggression (**Chapter 5**) and an empirical study on the metabolomics of aggression (**Chapter 6**).

SUMMARY

Both childhood disruptive behavior (DB) and the presence of parental mental disorders are independently associated with risk of long-term negative outcomes. To further extend this knowledge, the goal of Chapter 2 was to investigate whether 9-year-old children with DB and parents with a mental disorder had worse outcomes in adolescence compared to children with DB and parents without a mental disorder. In line with earlier research, child DB was related to all outcomes in adolescence. Paternal MD was related to criminality, aggression, truancy, poor school performance, and a cumulative risk index of poor functioning, and maternal MD to peer problems, rule breaking, and truancy. A subsample of children with DB was created to study whether the presence of parental mental disorders added additional risk of worse outcomes in children with DB. This appeared to be the case; paternal MD predicted adolescent criminality, consequences of antisocial behavior, truancy, poor school performance, and cumulative risk, whereas maternal MD predicted peer problems. Interestingly, paternal MD was a better predictor than maternal MD, regardless of child DB at age 9.

Chapter three covered the comorbidity between anxiety, depression, and DB. The first aim was to investigate whether anxiety and depression in childhood predicted DB in adolescence. The second aim was to what extent these relations were attributable to environmental and genetic confounding by means of a discordant co-twin design. Discordant co-twin designs allow to control very stringently for confounding because both monozygotic and dizygotic twins

typically share their rearing environment, while sharing 50 and 100% of their genetic material, respectively. Although significant in crude models, anxiety and depression in childhood did not predict DB in adolescence, after correcting for childhood DB. Cross-sectional co-twin analyses childhood indicated that the relation between anxiety and DB was fully explained by environmental and genetic confounding, while the relationship between depression and DB remained intact after correction. This suggests a more robust relationship between depression and DB, as compared to anxiety and DB. However, it should be noted that the relationship between depression is confined to childhood at most, and does not contribute to adolescent DB.

Chapter four focused on the Oppositional Defiant Disorder (ODD) behaviors of irritability and oppositionality. It has been shown before that irritability and oppositionality are correlated to different types of problems. To expand on this research, we wanted to investigate whether clinic-referred children and adolescents could be classified into mutually exclusive classes on the basis of their irritability or oppositionality symptoms, and whether the resulting classes would have clinical utility. Parent- and teacher-reported ODD symptoms at referral were used to classify 5- to 18-year-old youths into groups by means of cluster-based modeling. Three classes emerged with high, moderate, and low levels of both irritability and oppositionality. At referral, the High ODD class experienced the highest levels of mental health problems and DSM classifications. Importantly, all ODD classes defined at intake were predictive of diagnostic and treatment outcomes months later. Notably, the High ODD class had higher rates of clinician-based classifications of ODD and Conduct Disorder, and the lowest levels of pre- and posttreatment global functioning. Additionally, the Low ODD class exhibited higher rates of Generalized Anxiety Disorder and fear disorders. In sum, irritability and oppositionality co-occur in clinic-referred youths to such an extent that classification based on one of these behaviours does not add to clinical inference. Instead, overall ODD symptom severity at referral should be used as a guidance for treatment.

Chapter five consisted of a literature review on the genomics of aggression, focussing on a review of reviews of the genetics of human aggression, as well as a review on the literature on Genome-wide Association Studies (GWASs). The reviewed literature indicates that aggression in humans is heritable to a considerable extent, with behaviour genetics studies finding heritability estimates of aggression in children and adults of around 50%. Seventeen GWASs on

aggression and antisocial behaviour were recovered, reporting 817 genetic variants showing suggestive significance ($p \leq 1.0E-05$), including 10 genome-wide significant associations ($p \leq 5.0E-08$). Nominal associations ($5.0E-08 \leq p \leq 1E-05$) were found in gene-based tests for genes involved in immune, endocrine, and nervous systems. However, these associations were not replicated across GWASs. In sum, this review suggests considerable heritability of aggression and antisocial behaviour, but also clearly emphasizes that the actual biological basis of these heritability estimates remains to be uncovered.

Chapter six presents the first urinary metabolomics study on childhood aggression, using both community-residing twins as well as clinic-referred children. The analytical design consisted of three phases: a discovery phase in twins scoring low or high on aggression; a replication phase in twin pairs discordant for aggression; and a validation phase in clinical cases and matched twin controls. In the discovery phase, six biomarkers were significantly associated with childhood aggression, of which the association of O-phosphoserine, and gamma-L-glutamyl-L-alanine remained significant after multiple testing. Although non-significant, the directions of effect were congruent between the discovery and replication analyses for six biomarkers and two neurotransmitter ratios and the concentrations of six amines differed between low and high aggressive twins. In the validation analyses, the top biomarkers and neurotransmitter ratios, with congruent directions of effect, showed no significant associations with childhood aggression. Higher levels of O-phosphoserine could indicate a dysregulation of the serotonergic and dopaminergic system, specifically a lack of conversion from L-tryptophan to serotonin as well as from L-tyrosine to dopamine. Gamma-L-glutamyl-L-alanine could potentially indicate a role for oxidative stress in childhood aggression.

MAIN FINDINGS

1. Aggression and antisocial behavior were the best predictors for later aggression and aggression-related outcomes (**Chapters 2, 3, and 4**). The presence of psychopathology in fathers, not mothers, of children with disruptive behavior conferred an additional risk for long-term negative outcomes in adolescence (**Chapter 2**).
2. Subtyping, like on the basis of paternal mental disorders, can in some instances provide valuable insights (**Chapter 2**). However, especially in

individuals with significant problems (e.g., clinic-referred youths), multiple types of problems tend to co-occur next to one another. In these cases, instead of focusing on different types of behaviors, a focus on overall problem severity is more likely to give a reliable indication of prognosis and the amount of care required (**Chapter 4**).

3. This thesis indicates that is important to consider sample characteristics (e.g., community, at-risk, clinical) and the aim of the predictions (e.g., identification of at-risk individuals, diagnostics, gaining a deeper understanding of the etiology/development of aggression; **Chapters, 2, 4, and 6**).
4. Behavioral genetics research, which divides twin's individual differences in genetic and environmental components, indicates substantial heritability of aggression and antisocial behavior (**Chapter 5**). This in turn implies considerable biological differences related to aggressive behavior. However, these biological differences are not reflected in current, more direct measures of biology, specifically: Genome-wide Associations Studies and Metabolomics (**Chapters 5 and 6**).

GENERAL DISCUSSION

First, although aggression correlates with a multitude of problems, there is substantial variability to what extent aggression is driven by these problems. In line with the (unnuanced) maxim that past behavior predicts future behavior (e.g., Colins et al., 2015; Kennealy, Skeem, Walters, & Camp, 2010), this thesis confirmed that the overall severity of aggression and antisocial behavior is a powerful predictor of aggression and aggression-related outcomes in both clinical and community settings, specifically disruptive behavior (DB) in Chapters 2 and 3, and Oppositional Defiant symptoms in Chapter 4. Although this finding proves valuable for risk assessment, it only partially explains why some youths remain aggressive (i.e., because they displayed aggression previously) nor provides clues on treatment or prevention.

A risk factor which does seem to provide additional clues was found in Chapter 2; the presence of paternal (not maternal) mental disorders conferred additional risk for worse outcomes in adolescence in addition to DB. This finding is important because it provides some insight in the way aggression is influenced, in this case; suboptimal parenting practices and genetic risk, and because it shows the importance of fathers as compared to mothers. Interestingly, most of

the research has focused on mothers (e.g., Kim-Cohen et al., 2005) instead of fathers. It is already known that parental psychopathology impairs parenting practices in several ways, reducing positive engagement activities, warmth, responsiveness, and control, as well as interfering in more indirect activities like selecting childcare or arranging goods and services for their child (Barker, Iles, & Ramchandani, 2017). Furthermore parental involvement, which is affected considerably by parental psychopathology, was found to be an important moderator of treatment effectiveness of aggression (Hendriks, Bartels, Colins, & Finkenauer, 2018). So, the presence of parental mental disorders, particularly mental disorders in the father, could provide some clues on how to ameliorate some of its negative long-term outcomes.

Second, the given (e.g., Dodge & Coie, 1987; Klahr & Burt, 2014; Moffitt, 1993) that some constructs or subtypes are better predictors of functioning raises the question whether researchers should focus on subtyping aggression and antisocial behavior or focus on its heterogeneity. Studies on subtypes of aggression and antisocial behavior have provided us answers to some very interesting questions. An example from this thesis concerns Oppositional Defiant Disorder (ODD) behaviors, which can be divided into at least two dimensions: an irritable dimension, consisting of touchy and angry behavior, and an oppositional dimension, consisting of hurtful and headstrong behavior. Irritability is mainly associated with affective problems, especially depression and anxiety (Hipwell et al., 2011; Vidal-Ribas et al., 2016), whereas oppositionality is correlated with symptoms of Attention Deficit Hyperactivity Disorder (ADHD) and Conduct Disorder (CD), as well as violent and non-violent delinquency. So, in this case, and multiple others, subtypes of aggression and antisocial behavior do provide valuable information to some extent. However, a focus on subtyping brings along some limitations.

One considerable limitation is the co-occurrence of different types of problem behavior. The more severe the problems of a child or adolescent, the more various kinds of subtyping approaches or classifications seem to lose their distinctiveness. To illustrate, in our clinic-referred sample no ODD classes were found which were solely high in irritability or solely high in oppositionality (Chapter 3), while these "pure" classes were found in community samples with substantially lower levels of problem behavior (Althoff et al., 2014; Herzhoff & Tackett, 2016; Kuny et al., 2013; Wesselhoeft et al., 2019). This overlap or intercorrelation of aggression with a multitude of problems corresponds with clinical reality in which comorbidity is

rule, not exception, and in which patients frequently change in their diagnostical classifications over time. This heterogeneity but relative stability of problems is captured in more recently introduced research constructs like the general psychopathology factor (i.e., p factor). This p factor reflects an overall index of severity of psychopathology (Caspi et al., 2014), making it a transdiagnostic construct which transcends conventional psychiatric classifications. This suggests that instead of solely focusing on subtypes, a focus on overall problem severity could provide a more reliable indication of prognosis and the amount of care required.

Third, what constitutes a reliable predictor of functioning in one setting could have considerably less predictive qualities in another setting. We have already mentioned that we discovered that irritability and oppositionality can be used to classify individuals in a community setting (Althoff et al., 2014; Herzhoff & Tackett, 2016; Kuny et al., 2013; Wesselhoeft et al., 2019), but not in a clinic-referred setting (Chapter 3). We also found that the mere presence of parental mental disorders confers a considerable risk of poor outcomes in adolescence in community-residing twins. However other research indicated that prevalence of parental mental disorders is substantially higher in clinic-referred samples (e.g., 40% of mothers and 30% of fathers; Wesseldijk et al., 2018), which is likely to be even higher because of considerable non-response (30-40%). So, while parental mental disorders are a very potent predictor of future outcome in community settings, this differentiating potential could well be less valuable in a clinical setting comprising of severe and complex patients, most of whom experience severe family problems. Instead of asking whether there is a parental mental disorder present or not, this setting would more likely require a shift to what kind of parental mental disorder is present, and its severity.

Fourth, behavioral genetics research implies considerable heritability of aggressive and antisocial behavior (Chapter 4). However, direct measures of biology do not reflect this estimate, following from our review on Genome-wide Association studies of aggression and antisocial behavior (Chapter 4), as well as the metabolomics study in Chapter 5. Very few significant effects of biological measures are found which contribute to tiny percentages of explained variance. Furthermore, while psychopathology as well as aggression have a genetic basis, it could be difficult to discern actual biological correlates because the same behavior (e.g., aggression) could have different “push” and “pull factors” per individual. Interestingly, neighborhood characteristics seem to influence heritability estimates

of aggressive and antisocial behavior, with higher socioeconomic neighborhood status correlating with higher heritability estimates (Tuvblad et al., 2006; Burt et al., 2016, Hendriks et al., 2020). In other words, aggressive behavior can be exhibited across individuals, but in a "good" neighborhood genetics are likely to exert a bigger influence, while the environment exerts a bigger influence in "bad" neighborhoods. The heterogeneity of aggressive behavior potentially complicates matters even further. To illustrate, there are indications that physical aggression has higher heritability estimates as compared to more broader concepts of aggression and antisocial behavior (Waltes, et al., 2016).

The discovery of actual biological bases of problem behavior, like aggression, becomes even more challenging when considering theories like differential susceptibility in which a sizeable minority of the population are more sensitive to environmental input: for better *and* for worse (Belsky, Bakermans-Kranenburg, & IJzendoorn, 2007). This means that, amongst others, genetic variants that are associated with poor outcomes in suboptimal situations can be associated with good outcomes in optimal situations. If this theory holds, this would, unfortunately, mean the need for larger sample sizes to discover functional genetic variants; sample size increases of 50 percent are mentioned to achieve similar statistical power as in conventional research (Del Giudice, 2017). But, more importantly, this would also mean that genetical risk markers cannot be used to make accurate individual predictions about risk without considering environmental input. The same genes which are associated with negative outcomes in individuals which were exposed to suboptimal environments are in other instances associated with positive outcomes in individuals which were exposed to optimal environments.

Another prominent critique is on the way aggression is measured in behavioral genetics research; with most of the time a parent rating the behavior of both twins. Heritability decreases substantially when actual observations or tasks are used as compared to a single rater for both twins (Tuvblad & Baker, 2011). Interestingly, only two studies exist which used an experimental paradigm to induce aggressive behaviors twins. One study in 7- to 9-year-old twins showed considerable influence of unique environmental influences (74% CI: 0.63–0.90), moderate influences of genetics (A= 20%, CI: 0–37), and a small effect of the shared environment (C = 6%, CI: 0–34; Achterberg, van Duijvenvoorde, van der Meulen, Bakermans-Kranenburg, & Crone, 2018), while a study in adult twins even showed a 100% unique environmental influence on aggression in the case

of increasing provocation (Dinić et al., 2020). These studies provide preliminary evidence of the importance of multiple measurements as well as environmental factors in provoked aggressive behavior.

Another, more metaphysical critique is the medical lens through which human behavior is perceived in this field of research. There is always some sort of moral judgement when (problematic) human behavior is defined as well as a specific cultural context in which this judgment is passed, whether it be a teacher rating a student's aggression or a psychiatrist diagnosing an antisocial personality disorder in a delinquent. This is very different than other medical disciplines in which it is more clear that a certain aspect of human physiology is not functioning as intended. In some instances, a lab test gives a definitive and reliable diagnosis, while the observations of the doctor are of secondary value. This is in stark contrast with psychiatry in which observations are key and where cultural knowledge is necessary. To illustrate, the expression of psychosis as well as its perception as a disease varies across the world (Kendler, Zachar, & Craver, 2019). Feelings of extreme guilt are very prevalent in western countries and hypothesized to be a byproduct of Christianity (Bhavsar & Bhugra, 2008), while in pre-industrial societies the delusion that one transforms into all different kinds of animals is very prevalent, which could be attributed to animist beliefs and the local flora and fauna (Garlipp, Gödecke-Koch, Dietrich, & Haltenhof, 2004). Importantly, these variations in expression do not negate that a common underlying biological agent is not present, of course, some common elements can be found, like the given that delusions and hallucination are present in some form. Rather, they do point out that finding a biological cause for aggression is potentially more difficult because it is a more universal human behavior than psychosis, and is far more context-dependent.

Strengths and limitations

The studies in this thesis possessed several notable strengths. First, the use of multiple information sources (i.e., self-report, parents, teachers, clinicians, registries). Second, the studies spanned multiple settings, specifically clinic-referred and community samples. Third, state-of-the-art technologies were used, like the metabolomics approach to study childhood aggression and the machine learning-assisted literature search. Fourth, several studies possessed follow-up measurements, some spanning almost a decade. Fifth, the recruitment procedure in the CATSS and ODD papers substantially reduced selection bias. In case of

the CATSS paper all twins that were born in Sweden were approached, while in the ODD paper data were used which were collected as an integral part of a clinical protocol.

There are also several limitations that should be noted. First, the definition of aggression and antisocial behavior varied considerably across studies, (e.g., ODD and CD symptoms, ODD symptoms, aggressive behavior, aggressive and antisocial behavior), which hinders our ability to make precise comparisons. Second, only cross-sectional data were used in case of the metabolomics paper and the review of GWASs. It could for example be the case that some genetic variants are developmentally sensitive. Hypothetically, a gene could exert influence on aggression in 3-year-olds, while this wouldn't be the case in 15-year-olds. Third, this thesis focused primarily on risk factors, while it is known that protective factors like above-average intelligence, low impulsivity, living in a non-deprived non-violent neighborhood, and good family functioning can considerably lower the risk of developing aggression and/or antisocial behavior (Losel & Farrington, 2012).

Clinical implications

This thesis clearly suggests the importance of considering the specific setting in which risk assessments or predictions are made. Considerable research has been conducted on aggression and antisocial behavior in multiple settings (e.g., community and clinical). This thesis showed for example that the presence of parental mental disorders in childhood, especially those in fathers, can be a potent risk factor for poor psychosocial functioning in adolescence for children with DB. Although valuable, clinicians should be aware that findings from relatively high-functioning community samples sometimes find their way into clinical practice, while these would not necessarily hold up in clinical reality. To illustrate, very much to my surprise, physical exercise is not causally related to decreases in anxiety and depression in community-residing individuals (De Moor, Boomsma, Stubbe, Willemsen, & de Geus, 2008). However, randomized controlled trials (RCTs) targeting depressive individuals clearly indicate physical exercise to be effective in treating depression, with effect sizes being comparable to psychotherapy and antidepressants (Kvam, Kleppe, Nordhus, & Hovland, 2016). This is a powerful example of the ability of mental health professionals to initiate behavioral change in patients. Although community findings suggest that these depressive and anxious individuals would not have initiated physical

exercise by themselves, the same individuals did engage in this very beneficial behavior when offered in a care context. Another important implication is that the severity of problems should be a leading principle of diagnostics and treatment in clinical populations, not specific behavioral subtypes. If there is a focus on specific problems, these should be ones which are amendable to treatment or related to constructs which are amendable to treatment, for example parental mental disorders (Chapter 2).

Directions for future research

First, focussing on multiple biological systems, instead of one single system at a time, could give us a better indication what is happening biologically on an individual level. It is known that biological systems simultaneously interact with one another, therefore, such an approach could yield more robust results because it allows to study the aggregation and interaction of multiple biological system. In line with this idea, genetic, epigenetic, and metabolomic data which were collected within the ACTION framework are currently being combined into a cross-omics approach.

Another suggestion for future research would be an increased focused within twin research on experimental studies as well as randomized controlled trials. Twin research allows for very stringent controls for genetic and environmental confounding. Unfortunately, most of the literature, including chapter 4 of this thesis, has focused on observational studies. An increased focus on experiments and randomized controlled trials would allow for increased causal inference regarding characteristics that precipitate antisocial behavior and effectiveness of potential treatments, while maintaining the very stringent environmental and genetic controls which are characteristic of twin research. It should be noted that these approaches would require extensive recruitment efforts when studying high aggression and antisocial behavior. Twins are already relatively rare (15.9 twin births per 1000 births; Glasner, Van Beijsterveldt, Willemsen, & Boomsma, 2013), moreover including sufficient numbers of relevant individuals is greatly exacerbated by the fact that youths (and their families) who are high in aggression and antisocial behavior are less likely to participate in research in the first place, and are far more likely to drop out than their non-aggressive counterparts.

The increasing focus on aggression and antisociality as behaviors which are displayed in all individuals certainly has its merits to some extent, and can explain why in extreme situations (e.g., war) a lot of people can engage in very

serious aggressive acts. However, under normal circumstances only a very small percentage of the population causes the majority of problems, to illustrate; 1% of Sweden's population is responsible for 63% of all violent crime convictions (Falk et al., 2014). In this regard antisocial careers and academic careers aren't that different in their distribution of output (Laherrere & Sornette, 1998); a minority of individuals is responsible for a majority of the work done (i.e., highly cited researchers and childhood-onset chronic offenders). On the other hand, a majority gets a minority of the work done (i.e., PhD students/postdocs who quit science and children/adolescents who display developmentally normative antisocial behavior). Consequently, to achieve the highest gains in terms of societal costs and suffering, researchers should focus on the developmental trajectories of this elite of antisocial "high-achievers", not the average individual.

SUPPLEMENTARY MATERIALS

SUPPLEMENT TO CHAPTER 2

Children with early-onset disruptive behavior: parental mental disorders predict poor psychosocial functioning in adolescence

Chapter 2, Supplement 1. ICD codes of parental mental disorders
ICD-10
F10 Mental and behavioural disorders due to use of alcohol, except x.5
F11 Mental and behavioural disorders due to use of opioids, except x.5
F12 Mental and behavioural disorders due to use of cannabinoids, except, x.5
F13 Mental and behavioural disorders due to use of sedatives or hypnotics, except x.5
F14 Mental and behavioural disorders due to use of cocaine, except x.5
F15 Mental and behavioural disorders due to use of other stimulants, including caffeine, except x.5
F16 Mental and behavioural disorders due to use of hallucinogens, except x.5
F17 Mental and behavioural disorders due to use of tobacco, except x.5
F18 Mental and behavioural disorders due to use of volatile solvents, except x.5
F19 Mental and behavioural disorders due to multiple drug use and use of other psychoactive substances, except x.5
F20 Schizophrenia
F21 Schizotypal disorder
F22 Persistent delusional disorders
F23 Acute and transient psychotic disorders
F24 Induced delusional disorder
F25 Schizoaffective disorders
F28 Other nonorganic psychotic disorders
F29 Unspecified nonorganic psychosis
F30 Manic episode
F31 Bipolar affective disorder
F32 Depressive episode
F33 Recurrent depressive disorder
F34 Persistent mood [affective] disorders
F38 Other mood [affective] disorders
F39 Unspecified mood [affective] disorder

F40 Phobic anxiety disorders
F41 Other anxiety disorders
F42 Obsessive-compulsive disorder
F43 Reaction to severe stress, and adjustment disorders
F44 Dissociative [conversion] disorders
F45 Somatoform disorders
F48 Other neurotic disorders
F50.0 Anorexia nervosa
F50.1 Atypical anorexia nervosa
F50.2 Bulimia nervosa
F50.3 Atypical bulimia nervosa
F50.9 Eating disorder, unspecified
F51 Nonorganic sleep disorders
F60 Specific personality disorders
F60.0 Paranoid personality disorder
F60.1 Schizoid personality disorder
F60.2 Dissocial personality disorder
F60.3 Emotionally unstable personality disorder
F60.4 Histrionic personality disorder
F60.5 Anankastic personality disorder
F60.6 Anxious [avoidant] personality disorder
F60.7 Dependent personality disorder
F60.8 Other specific personality disorders
F60.9 Personality disorder, unspecified
F61 Mixed and other personality disorders
F63 Habit and impulse disorders
F64 Gender identity disorders
F70 Mild mental retardation
F71 Moderate mental retardation
F72 Severe mental retardation
F73 Profound mental retardation
F78 Other mental retardation
F79 Unspecified mental retardation
F80 Specific developmental disorders of speech and language
F81 Specific developmental disorders of scholastic skills
F82 Specific developmental disorder of motor function
F83 Mixed specific developmental disorders

F84 Pervasive developmental disorders
F84.0 Childhood autism
F84.1 Atypical autism
F84.3 Other childhood disintegrative disorder
F84.4 Overactive disorder associated with mental retardation and stereotyped movements
F84.5 Asperger's syndrome
F84.8 Other pervasive developmental disorders
F84.9 Pervasive developmental disorder, unspecified
F88 Other disorders of psychological development
F89 Unspecified disorder of psychological development
F90 Hyperkinetic disorders
F91 Conduct disorders
F91.0 Conduct disorder confined to the family context
F91.1 Unsocialised conduct disorder
F91.2 Socialised conduct disorder
F91.3 Oppositional defiant disorder
F91.8 Other conduct disorders
F91.9 Conduct disorder, unspecified
F92 Mixed disorders of conduct and emotions
F92.0 Depressive conduct disorder
F92.8 Other mixed disorders of conduct and emotions
F92.9 Mixed disorder of conduct and emotions, unspecified
F93 Emotional disorders with onset specific to childhood
F94 Disorders of social functioning with onset specific to childhood and adolescence
F95 Tic disorders
F98 Other behavioural and emotional disorders with onset usually occurring in childhood and adolescence

ICD-9
295.0 Simple type
295.1 Disorganised type
295.2 Catatonic type
295.3 Paranoid type
295.4 Acute schizophrenic episode
295.5 Latent schizophrenia

295.6 Residual schizophrenia
295.7 Schizo-affective type
295.8 Other specified types of schizophrenia
295.9 Unspecified schizophrenia

296.0 Manic disorder, single episode
296.1 Manic disorder, recurrent episode
296.2 Major depressive disorder, single episode
296.3 Major depressive disorder, recurrent episode
296.4 Bipolar affective disorder, manic
296.5 Bipolar affective disorder, depressed
296.6 Bipolar affective disorder, mixed
296.7 Bipolar affective disorder, unspecified
296.8 Manic-depressive psychosis, other and unspecified
296.9 Other and unspecified affective psychoses
297 Paranoid states
298 Other nonorganic psychoses
299.0 Infantile autism
299.1 Disintegrative psychosis
299.8 Other specified early childhood psychoses
299.9 Unspecified
300.0 Anxiety states
300.1 Hysteria
300.2 Phobic disorders
300.3 Obsessive-compulsive disorders
300.4 Neurotic depression
300.5 Neurasthenia
300.6 Depersonalisation syndrome
300.7 Hypochondriasis
300.8 Other neurotic disorders
300.9 Unspecified neurotic disorder
301 Personality disorders
301.0 Paranoid personality disorder
301.1 Affective personality disorder
301.2 Schizoid personality disorder
301.3 Explosive personality disorder
301.4 Compulsive personality disorder

301.5 Histrionic personality disorder
301.6 Dependent personality disorder
301.7 Antisocial personality disorder
301.8 Other personality disorders
301.81 Narcissistic personality
301.82 Avoidant personality
301.83 Borderline personality
301.84 Passive-aggressive personality
301.89 Other
301.9 Unspecified personality disorder
303 Alcohol dependence syndrome
304 Drug dependence
305.0 Alcohol abuse
305.9 Other, mixed, or unspecified drug abuse
307.1 Anorexia nervosa
307.2 Tics
307.3 Stereotyped repetitive movements
307.4 Specific disorders of sleep of nonorganic origin
307.50 Eating disorder, unspecified
307.51 Bulimia
307.52 Pica
307.53 Psychogenic rumination
307.54 Psychogenic vomiting
307.59 Other
311 Depressive disorder, not elsewhere classified
312 Disturbance of conduct not elsewhere classified
312.0 Undersocialised conduct disorder, aggressive type
312.00 Unspecified
312.01 Mild
312.02 Moderate
312.03 Severe
312.1 Undersocialised conduct disorder, unaggressive type
312.10 Unspecified
312.11 Mild
312.12 Moderate
312.13 Severe
312.2 Socialised conduct disorder

312.20 Unspecified
312.21 Mild
312.22 Moderate
312.23 Severe
312.3 Disorders of impulse control, not elsewhere classified
312.4 Mixed disturbance of conduct and emotions
312.8 Other specified disturbances of conduct, not elsewhere classified
312.81 Conduct disorder, childhood onset type
312.82 Conduct disorder, adolescent onset type
312.89 Other conduct disorder
312.9 Unspecified disturbance of conduct
313.8 Other or mixed emotional disturbances of childhood or adolescence
313.81 Oppositional disorder
313.82 Identity disorder
313.83 Academic underachievement disorder
313.89 Other
313.9 Unspecified emotional disturbance of childhood
314.0 Attention deficit disorder
314.00 Without mention of hyperactivity
314.01 With hyperactivity
314.1 Hyperkinesis with developmental delay
314.2 Hyperkinetic conduct disorder
314.8 Other specified manifestations of hyperkinetic syndrome
314.9 Unspecified hyperkinetic syndrome
317 Mild mental retardation
318 Other specified mental retardation
318.0 Moderate mental retardation
318.1 Severe mental retardation
318.2 Profound mental retardation
319 Unspecified mental retardation
ICD-8
291 Alcoholic psychosis
295.0 Simple type
295.1 Hebephrenic type
295.2 Catatonic type
295.3 Paranoid type
295.4 Acute schizophrenia episode

295.5 Latent schizophrenia
295.6 Residual schizophrenia
295.7 Schizo-affective type
295.8 Other
295.9 Unspecified type
296.0 Involutional melancholia
296.1 Manic-depression psychosis, manic type
296.2 Manic depressive psychosis, depressed type
296.3 Manic-depressive psychosis, circular type
296.8 Other
296.9 Unspecified
297 Paranoid states
298 Other psychoses
300 Neuroses
300.0 Anxiety neurosis
300.1 Hysterical neurosis
300.2 Phobic neurosis
300.3 Obsessive compulsive neurosis
300.4 Depressive neurosis
300.5 Neurasthenia
300.6 Depersonalisation syndrome
300.7 Hypochondriacal neurosis
300.8 Other
300.9 Unspecified neurosis
301 Personality disorders
301.0 Paranoid
301.1 Affective
301.2 Schizoid
301.3 Explosive
301.4 Anankastic
301.5 Hysterical
301.6 Asthenic
301.7 Antisocial
301.8 Other
301.9 Unspecified
303 Alcoholism
304 Drug dependence

308 Behaviour disorders of childhood
310 Borderline mental retardation
311 Mild mental retardation
312 Moderate mental retardation
313 Severe mental retardation
314 Profound mental retardation
315 Unspecified mental retardation

Chapter 2, Supplement 2. Additional information on measures

Outcome measures at age 15

Self-reported aggression

Aggressive behavior was assessed using the 23-item Reactive and Proactive Aggression Questionnaire (RPQ) (Raine et al., 2006). The RPQ includes 11 items that focus on reactive aggression (e.g., "Reacted angrily when provoked by others", "Gotten angry when frustrated"), and 12 items that focus on proactive aggression (e.g., "Had fights with others to show who was on top", "Taken things from other students"). The items are coded as 0 ("never"), 1 ("sometimes"), or 2 ("often").

Self-reported crime

The Self-reported Delinquency Scale (SRD; Ring, 1999) was used to assess the frequency of 13 non-violent criminal acts (e.g., vandalism, car theft, burglary, drug dealing) and nine violent criminal acts (e.g., hurting persons, hurting animals, sexual offenses). Each item is coded on a 6-point Likert scale, ranging from 0 ("never") to 5 ("more than 10 times").

Self-reported alcohol misuse

Alcohol misuse was assessed using the Self-reported Alcohol and Drug Use (Englund, 2016). First, a dichotomous variable "Frequent Alcohol Consumption" (no/yes) was created based upon the question: "Have you been drinking beer, wine or liquor last month?". A second question was asked concerning frequency of intoxication "How often do you feel drunk when you drink alcohol?", which was rated on a 6-point Likert scale ranging from 0 ("I don't drink") to 5 ("every time"). Based upon this second question, a dichotomous variable "Frequent Alcohol Intoxication" was created and differentiated between those who were not or rarely intoxicated (score 0-3) and those who were (very) often intoxicated (score 4-5). Finally, "Alcohol Misuse" was defined as being above the cut-off for

Frequent Alcohol Consumption and/or Frequent Alcohol Intoxication (of note, using this approach about 30% of the sample were identified as misusing alcohol).

Self-reported truancy

Following prior work (Norén Selinus et al., 2015), truancy of the child was assessed using one SRD item ("Did you ever skip school"), with scores ranging from 0 ("never") to 4 ("more than 10 times").

Parent-reported conduct problems

Conduct problems of the child were assessed using the Conduct Problems subscale of the Strengths and Difficulties Questionnaire (SDQ).(Goodman, 1997) SDQ items (Lansford et al.) scored on this and the other SDQ scales mentioned below range from 0 ("not true"), 1 ("somewhat true") and 2 ("certainly true").

Parent-reported emotional problems

Emotional problems of the child were assessed using the 5-item (e.g., "Often unhappy, down-hearted or tearful") Emotional Problems subscale of the SDQ parent version.

Parent-reported peer problems

Peer problems of the child were assessed through the 5-item (Lansford et al.)") Peer Problems subscale of the SDQ parent version.

Parent-reported prosocial behavior

Prosocial behavior of the child was assessed through the 5-item (Lansford et al.) Prosocial Behavior subscale of the SDQ parent version. Of note, a higher prosocial behavior score is indicative of less problems.

Outcome measures at age 18

Self-reported aggression

Aggression was assessed using the 11-item Aggression subscale of the Life History of Aggression Questionnaire.(Coccaro et al., 1997) Youth were asked how many times in their lives they had committed certain aggressive acts (Lansford et al.). Answers were given on a 6-point Likert scale ranging from 0 ("no event") to 5 ("more times than I can count").

Self-reported crime and truancy

Self-reported crime and truancy were assessed using the same Self-reported Delinquency Scale as at age 15 years (see outcome measures at age 15).

Self-reported alcohol misuse

Alcohol misuse was assessed using the Alcohol Use Disorders Identification Test (AUDIT). (Saunders et al., 1993) The AUDIT covers alcohol consumption,

drinking behavior (dependence), and alcohol-related problems. The first eight items have five response categories, and are coded from 0 to 4. Items nine and 10 have three response categories and are coded as: 0, 2 or 4. An example of a question concerning alcohol-related problems is: "How often during the last year did you have a feeling of guilt or remorse after drinking", with answers ranging from 0 ("never") to 4 ("daily or almost daily"). The cutoff for alcohol misuse for women is set at a value of 6 or higher, for men at 8 or higher.(Saunders et al., 1993)

Self-reported consequences of antisocial behavior

The 4-item Consequences of Antisocial Behavior subscale of the Life History of Aggression Questionnaire measures social consequences due to antisocial behavior of the reporter (Lansford et al.).

Parent-reported aggression

Aggression was assessed by means of the 16-item Aggressive Behavior subscale of the Adult Behavior Checklist (ABCL)(Achenbach & Rescorla, 2003) parent version. Parents rated aggression of their child over the last 6 months (Lansford et al.) on a 3-point Likert scale ranging from 0 ("not true") to 2 ("very true or often true").

Parent-reported rule-breaking behavior

Rule-breaking behavior was assessed by the 13-item ABCL Rule-breaking Behavior subscale (Lansford et al.).

Parent-reported emotional problems

Emotional problems were assessed by the 14-item ABCL Anxious/Depressed subscale (Lansford et al.).

Registered school performance

School performance of the child was assessed using the sum of the final grades of 16 subjects (e.g., math, English) in primary school. The grades were obtained through the National School Register. Swedish school grades range from 0 (equivalent to an F) to 20 (equivalent to an A). The total score on all 16 subjects ranged from 0 (equivalent to an F on all subjects) to 320 (equivalent to an A on all subjects).

Chapter 2, Supplement 3. Internalizing and externalizing mental disorders

It could be the case that the "what-question" (ie, Is there an internalizing or externalizing MD present in the parents?) might be more important than the "who-question" (ie, Does the mother or the father have a MD?), especially since a higher prevalence of externalizing disorders in fathers than in mothers might explain why paternal disorder was most often related to the reported antisocial

outcomes in the subsample of children with DB at age 18. We differentiated between parental external disorders (ie, at least one parent had an externalizing MD) and internalizing disorders (ie, at least one parent had an internalizing MD) and included these two predictors in a model, together with the control variables (for details see Table S6). Results showed that when predicting outcomes at age 18, parental internalizing disorder (6.7% in the sample of children with DB at age 18) was positively related to consequences of antisocial behaviour (OR = 1.80; 95% CI = 1.06; 3.05) and truancy (OR = 2.02; 95% CI = 1.14; 3.57), whereas parental externalizing disorder (3.0%) was positively related to violent criminality (OR = 2.94; 95% CI = 1.28; 6.77), aggression (OR = 2.58; 95% CI = 1.24; 5.35), alcohol misuse (OR = 2.34; 95% CI = 1.03; 5.32), and the cumulative risk index (OR = 1.38; 95% CI = 1.15; 1.67).

However, these analyses do not rule out the possibility that prospective links between externalizing and internalizing disorders and outcomes differ across mothers and fathers. To explore this possibility, we also tested a model with four predictors (paternal externalizing disorder, 1.8%; paternal internalizing disorder, 2.4%; maternal externalizing disorder, 1.7%; and maternal internalizing disorder, 4.4%;) together with the control variables. Results showed (i) that paternal externalizing disorder was related to violent criminality (OR = 4.10; 95% CI = 1.19; 14.14) and the cumulative risk index (OR = 1.39; 95% CI = 1.14; 1.71); (ii) paternal internalizing disorder to truancy (OR = 3.13; 95% CI = 1.41; 6.94) and poor school performance (OR = 2.42; 95% CI = 1.05; 5.73), and (iii) that maternal externalizing disorder was positively related to emotional problems (OR = 3.90; 95% CI = 1.11; 13.69), for details see Table S7. In short, the outcomes of these analyses suggest that the "what-" and "who-question" are equally important.

Chapter 2, Table S1 Overview of Disruptive Behavior Items

Oppositional Defiant Disorder items
Gate items
Has there ever been a time when s/he would be angry to the extent that s/he cannot be reached?
Does s/he often argue with adults?
Does s/he often tease others by deliberately doing things that are perceived as provocative?
Is s/he easily offended, or disturbed by others?
Is s/he easily teased?

Chapter 2, Table S1 Continued.

Additional items
Has there ever been a time when s/he would be angry to the extent that s/he is out of control without there being any particular triggering event?
Has there ever been a time when s/he would be angry to the extent that s/he is out of control in connection with changes?
Does s/he often lose temper?
Does s/he refuse following other people's directives?
Is s/he often vindictive or cruel?
Does s/he often treat significant others badly or without respect?
Does s/he often blame others for own mistakes or bad actions?
Conduct Disorder items
Gate items
Has s/he ever deliberately been physically cruel to anybody?
Does s/he often start fights?
Does s/he often lie or cheat?
Does s/he steal things at home or outside home?
Has s/he ever engaged in shoplifting?
Additional items
Does s/he often threaten, harass or humiliate others?
Is s/he cruel to insects?
Has s/he ever started a fire?
Has s/he ever sexually abused other children?
Has s/he ever been detained by the police?
Has s/he ever used a deadly weapon?
Has s/he ever robbed anyone or else unlawfully acquired other people's property by means of directs threats?
Has s/he ever purposely attempted to destroy other people's property?
Has s/he ever broken into someone else's home, premises or car?
Is s/he often out late at night without consent (beginning before 13 years of age)?
Has s/he ever ran away from home and stayed away over night at least two times (or one time if it was for an extended period of time)?
Is s/he often absconding (beginning before 13 years of age)?

Note: The additional items were administered if the parents endorsed one or more of the gate items with "yes to some extent" or "yes".

Chapter 2, Table S2 Frequencies and Percentages of Membership of Disorder Category Membership for Mothers at Waves 15 and 18 Years

	Wave 15 Years (n = 195)		Wave 18 Years (n = 84)	
Disorder category	Frequency	Percentage	Frequency	Percentage
Anxiety, obsessive compulsive, dissociative, and somatoform disorders	74	37.9	38	45.2
Depressive disorders	72	36.9	23	27.4
Substance abuse and dependence	27	13.8	17	20.2
Psychotic disorders	24	12.3	8	9.5
Personality disorders	10	5.1	7	8.3
Reactions to severe stress and PTSD	38	19.5	15	17.9
Other[a]	18	9.2	7	8.3

Note: Frequencies and Percentages refer to having at least one disorder in the respective disorder category. This implies, for example, that a mother who has a borderline personality disorder was counted as having a Personality Disorder, whether or not she met criteria for other personality disorders. The sample sizes refer to the number of mothers in each group and therefore deviates from the number of children exposed to maternal MD in Tables 1 and 2 of the main text. PTSD = post-traumatic stress disorder.

[a] Disorder categories with very low prevalences (≤ 5 or lower) were included in the "Other" disorder category to guarantee the confidentially of the parents and child.

Chapter 2, Table S3 Frequencies and Percentages of Disorder Category Membership for Fathers at Waves 15 and 18 Years

	Wave 15 Years (n = 170)		Wave 18 Years (n = 77)	
Disorder category	Frequency	Percentage	Frequency	Percentage
Anxiety, obsessive compulsive, dissociative, and somatoform disorders	45	26.5	15	19.5
Depressive disorders	33	19.4	22	28.6
Substance abuse and dependence	62	36.5	26	33.8
Psychotic disorders	26	15.3	13	16.9
Personality disorders	6	3.5	6	7.8
Reactions to severe stress and PTSD	31	18.2	15	19.5
Other[a]	13	7.6	7	9.1

Note: Frequencies and Percentages refer to having at least one disorder in the respective disorder category. This implies, for example, that a father who has a major depressive disorder was counted as having a Depressive Disorder, whether or not he met criteria for other depressive disorders, such as Dysthymic Disorder. The sample sizes refer to the number of fathers in each group and therefore deviates from the number of children exposed to paternal MD in Tables 1 and 2 in the main text. PTSD = post-traumatic stress disorder.

[a] Disorder categories with very low prevalences (≤ 5 or lower) were included in the "Other" disorder category to guarantee the confidentially of the parent and child.

Chapter 2, Table S4 Dichotomous Cutoff Values for Follow-up at Age 15 Years and Age 18 Years

Child age at assessment	Variable	Theoretical range	Cut point
15 years			
	Nonviolent crime (SR)	0-5	≥1
	Violent crime (SR)	0-5	≥1
	Proactive aggression (SR)	0-24	≥2
	Reactive aggression (SR)	0-22	≥7
	Truancy (SR)	0-4	≥1
	Frequency of alcohol consumption beer (SR)	0-1	≥1
	Frequency of alcohol consumption other (SR)	0-1	≥1
	Frequency of alcohol intoxication (SR)	0-5	≥3
	Conduct problems (PR)	0-10	≥3
	Emotional problems (PR)	0-10	≥5
	Peer problems (PR)	0-10	≥3
	Prosocial behavior (PR)	0-10	≥6
18 Years			
	Nonviolent crime (SR)	0-5	≥1
	Violent crime (SR)	0-5	≥1
	Aggression (SR)	0-55	≥10
	Consequences of aggression (SR)	0-20	≥1
	Truancy (SR)	0-4	≥3
	Alcohol misuse (SR)	0-40	boys: ≥8, girls: ≥6
	Rule-breaking behavior (PR)	0-26	≥14
	Aggression (PR)	0-32	≥10
	Emotional problems (PR)	0-28	≥17
	School performance (Reg.)	0-320	≥210

Note: PR = parent-reported; Reg. = registry; SR = self-reported.

Chapter 2, Table S5 Odds Ratios and 95% Confidence Intervals of the Fixed Part of the Crude and Adjusted Main Effects Models at Follow-up 15 Years in a Subsample of Children With Disruptive Behavior

		Criminality		Aggression		Problems						
		Violent (SR)	Nonviolent (SR)	Proactive (SR)	Reactive (SR)	Conduct (PR)	Emotional (PR)	Peer (PR)	Alcohol (SR)	Truancy (SR)	Low prosocial (PR)	Cumulative risk[a]
Predictor	Model	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Paternal MD	3	1.35	1.18	0.91	1.02	1.29	1.23	1.34	1.12	1.16	0.89	1.09
		[0.88, 2.08]	[0.78, 1.77]	[0.55, 1.49]	[0.66, 1.56]	[0.86, 1.92]	[0.79, 1.93]	[0.92, 1.95]	[0.71, 1.76]	[0.78, 1.72]	[0.55, 1.43]	[0.94, 1.27]
	4	1.36	1.17	0.91	1.02	1.28	1.26	1.29	1.13	1.13	0.88	1.09
		[0.88, 2.10]	[0.78, 1.76]	[0.55, 1.50]	[0.67, 1.57]	[0.86, 1.92]	[0.81, 1.97]	[0.89, 1.88]	[0.71, 1.78]	[0.76, 1.68]	[0.54, 1.42]	[0.93, 1.27]
Maternal MD	3	0.91	1.07	0.95	0.92	1.05	0.77	**1.64****	0.93	1.36	1.16	1.04
		[0.57, 1.45]	[0.71, 1.60]	[0.58, 1.56]	[0.61, 1.39]	[0.71, 1.54]	[0.49, 1.21]	[1.13, 2.38]	[0.60, 1.42]	[0.93, 2.00]	[0.75, 1.80]	[0.90, 1.20]
	4	0.89	1.05	0.96	0.92	1.03	0.76	**1.62***	0.92	1.35	1.17	1.03
		[0.55, 1.42]	[0.70, 1.58]	[0.58, 1.57]	[0.61, 1.39]	[0.70, 1.51]	[0.48, 1.20]	[1.12, 2.34]	[0.60, 1.42]	[0.92, 1.99]	[0.76, 1.82]	[0.89, 1.20]

Note: n = 2215. Model 1 = crude model including the control variables: gender child, parental education level, maternal age at birth, and paternal age at birth; Model 2 = adjusted model including paternal MD, maternal MD, and the same control variables as model 1. MD = mental disorder; *OR* = odd ratio; PR = parent-reported; SR = self-reported. Boldface values indicate statistical significance.

[a] Cumulative risk was predicted through negative binomial regressions.

*p <.05; **p <.01.

Chapter 2, Table S6 Odds Ratios and 95% Confidence Intervals of the Fixed Part of the Crude and Adjusted Main Effects Models at Follow-up 18 Years in a Subsample of Children with Disruptive Behavior With Parental Internalizing and Parental Externalizing Disorders as Predictors

		Criminality		Aggression		Problems						
		Violent (SR)	Nonviolent (SR)	Aggression (SR)	Aggression (PR)	Conseq. of antisocial behavior (SR)	Rule-break. (PR)	Emotional (PR)	Alcohol (SR)	Truancy (SR)	Poor school performance (Reg.)	Cumulative risk[a]
Predictor	Model	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Internalizing MD	1	1.54 [0.90, 2.62]	1.44 [0.81, 2.56]	1.14 [0.66, 1.95]	1.04 [0.67, 1.63]	**1.90*** [1.12, 3.22]	1.34 [0.76, 2.34]	1.03 [0.61, 1.74]	1.30 [0.73, 2.32]	**2.09*** [1.19, 3.69]	1.69 [0.91, 3.13]	**1.21*** [1.03, 1.43]
	2	1.38 [0.82, 2.32]	1.36 [0.77, 2.42]	1.01 [0.59, 1.75]	1.00 [0.63, 1.60]	**1.80*** [1.06, 3.05]	1.30 [0.75, 2.24]	0.94 [0.55, 1.59]	1.18 [0.65, 2.14]	**2.02*** [1.14, 3.57]	1.59 [0.84, 2.99]	1.16 [0.99, 1.36]
Externalizing MD	1	**3.16**** [1.35, 7.38]	1.87 [0.77, 4.52]	**2.59*** [1.25, 5.35]	1.42 [0.69, 2.91]	1.87 [0.81, 4.35]	1.39 [0.63, 3.07]	**2.24*** [1.01, 4.98]	**2.44*** [1.09, 5.43]	1.68 [0.70, 4.01]	1.95 [0.81, 4.68]	**1.44**** [1.18, 1.75]
	2	**2.94*** [1.28, 6.77]	1.74 [0.73, 4.18]	**2.58*** [1.24, 5.35]	1.42 [0.67, 2.98]	1.62 [0.71, 3.70]	1.30 [0.60, 2.78]	2.27 [1.00, 5.18]	**2.34*** [1.03, 5.32]	1.42 [0.59, 3.41]	1.73 [0.70, 4.28]	**1.38**** [1.15, 1.67]

Note: n = 1190. Model 1 = crude model including the control variables: gender child, parental education level, maternal age at birth, and paternal age at birth; Model 2 = adjusted model including internalizing MD, externalizing MD, and the same control variables as model 1. Internalizing MDs refers to having one or more disorders in one or more of the following disorders: anxiety, depressive, bipolar, OCD, eating, and post-traumatic stress, Externalizing MDs refers to having one or more disorders in one or more of the of the following disorders: substance abuse, behavior, and antisocial personality. Conseq. = consequences; MD = mental disorder; OR = odd ratio; PR = parent-reported; Reg. = registry; Rule-break. = rule-breaking; SR = self-reported. Boldface values indicate statistical significance.

[a] Cumulative risk was predicted through negative binomial regressions.

*p <.05; **p <.01.

Chapter 2, Table S7 Odds Ratios and 95% Confidence Intervals of the Fixed Part of the Crude and Adjusted Main Effects Models at Follow-up 18 Years in a Subsample of Children with Disruptive Behavior With Paternal and Maternal Internalizing and Paternal and Maternal Externalizing Disorders as Predictors

		Criminality		Aggression		Problems						
		Violent (SR)	Nonviolent (SR)	Aggression (SR)	Aggression (PR)	Conseq. of antisocial behavior (SR)	Rule-break. (PR)	Emotional (PR)	Alcohol (SR)	Truancy (SR)	Poor school performance (Reg.)	Cumulative risk[a]
Predictor	Model	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Internalizing MD Father	1	1.15 [0.51, 2.61]	1.65 [0.68, 4.00]	1.40 [0.64, 3.04]	1.05 [0.50, 2.21]	1.61 [0.76, 3.43]	1.02 [0.39, 2.71]	0.62 [0.25, 1.53]	1.03 [0.42, 2.53]	**3.14**** [1.42, 6.95]	**2.46*** [1.05, 5.79]	1.20 [0.93, 1.55]
	2	1.08 [0.48, 2.44]	1.55 [0.62, 3.85]	1.34 [0.61, 2.94]	0.99 [0.47, 2.10]	1.57 [0.73, 3.40]	1.01 [0.38, 2.70]	0.64 [0.26, 1.58]	0.98 [0.39, 2.45]	**3.13**** [1.41, 6.94]	**2.42*** [1.02, 5.73]	1.18 [0.92, 1.52]
Internalizing MD Mother	1	1.71 [0.87, 3.35]	1.24 [0.60, 2.56]	0.96 [0.46, 1.98]	1.10 [0.64, 1.87]	1.96 [0.99, 3.88]	1.48 [0.77, 2.86]	1.40 [0.76, 2.59]	1.40 [0.67, 2.93]	1.65 [0.78, 3.49]	1.24 [0.54, 2.83]	1.20 [0.98, 1.48]
	2	1.49 [0.77, 2.89]	1.12 [0.54, 2.33]	0.83 [0.39, 1.76]	1.12 [0.62, 2.00]	1.80 [0.90, 3.62]	1.37 [0.73, 2.58]	1.20 [0.63, 2.29]	1.21 [0.56, 2.63]	1.64 [0.77, 3.46]	1.14 [0.48, 2.73]	1.14 [0.93, 1.40]
Externalizing MD Father	1	**4.87*** [1.42, 16.64]	**3.68*** [1.08, 12.52]	**2.57*** [1.11, 5.94]	1.96 [0.76, 5.05]	2.20 [0.81, 5.93]	1.51 [0.49, 4.67]	1.16 [0.44, 3.06]	**3.00*** [1.12, 8.07]	1.44 [0.50, 4.19]	2.00 [0.68, 5.87]	**1.50**** [1.26, 1.78]
	2	**4.10*** [1.19, 14.14]	3.35 [0.94, 11.96]	2.37 [0.95, 5.89]	2.17 [0.82, 5.73]	1.80 [0.62, 5.24]	1.31 [0.48, 3.59]	0.85 [0.26, 2.77]	2.61 [0.98, 6.97]	1.20 [0.38, 3.79]	1.71 [0.54, 5.45]	**1.39**** [1.14, 1.71]
Externalizing MD Mother	1	2.41 [0.80, 7.28]	1.54 [0.45, 5.26]	1.75 [0.62, 4.98]	0.73 [0.32, 1.67]	1.89 [0.59, 6.06]	1.73 [0.59, 5.09]	**4.01*** [1.23, 13.04]	2.38 [0.72, 7.82]	1.21 [0.33, 4.52]	1.57 [0.48, 5.21]	1.35 [0.99, 1.86]
	2	1.62 [0.54, 4.83]	1.18 [0.36, 3.88]	1.54 [0.47, 5.04]	0.57 [0.21, 1.57]	1.34 [0.42, 4.23]	1.44 [0.55, 3.75]	**3.90*** [1.11, 13.69]	1.82 [0.53, 6.26]	1.01 [0.26, 3.94]	1.34 [0.35, 5.12]	1.19 [0.86, 1.65]

Note: n = 1190. Model 1 = crude model including the control variables: gender child, parental education level, maternal age at birth, and paternal age at birth; Model 2 = adjusted model including maternal/paternal internalizing MD, maternal/paternal externalizing MD, and the same control variables as model 1. Internalizing MDs refers to having one or more disorders in one or more of the following disorders: anxiety, depressive, bipolar, OCD, eating, and post-traumatic stress, Externalizing MDs refers to having one or more disorders in one or more of the following disorders: substance abuse, behavior, and antisocial personality. CI = confidence interval; Conseq. = consequences; MD = mental disorder; *OR* = odd ratio; PR = parent-reported; Reg. = registry; Rule-break. = rule-breaking; SR = self-reported. Boldface values indicate statistical significance.

[a] Cumulative risk was predicted through negative binomial regressions.

*p <.05; **p <.01.

SUPPLEMENT TO CHAPTER 3

Associations between anxiety, depression, and disruptive behavior spanning childhood and adolescence

Chapter 3, Supplement 1. Additional information on measures

Chapter 3, Table S1 Overview of Disruptive Behavior Items

Oppositional Defiant Disorder items
Gate items
Has there ever been a time when s/he would be angry to the extent that s/he cannot be reached?
Does s/he often argue with adults?
Does s/he often tease others by deliberately doing things that are perceived as provocative?
Is s/he easily offended, or disturbed by others?
Is s/he easily teased?
Additional items
Has there ever been a time when s/he would be angry to the extent that s/he is out of control without there being any particular triggering event?
Has there ever been a time when s/he would be angry to the extent that s/he is out of control in connection with changes?
Does s/he often lose temper?
Does s/he refuse following other people's directives?
Is s/he often vindictive or cruel?
Does s/he often treat significant others badly or without respect?
Does s/he often blame others for own mistakes or bad actions?
Conduct Disorder items
Gate items
Has s/he ever deliberately been physically cruel to anybody?
Does s/he often start fights?
Does s/he often lie or cheat?
Does s/he steal things at home or outside home?
Has s/he ever engaged in shoplifting?
Additional items
Does s/he often threaten, harass or humiliate others?
Is s/he cruel to insects?
Is s/he cruel to other animals?
Has s/he ever started a fire?

Chapter 3, Table S1 Continued.

Has s/he ever sexually abused other children?
Has s/he ever been detained by the police?
Has s/he ever used a deadly weapon?
Has s/he ever robbed anyone or else unlawfully acquired other people's property by means of directs threats?
Has s/he ever purposely attempted to destroy other people's property?
Has s/he ever broken into someone else's home, premises or car?
Is s/he often out late at night without consent (beginning before 13 years of age)?
Has s/he ever ran away from home and stayed away over night at least two times (or one time if it was for an extended period of time)?
Is s/he often absconding (beginning before 13 years of age)?

Note. The additional items were administered if the parents endorsed one or more of the gate items with "yes to some extent" or "yes".

Disruptive behavior at age 15 years

Self-reported aggression

Aggressive behavior was assessed using the 23-item Reactive and Proactive Aggression Questionnaire (RPQ).(Raine et al., 2006) The RPQ includes 11 items that focus on reactive aggression (e.g., "Reacted angrily when provoked by others", "Gotten angry when frustrated"), and 12 items that focus on proactive aggression (e.g., "Had fights with others to show who was on top", "Taken things from other students"). The items are coded as 0 ("never"), 1 ("sometimes"), or 2 ("often").

Self-reported crime

The Self-reported Delinquency Scale (SRD)(Ring, 1999) was used to assess the frequency of 13 non-violent criminal acts (e.g., vandalism, car theft, burglary, drug dealing) and nine violent criminal acts (e.g., hurting persons, hurting animals, sexual offenses). Each item is coded on a 6-point Likert scale, ranging from 0 ("never") to 5 ("more than 10 times").

Parent- and self-reported conduct problems

Conduct problems of the twin were assessed using the Conduct Problems subscale of the Strengths and Difficulties Questionnaire (SDQ).(Goodman, 1997) SDQ items (e.g., "Often has temper tantrums or hot tempers") scored on this and the other SDQ scales mentioned below range from 0 ("not true"), 1 ("somewhat true") and 2 ("certainly true").

Bullying perpetration

The Bullying Perpetration subscale of the Revised Olweus Bully/Victim Questionnaire (OBVQ; Olweus, 1996) was used to measure self-reported bullying behavior (Solberg and Olweus, 2003). The scale consists of nine questions covering various types of bullying behaviors (e.g., "I called another student(s) mean names and made fun of or teased him or her in a hurtful way."). Answers ranged from 1 ("It has not happened in the last couple of months") to 5 ("Several times a week"). In line with prior work (Solberg and Olweus, 2003), being a bully perpetrator was defined as answering one or more of these questions with a 3 or higher ("2 or 3 times a month").

Disruptive behavior at age 18 years

Self-reported aggression

Aggression was assessed using the 11-item Aggression subscale of the Life History of Aggression Questionnaire.(Coccaro et al., 1997) Youth were asked how many times in their lives they had committed certain aggressive acts (e.g., "Gotten into verbal fights or arguments with other people"). Answers were given on a 6-point Likert scale ranging from 0 ("no event") to 5 ("more times than I can count").

Self-reported crime

Self-reported crime was assessed using the same Self-reported Delinquency Scale as at age 15 years (see outcome measures at age 15).

Self-reported consequences of antisocial behavior

The 4-item Consequences of Antisocial Behavior subscale of the Life History of Aggression Questionnaire measures social consequences due to antisocial behavior of the reporter (e.g., "Had discipline problems in schools that resulted in a reprimand by the school principal or in suspensions or expulsion").

Parent-reported aggression

Aggression was assessed by means of the 16-item Aggressive Behavior subscale of the Adult Behavior Checklist (ABCL)(Achenbach & Rescorla, 2003) parent version. Parents rated aggression of their twin over the last 6 months (e.g., "Physically attacks people" and "Argues a lot") on a 3-point Likert scale ranging from 0 ("not true") to 2 ("very true or often true").

Parent-reported rule-breaking behavior

Rule-breaking behavior was assessed by the 13-item ABCL Rule-breaking Behavior subscale (e.g., "Breaks rules at work or elsewhere" or "Lying or cheating").

Chapter 3, Supplement 2. Analyses with dichotomous measures of anxiety and depression

At baseline 9, crude models indicated that anxiety (IRR = 3.63; 95% CI: 3.39), and depressive disorders (IRR = 4.18; 95% CI: 3.93, 4.45), as well as 3.89), were significantly related to DB (p's < .001). When included simultaneously in an adjusted model, both anxiety (IRR = 2.35; 95% CI: 2.16, 2.56), and depressive disorders (IRR = 3.19; 95% CI: 2.96, 3.44) retained their associations with DB (p's < .001).

Longitudinally, crude models indicated that DB at 9 years was predictive (p's < .001) of DB at 15 (IRR = 1.11; 95% CI: 1.09, 1.13) and 18 years (IRR = 1.17; 95% CI: 1.03, 1.06). Similar crude models indicated no significant predictive effects on DB for anxiety disorders at 15 and 18 years. For depressive disorders significant predictive effects were found on DB at 15 (IRR = 1.33; 95% CI: 1.09, 1.63; p = .006) and 18 years (IRR = 1.75; 95% CI: 1.29, 2.38; p < .001).

When DB, anxiety, and depressive disorders at 9 years were included simultaneously in one model, DB retained its predictive associations (p's < .001) with DB on 15 years (IRR = 1.09; 95% CI: 1.08, 1.10), and 18 years (IRR = 1.09; 95% CI: 1.06, 1.12). Both anxiety and depressive disorders lost their association with DB at 15 years and age 18 years. Interaction models did not indicate significant interactions between DB and anxiety and depressive disorders at 15 years, and for anxiety disorders at 18 years. At 18 years, a significant interaction emerged between depressive disorders and DB (IRR = 0.88; 95% CI: 0.81, 0.96), however it should be noted that this interaction was underpowered (i.e., only seven 18-year-olds had a depression at baseline 9 years vs. 779 without a depression).

SUPPLEMENT TO CHAPTER 4

Classes of oppositional defiant disorder behavior in clinic-referred children and adolescents: concurrent features and outcomes

Chapter 4, Table S1 Oppositional Defiant Behavior Items of the Development and Well-Being Assessment (DAWBA)

Dimensions	Parent-version	Teacher-version
Irritable	Had temper outbursts?	Temper tantrums or hot tempers
	Been touchy or easily annoyed?	Easily annoyed by others
	Been angry and resentful?	Angry and resentful
Oppositional	Seemed to do things to annoy other people on purpose?	Deliberately does things to annoy others
	Blamed others for his/her own mistakes or bad behaviour?	Blames others for his/her own mistakes
	Argued with grown-ups?	Argues a lot with adults
	Taken no notice of rules, or refused to do as s/he is told?	Disobedient at school
	Been spiteful?	Spiteful
	Tried to get back at someone	Tried to get back at someone

Chapter 4, Table S2 Steps in the Symptom-based ODD behavior Latent Class Analysis in Children Aged 5 through 11 (n = 1499)

Analysis	LL	BIC	BIC (sample adjusted)	AIC	Entropy	Bootstrap p-value
1-class	-8973.139	18026.717	17991.773	17968.279		
2-class	-6446.922	13040.095	12976.560	12933.844	0.847	
2-class (corrected for bivariate residuals)	Model did not converge					
2-class (corrected for bivariate residuals) no sex						
3-class	-6190.631	12607.951	12509.473	12443.262	0.822	
3-class (corrected for bivariate residuals)	-6101.663	12481.203	12360.488	12279.326	0.786	
3-class (corrected for bivariate residuals) no sex	-6105.268	12473.788	12359.426	12282.536	0.789	<.001
4-class	-6148.064	12603.255	12469.833	12380.128	0.788	
4-class (corrected for bivariate residuals)	-6096.880	12537.450	12388.144	12287.760	0.760	
4-class (corrected for bivariate residuals) no sex	-6101.081	12523.915	12384.140	12290.163	0.752	<.001
5-class	-6112.966	12613.498	12445.132	12331.933	0.752	

Note. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; LL = Log-likelihood

Chapter 4, Table S3 Steps in the Symptom-based ODD behavior Latent Class Analysis in Children Aged 12 through 18 (n = 686)

Analysis	LL	BIC	BIC (sample adjusted)	AIC	Entropy	Bootstrap p-value
1-class	-4200.938	8473.717	8438.790	8423.877		
2-class	-2914.504	5959.626	5896.123	5869.008	0.865	
2-class (corrected for bivariate residuals)	-2736.718	5675.893	5577.464	5535.436	0.868	
2-class (corrected for bivariate residuals) no sex	-2736.730	5669.387	5574.133	5533.461	0.866	<.001
3-class	-2788.057	5778.571	5680.141	5638.114	0.838	
3-class (corrected for bivariate residuals)	-2720.288	5688.749	5568.093	5516.576	0.798	
3-class (corrected for bivariate residuals) no sex	-2720.447	5676.005	5561.700	5512.893	0.794	<.001
4-class	-2756.396	5787.089	5653.733	5596.792	0.797	
4-class (corrected for bivariate residuals)						
4-class (corrected for bivariate residuals) no sex						
5-class	-2730.075	5806.287	5638.004	5566.150	0.821	

Note. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; LL = Log-likelihood

Chapter 4, Table S4 Steps in the Symptom-based ODD Behavior Latent Class Analysis

Analysis	LL	BIC	BIC (sample adjusted)	AIC	Entropy	Bootstrap p-value
1-class	-18918.397	37936.757	37895.454	37862.795		
2-class	-9412.654	18986.786	18920.066	18867.309	0.850	
2-class (corrected for bivariate residuals)						
2-class (corrected for bivariate residuals) age-only						
2-class (corrected for bivariate residuals) sex-only						
2-class (corrected for bivariate residuals) no covariates						
3-class	-9044.693	18343.135	18238.289	18155.386	0.816	

Chapter 4, Table S4 Continued.

Analysis	LL	BIC	BIC (sample adjusted)	AIC	Entropy	Bootstrap p-value
3-class (corrected for bivariate residuals)						
3-class (corrected for bivariate residuals) age-only						
3-class (corrected for bivariate residuals) sex-only						
3-class (corrected for bivariate residuals) no covariates						
4-class	-8965.953	18277.927	18134.956	18021.906	0.861	
4-class (corrected for bivariate residuals)						
4-class (corrected for bivariate residuals) age-only						
4-class (corrected for bivariate residuals) sex-only						
4-class (corrected for bivariate residuals) no covariates						
5-class	-8909.591	18257.476	18076.379	17933.181	0.792	
5-class (corrected for bivariate residuals)	-8832.732	18172.963	17963.272	17797.464	0.745	
5-class (corrected for bivariate residuals) age-only	-8837.797	18152.336	17955.353	17799.595	0.743	0.2174
5-class (corrected for bivariate residuals) sex-only	-8863.570	18203.881	18006.898	17851.140	0.750	
5-class (corrected for bivariate residuals) no covariates	-8878.032	18202.048	18017.774	17872.065	0.708	
6-class	-8879.034	18288.634	18069.411	17896.067	0.775	

Note. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; LL = Log-likelihood.

Chapter 4, Table S5 Steps in the DSM-based ODD Latent Class Analysis

Analysis	LL	BIC	BIC (sample adjusted)	AIC	Entropy	Bootstrap p-value
1-class	-19997.511	40094.984	40053.681	40021.022		
2-class	-8823.071	17807.618	17740.898	17688.142	0.917	
2-class (corrected for bivariate residuals)	Model did not converge					
2-class (corrected for bivariate residuals) age-only						
2-class (corrected for bivariate residuals) sex-only						
2-class (corrected for bivariate residuals) no covariates						
3-class	-8159.108	16571.965	16467.119	16384.216	0.899	
3-class (corrected for bivariate residuals)	-7988.821	16339.042	16189.717	16071.642	0.832	
3-class (corrected for bivariate residuals) age-only	-7995.788	16337.598	16194.627	16081.577	0.821	
3-class (corrected for bivariate residuals) sex-only	-7989.519	16325.059	16182.088	16069.037	0.820	
3-class (corrected for bivariate residuals) no covariates	-7996.763	16324.168	16187.551	16079.525	0.821	<.001
4-class	-8067.291	16480.604	16337.633	16224.582	0.851	
4-class (corrected for bivariate residuals)	-7998.807	16389.771	16227.737	16099.614	0.835	
4-class (corrected for bivariate residuals) age-only	-8005.390	16379.870	16227.368	16106.781	0.834	
4-class (corrected for bivariate residuals) sex-only	-7999.153	16367.396	16214.893	16094.306	0.833	
4-class (corrected for bivariate residuals) no covariates	-8006.650	16359.322	16216.351	16103.300	0.832	<.001
5-class	-8015.251	16468.797	16287.700	16144.503	0.829	
5-class (corrected for bivariate residuals)	-7978.415	16433.571	16236.589	16080.830	0.816	
5-class (corrected for bivariate residuals) age-only	-7985.638	16417.259	16232.985	16087.275	0.805	
5-class (corrected for bivariate residuals) sex-only	-7982.488	16410.959	16226.685	16080.976	0.815	
5-class (corrected for bivariate residuals) no covariates	-7991.713	16398.652	16227.086	16091.426	0.808	<.001
6-class	-7980.686	16491.939	16272.716	16099.372	0.827	

Note. AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; LL = Log-likelihood.

Chapter 4, Table S6 Means and Standard Deviations of the DSM-based ODD Classes on Highest Prevailing Parent- and Teacher-reported SDQ Scores

	ODD classes		
	High ODD	Moderate ODD	Low ODD
	(n = 565)	(n = 693)	(n = 906)
SDQ Total Problems (SD)	23.29 (5.28)	20.51 (4.83)	18.28 (4.71)
SDQ Emotional Problems (SD)	6.05 (2.57)	5.69 (2.55)	5.75 (2.50)
SDQ Conduct Problems (SD)	5.75 (1.90)	4.21 (1.70)	3.28 (1.66)
SDQ Hyperactivity (SD)	7.77 (2.14)	7.39 (2.25)	6.51 (2.53)
SDQ Peer Problems (SD)	4.55 (2.20)	4.05 (2.28)	3.55 (2.16)
SDQ Prosocial (SD)	6.27 (1.99)	7.00 (1.97)	7.57 (1.83)

Note. N = 2164. SDQ = strengths and difficulties questionnaire.

Chapter 4, Table S7 Prevalence of DAWBA Classifications of the DSM-based Oppositional Defiant Disorder Classes

	ODD classes		
	High ODD	Moderate ODD	Low ODD
	(n = 565)	(n = 693)	(n = 906)
ODD [n(% of class)]	493 (87.3%)	388 (56.0%)	78 (8.6%)
CD [n(% of class)]	154 (27.3%)	43 (6.2%)	22 (2.4%)
ADHD [n(% of class)]	318 (56.3%)	314 (45.3%)	216 (23.8%)
Depressive disorders [n(% of class)]	102 (18.1%)	93 (13.4%)	138 (15.2%)
Generalized anxiety [n(% of class)]	114 (20.2%)	107 (15.4%)	134 (14.8%)
Fear disorders [n(% of class)]	131 (23.2%)	136 (19.6%)	184 (20.3%)
Autism spectrum disorders [n(% of class)]	39 (6.9%)	36 (5.2%)	24 (2.6%)

Note. N = 2164. ADHD = attention deficit hyperactivity disorder; CD = conduct disorder; ODD = oppositional defiant disorder.

Chapter 4, Table S8 Prevalence of Clinical Classifications of the DSM-based Oppositional Defiant Disorder Classes

	ODD class		
	High ODD	Moderate ODD	Low ODD
	(n = 540)	(n = 653)	(n = 848)
ODD [n(% of class)]	78 (14.4%)	57 (8.7%)	42 (5.0%)
CD [n(% of class)]	41 (7.6%)	15 (2.3%)	13 (1.5%)
ADHD [n(% of class)]	206 (38.1%)	249 (38.1%)	300 (35.4%)
Depressive disorders [n(% of class)]	34 (6.3%)	38 (5.8%)	65 (7.7%)
Generalized anxiety [n(% of class)]	13 (2.4%)	23 (3.5%)	56 (6.6%)
Fear disorders [n(% of class)]	7 (1.3%)	15 (2.3%)	39 (4.6%)
Autism spectrum disorder [n(% of class)]	131 (24.3%)	169 (25.9%)	186 (21.9%)

Note. N = 2041. ADHD = attention deficit hyperactivity disorder; CD = conduct disorder; ODD = oppositional defiant disorder.

Chapter 4, Supplement 1. Detailed measures

Clustering Variables

ODD symptoms were measured by the Dutch parent and teacher versions of the DAWBA, a widely-used computerized diagnostic interview which generates DSM-IV classifications. The parent version of the DAWBA has a gate-item which inquires if the child had exhibited any ODD-related symptoms in the last six months (i.e., "Not doing what they are told, being irritable or annoying, having temper outbursts, and so on"). The response on this gate item ranges from 0 (on average less difficult or problematic than other children), to 1 (about average) to 2 (on average more difficult or more problematic). If the parent endorses this gate-item with a 2, the ODD part of the DAWBA is activated, which inquires after the occurrence of the eight DSM-IV ODD symptoms in the last six months. The ODD part of the DAWBA is also activated when the parent indicates a score of 3 or higher on the SDQ conduct problems scale, which consists of five questions, and which is an integral part of the DAWBA. The teacher version of the DAWBA always directly asks teachers about all eight DSM-IV ODD symptoms. Of note, the Dutch version of the DAWBA separates the original DSM criterion of "vindictive and spiteful" into two different questions (see Table S1), resulting in a total of nine ODD symptoms. Assessment of impairment and persistence is considered a crucial diagnostic criterion for identifying individuals whose psychiatric disorders are of clinical significance.

Therefore, the DAWBA also asks parents and teachers whether ODD symptoms have resulted in impairment in various developmental contexts (e.g., "Has his/her awkward behavior interfered with making and keeping friends") and whether these symptoms have been present for more than 6 months. The impairment questions are rated from 0 ("Not at all") to 3 ("A great deal") and the persistence question is rated by 0 ("No") or 1 ("Yes").

Whereas prior research on ODD classes merely considered if ODD symptoms were present or absent (from here onwards referred to as the *symptom approach*), the present study also considered symptom persistence (6 months ≤) and impairment (from here onwards referred to as the *DSM app*roach). Specifically, according to this first approach an ODD symptom was coded as 1 ("behavior present") when endorsed as 1 ("A little more than others") or higher (2: "A lot more others"), while a score of 0 ("Not more often than others") was dichotomized as 0 ("behavior absent") (0 = 0; 1, 2 = 1). For the DSM approach, more stringent criterion for ODD presence were used; a symptom was coded as 1 ("behavior present") when endorsed as 2 ("A lot more than others"), while lower scores were coded as 0 ("behavior absent") (0, 1 = 0; 2 = 1). In addition, the reported ODD symptom was required to be present for six months or longer, and to cause impairment according to parent- and/or teacher-ratings. In both the symptom and DSM-approach parent- and teacher-ratings were combined by using highest prevailing scores (i.e., if at least one informant indicated an ODD behavior to be present, the behavior was indicated as present). Finally, the nine DAWBA ODD symptoms will be used as clustering variables in the person-oriented analyses (i.e., latent class analysis) to assign youths to mutually exclusive classes.

External variables for cluster comparisons: concurrent features at referral

Dimensionally assessed mental health and other problems

The SDQ is a brief screening questionnaire that was completed as part of the DAWBA. The SDQ consists of 25 items which are scored on a 3-point Likert scale 0 ("not true"), 1 ("somewhat true") and 2 ("certainly true"), and is subdivided in 5 subscales: Conduct Problems, Emotional Problems, Hyperactivity, Peer Problems, and Prosocial Behavior. The Total Problems scale consists of all SDQ items, minus the Prosocial Behavior scale. Because items of the Conduct Problems scale were used as gate items for the ODD symptoms, this scale was

not used in class comparisons. Highest prevailing scores of parent-, teacher-, and, if applicable -youth self-report were used in the subsequent analyses.

Categorically assessed mental health problems

The DAWBA reports of parents, teachers, and youths who were at least 11 years of age, were used to generate *computer-generated DSM classifications* at referral. These classifications are based on predictions on the probability of the presence of various mental disorders. For each disorder, five categories are given, ranging from 0 (0.1 % of children in this category have the disorder in question) to 5 (70% of children in this category have the disorder in question). The categories were dichotomized into a "disorder absent" category ranging from values 0 to 3 (15% of children in this category have the disorder in question) and a "disorder present" category spanning values 4 (50% of children in this category have the disorder in question) and 5 (Goodman et al., 2011). To ease the interpretation of the results, and in line with previous recommendations (Stringaris & Goodman, 2009a), several DAWBA computer-generated DSM classifications were combined into disorder categories, from here onwards referred to as *DAWBA computer-generated DSM disorder categories*. Specifically, the category "depressive disorders" refers to the presence of major depressive disorder, dysthymic disorder, and/or depressive disorder not otherwise specified, whereas the category "fear disorders" refers to the presence of separation anxiety disorder, panic disorder, agoraphobia specific, and/or social phobia.

External variables for cluster comparisons: longitudinal features

Categorically assessed mental health problems

Psychiatric disorders, as defined by the DSM-IV, were determined at the end of a diagnostic process consisting of multidisciplinary psychiatric and psychological (semi-structured) evaluation conform clinical diagnostic guidelines by psychiatrists and psychologists. These evaluations took place on average 3.81 months (SD = 3.34) after completion of the DAWBA at referral. Any clinical classification, not just primary classifications, were included in the analyses. This was done to optimally use the classifications provided by the multidisciplinary team and because the DAWBA also provides multiple classifications per individual. From here onward, we refer to these disorders and disorder categories as *Multidisciplinary Team-based Classifications of DSM Disorders and Disorder Categories*.

Global functioning

DSM-based Global Assessment Functioning (GAF) scores give an indication of social, occupational, and psychological functioning of an individual, with a

score of "100" indicating extremely high functioning, while "1" indicates severe impairment (e.g., persistent danger of severely hurting self or others, suicidal acts). General functioning of the youth at the beginning and end of treatment was measured through clinician-rated GAF scores.

Chapter 4, Supplement 2. Latent class analysis model selection

The following steps were taken to select the best fitting latent class solution. First, models were selected on the basis of the Bayesian Information Criterion (BIC), which is considered to be the most reliable index of model fit in LCA after non-parametric bootstrapping.(Nylund, Asparouhov, & Muthén, 2007) Although other indices of model fit were also studied, including: entropy, loglikelihood, Akaike information criterion (AIC). In the second step, to control for local independence, the Pearson chi-squared test of model fit was used to determine if main effects between items should be included in the model. When the Pearson chi-squared test of model fit indicated significance ($p < .05$), the item-pair with the highest bivariate residuals was included as a direct effect (e.g., the item-pair vindictive and spiteful), and the model rerun. This process was repeated until the Pearson chi-squared index indicated non-significance.(Asparouhov & Muthén, 2015) The third step focussed on the influence of the covariates age and gender on the model, which was investigated by deleting the covariates in a stepwise manner. If exclusion of a covariate(s) resulted in a better model fit, the better fitting model was included in consequent analyses. The fourth step consisted of estimating model fit through non-parametric bootstrapping. The number of random starts perturbations varied per solution and was in each example increased until the best loglikelihood was replicated during the bootstrap runs. If a p-value was greater than .05 (indicating model fit) the model was chosen. When the p-value was lower than .05, the next most appropriate model was fitted, starting with step two.

Symptom-based latent class analysis: stability of age covariate

Because the symptom-based Latent Class Solution required age as a covariate, the robustness of age was investigated. This was done by running separate LCA's on two age groups: 11 years or younger (n = 1499), and 12 years or older (n = 686). Unfortunately, the five factor solution did not hold up with three classes found in the younger group and two-class solutions in the older group. Specifically, separate LCAs extracted three classes in children aged 11 or younger (n = 1499), and two classes in adolescents aged 12 or older (n = 686; see Tables S2-S3).

SUPPLEMENT TO CHAPTER 5

Genomics of human aggression: current state of genome-wide studies and an automated systematic review tool

Chapter 5, Supplement S1 Definitions of Aggression in Reviews

Concept	Definitions	References
Reactive/hostile/ affective/impulsive aggression	Angry or frustrated responses to a real or perceived threat (Tuvbald,Baker, 2011) Aggressive response to a perceived threat or provocation (Waltes et al., 2015)	Tuvblad&Baker, 2011 Craig et al, 2009; Waltes et al, 2015
Proactive/ instrumental/pre-mediated aggression	Planning, the motive of the act extends beyond harming the victim (Tuvbald,Baker, 2011) Planned antisocial behaviour that anticipates a reward or dominance over others (Waltes et al., 2015)	
Direct/physical aggression	Intentionally causing pain or harm to the victim	Tuvblad&Baker, 2011
Indirect/relational aggression	Relational social manipulation such as gossip and peer exclusion	
Chronic physical aggression	Tendency to use physical aggression more frequently than the large majority of a birth cohort over many years	Tremblay et al, 2018; Provencal et al., 2015
Externalizing behaviour	Behavior that directs problematic energy outward and is expressed as aggression, defiance, bullying, vandalism, theft, and other socially unacceptable actions	Anholt&Mackay, 2012 Dick et al, 2016
Aggression and anger-related traits associated with suicidal behaviour	Anger can be conceptualized as a core construct of related traits or variables inwardly and/or outwardly expressed such as aggression, rage, and hostility (Spielberger et al, 1985 cite: Baud, 2005) Aggression and anger-related traits are considered risk factors for suicidal behaviour	Baud, 2005
Aggression related phenotype	A dimensional trait including externalizing behaviour, anger, delinquency, criminality, violence or a diagnostic category (conduct disorder, oppositional defiant disorder, callous unemotional, and antisocial personality)	Fernandez-Castillo, Cormand, 2016

Concept	Definitions	References
Frustrative non-reward aggression	Behaviours that correspond to the withdrawal or prevention of reward	RDoC nomenclature Veroude et al, 2015
Defensive aggression	Behaviors caused by the perception of an immediate threat, which have the goal of eliminating the threat	
Offensive (or proactive) aggression	Instrumental behaviors aimed at achieving a positive goal, often in the face of competition or in the context of social hierarchies	
Aggression as behavior category in conduct disorder (CD)	CD is a developmental disorder characterized by a consistent pattern of externalizing behavior, developing during childhood or adolescence, where an individual displays aggression toward people or animals, destroys property, exhibits deceit by lying or stealing, and/or seriously violates societal rules or norms (DSM-V) Conduct disorder is a psychiatric disorder of childhood and adolescence characterized by aggression toward people and animals, destruction of property, deceitfulness or theft, and serious violation of rules (Salvatore & Dick, 2018)	DSM-V Salvatore et al, 2018
Antisocial behaviour	Refers to actions that violate social norms in ways that reflect the violation of others' rights	Moffit, 2005; Gard et al, 2018
Aggression as violence	*No definition is given*	Vassos et al, 2014

Chapter 5, Supplement S2. Search terms used to extract papers from databases

Search terms are reported for each subject and database respectively.

Pubmed

Searchterms for reviews on genetics of aggression:
((“Aggression/genetics”[Mesh] OR ((“genetics”[tiab] OR “Genetic Techniques”[mesh] OR “Genetic Phenomena”[mesh] OR “Genes”[mesh] OR “genes”[tiab] OR “gene”[tiab] OR “heredity”[tiab] OR “hereditary”[tiab] OR “Epigenomics”[mesh] OR epigenetic*[tiab] OR “Polymorphism, Genetic”[mesh] OR polymorphism*[tiab] OR “Genotype”[mesh] OR genotype*[tiab] OR “Genome”[mesh] OR genome*[tiab] OR “systems genetics approach”[tiab] OR “systems genetics”[tiab] OR “Genome-Wide Association Study”[Mesh] OR “genome wide association”[tw] OR “genomic wide association”[tw] OR “GWA Study”[tw] OR “GWA Studies”[tw] OR “GWAS”[tw] OR “GWASs”[tw] OR “epigenome wide association”[tw] OR ((“genome wide”[tw] OR “genomic wide”[tw]) AND “association”[tiab]) OR “genetic association”[tw] OR “Genetic Association Studies”[Mesh] OR “candidate genes”[tw] OR “candidate gene”[tw] OR “candidates genes”[tw] OR “SNP”[tw] OR “SNPS”[tw] OR “Polymorphism, Single Nucleotide”[Mesh] OR “single nucleotide polymorphisms”[tw] OR “single nucleotide polymorphism”[tw] OR “Polymorphism, Genetic”[Mesh] OR “Genetic Polymorphisms”[tw] OR “Genetic Polymorphism”[tw] OR “Genomic Structural Variation”[tw] OR “DNA Copy Number Variations”[tw] OR “Pharmacogenomic Variants”[tw] OR “Restriction Fragment Length Polymorphism “[tw] OR “Single-Stranded Conformational Polymorphism “[tw] OR “Genomic Structural Variations”[tw] OR “DNA Copy Number Variation”[tw] OR “Pharmacogenomic Variant”[tw] OR “Restriction Fragment Length Polymorphisms”[tw] OR “Single-Stranded Conformational Polymorphisms”[tw]) AND (“Aggression”[mesh:noexp] OR “aggression”[tiab] OR aggression*[tiab] OR “aggressive behavior”[tiab] OR “aggressive behaviour”[tiab] OR “aggressive behaviors”[tiab] OR “aggressive behaviours”[tiab] OR aggressive behavi*[tiab] OR “Anger”[mesh] OR “anger”[tiab] OR “Rage”[mesh] OR “angry”[tiab] OR “Hostility”[mesh] OR “hostility”[tiab] OR “hostile”[tiab] OR “Violence”[mesh:noexp] OR “violence”[tiab] OR “violent”[tiab] OR (violen*[tiab] AND (crime*[tiab] OR crimin*[tiab])) OR (aggress*[tiab] AND (crime*[tiab] OR crimin*[tiab])) OR

aggressive trait*[tiab] OR "hyperaggression"[tiab] OR hyperaggress*[tiab] OR "oppositional defiant disorder"[tiab] OR "oppositional defiant"[tiab] OR oppositional defiant*[tiab] OR "conduct disorder"[tiab] OR "conduct disorders"[tiab] OR conduct disorder*[tiab] OR "Antisocial Personality Disorder"[mesh] OR "antisocial personality disorder"[tiab] OR "antisocial personality disorders"[tiab] OR "anti-social personality disorder"[tiab] OR "anti-social personality disorders"[tiab] OR (aggressi*[tiab] AND ("proactive"[tiab] OR "reactive"[tiab] OR "impulsive"[tiab] OR "physical"[tiab])))) NOT ("Animals"[mesh] NOT "Humans"[mesh]) NOT ("Neoplasms"[mesh] OR "cancer"[tw] OR "tumour"[tw] OR "tumours"[tw] OR "tumor"[tw] OR "tumors"[tw] OR "aggressive treatment"[tiab] OR ("Mental Disorders"[mesh] NOT ("Attention Deficit and Disruptive Behavior Disorders"[Mesh] OR "Conduct Disorder"[Mesh] OR "Antisocial Personality Disorder"[Mesh])) OR "Nervous System Diseases"[mesh] OR "Congenital, Hereditary, and Neonatal Diseases and Abnormalities"[Mesh]) AND ("Review"[ptyp] OR "review"[tw] OR review*[tw] OR overview*[tw] OR "systematic"[sb]))

Searchterms for genome-wide studies:
(("Genome-Wide Association Study"[Mesh] OR "genome wide association"[tw] OR "genomic wide association"[tw] OR "GWA Study"[tw] OR "GWA Studies"[tw] OR "GWAS"[tw] OR "GWASs"[tw] OR "epigenome wide association"[tw] OR (("genome wide"[tw] OR "genomic wide"[tw]) AND "association"[tiab]) OR "genetic association"[tw] OR "Genetic Association Studies"[Mesh]) AND ("Aggression"[Mesh:noexp] OR "aggression"[tw] OR aggression*[tw] OR "aggressive behavior"[tw] OR "aggressive behaviour"[tw] OR "aggressive behaviors"[tw] OR "aggressive behaviours"[tw] OR aggressive behavi*[tw] OR "Anger"[mesh] OR "anger"[tw] OR "rage"[mesh] OR "angry"[tw] OR "Hostility"[mesh] OR "hostility"[tw] OR "hostile"[tw] OR "Violence"[Mesh:noexp] OR "violence"[tw] OR "violent"[tw] OR (violen*[tw] AND (crime*[tw] OR crimin*[tw])) OR (aggress*[tw] AND (crime*[tw] OR crimin*[tw])) OR aggressive trait*[tw] OR "hyperaggression"[tw] OR hyperaggress*[tw] OR "oppositional defiant disorder"[tw] OR "oppositional defiant"[tw] OR oppositional defiant*[tw] OR "conduct disorder"[tw] OR "conduct disorders"[tw] OR conduct disorder*[tw] OR "Antisocial Personality Disorder"[Mesh] OR "antisocial personality disorder"[tw] OR "antisocial personality disorders"[tw] OR "anti-social personality disorder"[tw] OR "anti-

social personality disorders"[tw] OR (aggressi*[tw] AND ("proactive"[tw] OR "reactive"[tw] OR "impulsive"[tw] OR "physical"[tw]))) NOT ("Animals"[mesh] NOT "Humans"[mesh]))

Embase

Searchterms for reviews on genetics of aggression:
(("genetics".ti,ab OR exp *"genetics"/ OR exp *"Genetic Procedure"/ OR exp *"Heredity"/ OR exp *"molecular genetic phenomena and functions"/ OR exp *"Gene"/ OR "genes".ti,ab OR "gene".ti,ab OR "heredity".ti,ab OR "hereditary".ti,ab OR *"Epigenetics"/ OR epigenetic*.ti,ab OR exp *"Genetic Polymorphism"/ OR polymorphism*.ti,ab OR exp *"Genotype"/ OR genotype*.ti,ab OR exp "Genome"/ OR genome*.ti,ab OR "systems genetics approach".ti,ab OR "systems genetics".ti,ab OR *"Genome-Wide Association Study"/ OR "genome wide association".mp OR "genomic wide association". mp OR "GWA Study".mp OR "GWA Studies".mp OR "GWAS".mp OR "GWASs".mp OR "epigenome wide association".mp OR (("genome wide".mp OR "genomic wide".mp) AND "association".ti,ab) OR "genetic association".mp OR *"Genetic Association Study"/ OR "candidate genes".mp OR "candidate gene".mp OR "candidates genes".mp OR "SNP".mp OR "SNPS".mp OR *"Single Nucleotide Polymorphism"/ OR "single nucleotide polymorphisms". mp OR "single nucleotide polymorphism".mp OR exp *"DNA Polymorphism"/ OR "Genetic Polymorphisms".mp OR "Genetic Polymorphism".mp OR "Genomic Structural Variation".mp OR "DNA Copy Number Variations". mp OR "Pharmacogenomic Variants".mp OR "Restriction Fragment Length Polymorphism ".mp OR "Single-Stranded Conformational Polymorphism ".mp OR "Genomic Structural Variations".mp OR "DNA Copy Number Variation". mp OR "Pharmacogenomic Variant".mp OR "Restriction Fragment Length Polymorphisms".mp OR "Single-Stranded Conformational Polymorphisms". mp) AND (exp *"Aggression"/ OR "aggression".ti,ab OR aggression*.ti,ab OR "aggressive behavior".ti,ab OR "aggressive behaviour".ti,ab OR "aggressive behaviors".ti,ab OR "aggressive behaviours".ti,ab OR aggressive behavi*.ti,ab OR exp *"Anger"/ OR "anger".ti,ab OR exp *"Rage"/ OR "angry".ti,ab OR exp "Hostility"/ OR "hostility".ti,ab OR "hostile".ti,ab OR *"Violence"/ OR "violence".ti,ab OR "violent".ti,ab OR (violen*.ti,ab AND (crime*.ti,ab OR crimin*.ti,ab)) OR (aggress*.ti,ab AND (crime*.ti,ab OR crimin*.ti,ab)) OR

aggressive trait*.ti,ab OR “hyperaggression”.ti,ab OR hyperaggress*.ti,ab OR *”oppositional defiant disorder”/ OR “oppositional defiant disorder”.ti,ab OR “oppositional defiant”.ti,ab OR oppositional defiant*.ti,ab OR *”conduct disorder”/ OR “conduct disorder”.ti,ab OR “conduct disorders”.ti,ab OR conduct disorder*.ti,ab OR *”Antisocial Personality Disorder”/ OR “antisocial personality disorder”.ti,ab OR “antisocial personality disorders”.ti,ab OR “anti-social personality disorder”.ti,ab OR “anti-social personality disorders”.ti,ab OR (aggressi*.ti,ab AND (“proactive”.ti,ab OR “reactive”.ti,ab OR “impulsive”.ti,ab OR “physical”.ti,ab))) AND exp “Humans”/ NOT (exp “Neoplasm”/ OR “cancer”.mp OR “tumour”.mp OR “tumours”.mp OR “tumor”.mp OR “tumors”.mp OR “aggressive treatment”.ti,ab OR (exp “Mental Disease”/ NOT (“Attention Deficit Disorder”/ OR “Conduct Disorder”/ OR “Antisocial Personality Disorder”/)) OR exp “Neurologic Disease”/ OR exp “Congenital Disorder”/) AND (exp “Review”/ OR “review”.mp OR review*.mp OR overview*.mp OR exp “systematic review”/)) NOT (conference review or conference abstract).pt

Searchterm for genome-wide studies
((“Genome-Wide Association Study”/ OR “genome wide association”.ti,ab OR “genomic wide association”.ti,ab OR “GWA Study”.ti,ab OR “GWA Studies”.ti,ab OR “GWAS”.ti,ab OR “GWASs”.ti,ab OR “epigenome wide association”.ti,ab OR ((“genome wide”.ti,ab OR “genomic wide”.ti,ab) AND “association”.ti,ab) OR “genetic association”.ti,ab OR “Genetic Association Study”/) AND (exp “Aggression”/ OR “aggression”.ti,ab OR aggression*.ti,ab OR “aggressive behavior”.ti,ab OR “aggressive behaviour”.ti,ab OR “aggressive behaviors”.ti,ab OR “aggressive behaviours”.ti,ab OR aggressive behavi*.ti,ab OR exp “Anger”/ OR “anger”.ti,ab OR exp “Rage”/ OR “angry”.ti,ab OR exp “Hostility”/ OR “hostility”.ti,ab OR “hostile”.ti,ab OR “Violence”/ OR “violence”.ti,ab OR “violent”.ti,ab OR (violen*.ti,ab AND (crime*.ti,ab OR crimin*.ti,ab)) OR (aggress*.ti,ab AND (crime*.ti,ab OR crimin*.ti,ab)) OR aggressive trait*.ti,ab OR “hyperaggression”.ti,ab OR hyperaggress*.ti,ab OR “oppositional defiant disorder”/ OR “oppositional defiant disorder”.ti,ab OR “oppositional defiant”.ti,ab OR oppositional defiant*.ti,ab OR “conduct disorder”/ OR “conduct disorder”.ti,ab OR “conduct disorders”.ti,ab OR conduct disorder*.ti,ab OR “Antisocial Personality Disorder”/ OR “antisocial personality disorder”.ti,ab OR “antisocial personality disorders”.ti,ab OR “anti-social personality disorder”.

ti,ab OR “anti-social personality disorders”.ti,ab OR (aggressi*.ti,ab AND (“proactive”.ti,ab OR “reactive”.ti,ab OR “impulsive”.ti,ab OR “physical”.ti,ab))) AND exp “Humans”/ NOT (conference review or conference abstract).pt)

Web of Science

Searchterms for reviews on genetics of aggression:
(ti=(“genetics” OR “genetics” OR “Genetic Procedure” OR “Heredity” OR “molecular genetic phenomena and functions” OR “Gene” OR “genes” OR “gene” OR “heredity” OR “hereditary” OR “Epigenetics” OR epigenetic* OR “Genetic Polymorphism” OR polymorphism* OR “Genotype” OR genotype* OR “Genome” OR genome* OR “systems genetics approach” OR “systems genetics” OR “Genome-Wide Association Study” OR “genome wide association” OR “genomic wide association” OR “GWA Study” OR “GWA Studies” OR “GWAS” OR “GWASs” OR “epigenome wide association” OR ((“genome wide” OR “genomic wide”) AND “association”) OR “genetic association” OR “Genetic Association Study” OR “candidate genes” OR “candidate gene” OR “candidates genes” OR “SNP” OR “SNPS” OR “Single Nucleotide Polymorphism” OR “single nucleotide polymorphisms” OR “single nucleotide polymorphism” OR “DNA Polymorphism” OR “Genetic Polymorphisms” OR “Genetic Polymorphism” OR “Genomic Structural Variation” OR “DNA Copy Number Variations” OR “Pharmacogenomic Variants” OR “Restriction Fragment Length Polymorphism “ OR “Single-Stranded Conformational Polymorphism “ OR “Genomic Structural Variations” OR “DNA Copy Number Variation” OR “Pharmacogenomic Variant” OR “Restriction Fragment Length Polymorphisms” OR “Single-Stranded Conformational Polymorphisms”) AND ts=(“Aggression” OR “aggression” OR aggression* OR “aggressive behavior” OR “aggressive behaviour” OR “aggressive behaviors” OR “aggressive behaviours” OR “aggressive behavi*” OR “Anger” OR “anger” OR “Rage” OR “angry” OR “Hostility” OR “hostility” OR “hostile” OR “Violence” OR “violence” OR “violent” OR (violen* AND (crime* OR crimin*)) OR (aggress* AND (crime* OR crimin*)) OR “aggressive trait*” OR “hyperaggression” OR hyperaggress* OR “oppositional defiant disorder” OR “oppositional defiant disorder” OR “oppositional defiant” OR “oppositional defiant*” OR “conduct disorder” OR “conduct disorder” OR “conduct disorders” OR “conduct disorder*” OR “Antisocial Personality Disorder” OR “antisocial

personality disorder" OR "antisocial personality disorders" OR "anti-social personality disorder" OR "anti-social personality disorders" OR (aggressi* AND ("proactive" OR "reactive" OR "impulsive" OR "physical"))) NOT ts=("Neoplasm" OR "cancer" OR "tumour" OR "tumours" OR "tumor" OR "tumors" OR "aggressive treatment" OR ("Mental Disease" NOT ("Attention Deficit Disorder" OR "Conduct Disorder" OR "Antisocial Personality Disorder")) OR Neurolog* OR Congenital* OR neonat* OR newborn*) AND ts=("Review" OR "review" OR review* OR overview* OR "systematic review") NOT ti=("veterinary" OR "rabbit" OR "rabbits" OR "animal" OR "animals" OR "mouse" OR "mice" OR "rodent" OR "rodents" OR "rat" OR "rats" OR "pig" OR "pigs" OR "porcine" OR "horse" OR "horses" OR "equine" OR "cow" OR "cows" OR "bovine" OR "goat" OR "goats" OR "sheep" OR "ovine" OR "canine" OR "dog" OR "dogs" OR "feline" OR "cat" OR "cats")) OR (ts=("genetics" OR "genetics" OR "Genetic Procedure" OR "Heredity" OR "molecular genetic phenomena and functions" OR "Gene" OR "genes" OR "gene" OR "heredity" OR "hereditary" OR "Epigenetics" OR epigenetic* OR "Genetic Polymorphism" OR polymorphism* OR "Genotype" OR genotype* OR "Genome" OR genome* OR "systems genetics approach" OR "systems genetics" OR "Genome-Wide Association Study" OR "genome wide association" OR "genomic wide association" OR "GWA Study" OR "GWA Studies" OR "GWAS" OR "GWASs" OR "epigenome wide association" OR (("genome wide" OR "genomic wide") AND "association") OR "genetic association" OR "Genetic Association Study" OR "candidate genes" OR "candidate gene" OR "candidates genes" OR "SNP" OR "SNPS" OR "Single Nucleotide Polymorphism" OR "single nucleotide polymorphisms" OR "single nucleotide polymorphism" OR "DNA Polymorphism" OR "Genetic Polymorphisms" OR "Genetic Polymorphism" OR "Genomic Structural Variation" OR "DNA Copy Number Variations" OR "Pharmacogenomic Variants" OR "Restriction Fragment Length Polymorphism " OR "Single-Stranded Conformational Polymorphism " OR "Genomic Structural Variations" OR "DNA Copy Number Variation" OR "Pharmacogenomic Variant" OR "Restriction Fragment Length Polymorphisms" OR "Single-Stranded Conformational Polymorphisms") AND ti=("Aggression" OR "aggression" OR aggression* OR "aggressive behavior" OR "aggressive behaviour" OR "aggressive behaviors" OR "aggressive behaviours" OR "aggressive behavi*" OR "Anger" OR "anger" OR "Rage" OR "angry" OR "Hostility" OR "hostility" OR "hostile" OR "Violence" OR "violence" OR

"violent" OR (violen* AND (crime* OR crimin*)) OR (aggress* AND (crime* OR crimin*)) OR "aggressive trait*" OR "hyperaggression" OR hyperaggress* OR "oppositional defiant disorder" OR "oppositional defiant disorder" OR "oppositional defiant" OR "oppositional defiant*" OR "conduct disorder" OR "conduct disorder" OR "conduct disorders" OR "conduct disorder*" OR "Antisocial Personality Disorder" OR "antisocial personality disorder" OR "antisocial personality disorders" OR "anti-social personality disorder" OR "anti-social personality disorders" OR (aggressi* AND ("proactive" OR "reactive" OR "impulsive" OR "physical"))) NOT ts=("Neoplasm" OR "cancer" OR "tumour" OR "tumours" OR "tumor" OR "tumors" OR "aggressive treatment" OR ("Mental Disease" NOT ("Attention Deficit Disorder" OR "Conduct Disorder" OR "Antisocial Personality Disorder")) OR Neurolog* OR Congenital* OR neonat* OR newborn*) AND ts=("Review" OR "review" OR review* OR overview* OR "systematic review") NOT ti=("veterinary" OR "rabbit" OR "rabbits" OR "animal" OR "animals" OR "mouse" OR "mice" OR "rodent" OR "rodents" OR "rat" OR "rats" OR "pig" OR "pigs" OR "porcine" OR "horse" OR "horses" OR "equine" OR "cow" OR "cows" OR "bovine" OR "goat" OR "goats" OR "sheep" OR "ovine" OR "canine" OR "dog" OR "dogs" OR "feline" OR "cat" OR "cats"))

NOT (conference review or conference abstract).pt

Searchterms for genome-wide studies:
(ts=("Genome-Wide Association Study" OR "genome wide association".mp OR "genomic wide association".mp OR "GWA Study".mp OR "GWA Studies".mp OR "GWAS".mp OR "GWASs".mp OR "epigenome wide association".mp OR (("genome wide".mp OR "genomic wide".mp) AND "association") OR "genetic association".mp OR "Genetic Association Study") AND ts=("Aggression" OR "aggression" OR aggression OR "aggressive behavior" OR "aggressive behaviour" OR "aggressive behaviors" OR "aggressive behaviours" OR aggressive behavi OR "Anger" OR "anger" OR "Rage" OR "angry" OR "Hostility" OR "hostility" OR "hostile" OR "Violence" OR "violence" OR "violent" OR ("violen*" AND ("crime*" OR "crimin*")) OR ("aggress*" AND ("crime*" OR "crimin*")) OR "aggressive trait*" OR "hyperaggression" OR "hyperaggress*" OR "oppositional defiant disorder" OR "oppositional defiant disorder*" OR "oppositional defiant*" OR "conduct disorder*" OR "Antisocial

Personalit*" OR "anti-social personalit*" OR (aggressi* AND ("proactive" OR "reactive" OR "impulsive" OR "physical"))) NOT ti=("veterinary" OR "rabbit" OR "rabbits" OR "animal" OR "animals" OR "mouse" OR "mice" OR "rodent" OR "rodents" OR "rat" OR "rats" OR "pig" OR "pigs" OR "porcine" OR "horse" OR "horses" OR "equine" OR "cow" OR "cows" OR "bovine" OR "goat" OR "goats" OR "sheep" OR "ovine" OR "canine" OR "dog" OR "dogs" OR "feline" OR "cat" OR "cats"))

Cochrane

Searchterms for reviews on genetics of aggression:
(("genetics" OR "genetics" OR "Genetic Procedure" OR "Heredity" OR "molecular genetic phenomena and functions" OR "Gene" OR "genes" OR "gene" OR "heredity" OR "hereditary" OR "Epigenetics" OR epigenetic* OR "Genetic Polymorphism" OR polymorphism* OR "Genotype" OR genotype* OR "Genome" OR genome* OR "systems genetics approach" OR "systems genetics" OR "Genome-Wide Association Study" OR "genome wide association" OR "genomic wide association" OR "GWA Study" OR "GWA Studies" OR "GWAS" OR "GWASs" OR "epigenome wide association" OR (("genome wide" OR "genomic wide") AND "association") OR "genetic association" OR "Genetic Association Study" OR "candidate genes" OR "candidate gene" OR "candidates genes" OR "SNP" OR "SNPS" OR "Single Nucleotide Polymorphism" OR "single nucleotide polymorphisms" OR "single nucleotide polymorphism" OR "DNA Polymorphism" OR "Genetic Polymorphisms" OR "Genetic Polymorphism" OR "Genomic Structural Variation" OR "DNA Copy Number Variations" OR "Pharmacogenomic Variants" OR "Restriction Fragment Length Polymorphism " OR "Single-Stranded Conformational Polymorphism " OR "Genomic Structural Variations" OR "DNA Copy Number Variation" OR "Pharmacogenomic Variant" OR "Restriction Fragment Length Polymorphisms" OR "Single-Stranded Conformational Polymorphisms") AND ("Aggression" OR "aggression" OR aggression* OR "aggressive behavior" OR "aggressive behaviour" OR "aggressive behaviors" OR "aggressive behaviours" OR "aggressive behavi*" OR "Anger" OR "anger" OR "Rage" OR "angry" OR "Hostility" OR "hostility" OR "hostile" OR "Violence" OR "violence" OR "violent" OR (violen* AND (crime* OR crimin*)) OR (aggress* AND (crime* OR crimin*)) OR "aggressive trait*" OR "hyperaggression" OR hyperaggress*

OR “oppositional defiant disorder” OR “oppositional defiant disorder” OR “oppositional defiant” OR “oppositional defiant*” OR “conduct disorder” OR “conduct disorder” OR “conduct disorders” OR “conduct disorder*” OR “Antisocial Personality Disorder” OR “antisocial personality disorder” OR “antisocial personality disorders” OR “anti-social personality disorder” OR “anti-social personality disorders” OR (aggressi* AND (“proactive” OR “reactive” OR “impulsive” OR “physical”))) NOT (“Neoplasm” OR “cancer” OR “tumour” OR “tumours” OR “tumor” OR “tumors” OR “aggressive treatment” OR (“Mental Disease” NOT (“Attention Deficit Disorder” OR “Conduct Disorder” OR “Antisocial Personality Disorder”)) OR Neurolog* OR Congenital* OR neonat* OR newborn*))

Searchterms for genome-wide studies:
((“Genome-Wide Association Study” OR “genome wide association”.mp OR “genomic wide association”.mp OR “GWA Study”.mp OR “GWA Studies”.mp OR “GWAS”.mp OR “GWASs”.mp OR “epigenome wide association”.mp OR ((“genome wide”.mp OR “genomic wide”.mp) AND “association”) OR “genetic association”.mp OR “Genetic Association Study”) AND (“Aggression” OR “aggression” OR aggression OR “aggressive behavior” OR “aggressive behaviour” OR “aggressive behaviors” OR “aggressive behaviours” OR aggressive behavi OR “Anger” OR “anger” OR “Rage” OR “angry” OR “Hostility” OR “hostility” OR “hostile” OR “Violence” OR “violence” OR “violent” OR (“violen*” AND (“crime*” OR “crimin*”)) OR (“aggress*” AND (“crime*” OR “crimin*”)) OR “aggressive trait*” OR “hyperaggression” OR “hyperaggress*” OR “oppositional defiant disorder” OR “oppositional defiant disorder*” OR “oppositional defiant*” OR “conduct disorder*” OR “Antisocial Personalit*” OR “anti-social personalit*” OR (aggressi* AND (“proactive” OR “reactive” OR “impulsive” OR “physical”))):ti,ab,kw

PsycINFO

Searchterms for reviews on genetics of aggression:
TI((“genetics” OR “genetics” OR “Genetic Procedure” OR “Heredity” OR “molecular genetic phenomena and functions” OR “Gene” OR “genes” OR “gene” OR “heredity” OR “hereditary” OR “Epigenetics” OR epigenetic* OR “Genetic Polymorphism” OR polymorphism* OR “Genotype” OR genotype*

OR “Genome” OR genome* OR “systems genetics approach” OR “systems genetics” OR “Genome-Wide Association Study” OR “genome wide association” OR “genomic wide association” OR “GWA Study” OR “GWA Studies” OR “GWAS” OR “GWASs” OR “epigenome wide association” OR (((“genome wide” OR “genomic wide”) AND “association”) OR “genetic association” OR “Genetic Association Study” OR “candidate genes” OR “candidate gene” OR “candidates genes” OR “SNP” OR “SNPS” OR “Single Nucleotide Polymorphism” OR “single nucleotide polymorphisms” OR “single nucleotide polymorphism” OR “DNA Polymorphism” OR “Genetic Polymorphisms” OR “Genetic Polymorphism” OR “Genomic Structural Variation” OR “DNA Copy Number Variations” OR “Pharmacogenomic Variants” OR “Restriction Fragment Length Polymorphism “ OR “Single-Stranded Conformational Polymorphism “ OR “Genomic Structural Variations” OR “DNA Copy Number Variation” OR “Pharmacogenomic Variant” OR “Restriction Fragment Length Polymorphisms” OR “Single-Stranded Conformational Polymorphisms”) AND (“Aggression” OR “aggression” OR aggression* OR “aggressive behavior” OR “aggressive behaviour” OR “aggressive behaviors” OR “aggressive behaviours” OR “aggressive behavi*” OR “Anger” OR “anger” OR “Rage” OR “angry” OR “Hostility” OR “hostility” OR “hostile” OR “Violence” OR “violence” OR “violent” OR (violen* AND (crime* OR crimin*)) OR (aggress* AND (crime* OR crimin*)) OR “aggressive trait*” OR “hyperaggression” OR hyperaggress* OR “oppositional defiant disorder” OR “oppositional defiant disorder” OR “oppositional defiant” OR “oppositional defiant*” OR “conduct disorder” OR “conduct disorder” OR “conduct disorders” OR “conduct disorder*” OR “Antisocial Personality Disorder” OR “antisocial personality disorder” OR “antisocial personality disorders” OR “anti-social personality disorder” OR “anti-social personality disorders” OR (aggressi* AND (“proactive” OR “reactive” OR “impulsive” OR “physical”))) NOT (“Neoplasm” OR “cancer” OR “tumour” OR “tumours” OR “tumor” OR “tumors” OR “aggressive treatment” OR (“Mental Disease” NOT (“Attention Deficit Disorder” OR “Conduct Disorder” OR “Antisocial Personality Disorder”)) OR Neurolog* OR Congenital* OR neonat* OR newborn*) AND (“Review” OR “review” OR review* OR overview* OR “systematic review”)) **OR** MJ((“genetics” OR “genetics” OR “Genetic Procedure” OR “Heredity” OR “molecular genetic phenomena and functions” OR “Gene” OR “genes” OR “gene” OR “heredity” OR “hereditary” OR “Epigenetics” OR epigenetic* OR “Genetic

Polymorphism" OR polymorphism* OR "Genotype" OR genotype* OR "Genome" OR genome* OR "systems genetics approach" OR "systems genetics" OR "Genome-Wide Association Study" OR "genome wide association" OR "genomic wide association" OR "GWA Study" OR "GWA Studies" OR "GWAS" OR "GWASs" OR "epigenome wide association" OR (("genome wide" OR "genomic wide") AND "association") OR "genetic association" OR "Genetic Association Study" OR "candidate genes" OR "candidate gene" OR "candidates genes" OR "SNP" OR "SNPS" OR "Single Nucleotide Polymorphism" OR "single nucleotide polymorphisms" OR "single nucleotide polymorphism" OR "DNA Polymorphism" OR "Genetic Polymorphisms" OR "Genetic Polymorphism" OR "Genomic Structural Variation" OR "DNA Copy Number Variations" OR "Pharmacogenomic Variants" OR "Restriction Fragment Length Polymorphism " OR "Single-Stranded Conformational Polymorphism " OR "Genomic Structural Variations" OR "DNA Copy Number Variation" OR "Pharmacogenomic Variant" OR "Restriction Fragment Length Polymorphisms" OR "Single-Stranded Conformational Polymorphisms") AND ("Aggression" OR "aggression" OR aggression* OR "aggressive behavior" OR "aggressive behaviour" OR "aggressive behaviors" OR "aggressive behaviours" OR "aggressive behavi*" OR "Anger" OR "anger" OR "Rage" OR "angry" OR "Hostility" OR "hostility" OR "hostile" OR "Violence" OR "violence" OR "violent" OR (violen* AND (crime* OR crimin*)) OR (aggress* AND (crime* OR crimin*)) OR "aggressive trait*" OR "hyperaggression" OR hyperaggress* OR "oppositional defiant disorder" OR "oppositional defiant disorder" OR "oppositional defiant" OR "oppositional defiant*" OR "conduct disorder" OR "conduct disorder" OR "conduct disorders" OR "conduct disorder*" OR "Antisocial Personality Disorder" OR "antisocial personality disorder" OR "antisocial personality disorders" OR "anti-social personality disorder" OR "anti-social personality disorders" OR (aggressi* AND ("proactive" OR "reactive" OR "impulsive" OR "physical"))) NOT ("Neoplasm" OR "cancer" OR "tumour" OR "tumours" OR "tumor" OR "tumors" OR "aggressive treatment" OR ("Mental Disease" NOT ("Attention Deficit Disorder" OR "Conduct Disorder" OR "Antisocial Personality Disorder")) OR Neurolog* OR Congenital* OR neonat* OR newborn*) AND ("Review" OR "review" OR review* OR overview* OR "systematic review")) OR AB(("genetics" OR "genetics" OR "Genetic Procedure" OR "Heredity" OR "molecular genetic phenomena and functions" OR "Gene" OR "genes" OR "gene" OR

"heredity" OR "hereditary" OR "Epigenetics" OR epigenetic* OR "Genetic Polymorphism" OR polymorphism* OR "Genotype" OR genotype* OR "Genome" OR genome* OR "systems genetics approach" OR "systems genetics" OR "Genome-Wide Association Study" OR "genome wide association" OR "genomic wide association" OR "GWA Study" OR "GWA Studies" OR "GWAS" OR "GWASs" OR "epigenome wide association" OR (("genome wide" OR "genomic wide") AND "association") OR "genetic association" OR "Genetic Association Study" OR "candidate genes" OR "candidate gene" OR "candidates genes" OR "SNP" OR "SNPS" OR "Single Nucleotide Polymorphism" OR "single nucleotide polymorphisms" OR "single nucleotide polymorphism" OR "DNA Polymorphism" OR "Genetic Polymorphisms" OR "Genetic Polymorphism" OR "Genomic Structural Variation" OR "DNA Copy Number Variations" OR "Pharmacogenomic Variants" OR "Restriction Fragment Length Polymorphism " OR "Single-Stranded Conformational Polymorphism " OR "Genomic Structural Variations" OR "DNA Copy Number Variation" OR "Pharmacogenomic Variant" OR "Restriction Fragment Length Polymorphisms" OR "Single-Stranded Conformational Polymorphisms") AND ("Aggression" OR "aggression" OR aggression* OR "aggressive behavior" OR "aggressive behaviour" OR "aggressive behaviors" OR "aggressive behaviours" OR "aggressive behavi*" OR "Anger" OR "anger" OR "Rage" OR "angry" OR "Hostility" OR "hostility" OR "hostile" OR "Violence" OR "violence" OR "violent" OR (violen* AND (crime* OR crimin*)) OR (aggress* AND (crime* OR crimin*)) OR "aggressive trait*" OR "hyperaggression" OR hyperaggress* OR "oppositional defiant disorder" OR "oppositional defiant disorder" OR "oppositional defiant" OR "oppositional defiant*" OR "conduct disorder" OR "conduct disorder" OR "conduct disorders" OR "conduct disorder*" OR "Antisocial Personality Disorder" OR "antisocial personality disorder" OR "antisocial personality disorders" OR "anti-social personality disorder" OR "anti-social personality disorders" OR (aggressi* AND ("proactive" OR "reactive" OR "impulsive" OR "physical"))) NOT ("Neoplasm" OR "cancer" OR "tumour" OR "tumours" OR "tumor" OR "tumors" OR "aggressive treatment" OR ("Mental Disease" NOT ("Attention Deficit Disorder" OR "Conduct Disorder" OR "Antisocial Personality Disorder")) OR Neurolog* OR Congenital* OR neonat* OR newborn*) AND ("Review" OR "review" OR review* OR overview* OR "systematic review"))

Searchterms for genome-wide studies:
TI((“Genome-Wide Association Study” OR “genome wide association”.mp OR “genomic wide association”.mp OR “GWA Study”.mp OR “GWA Studies”.mp OR “GWAS”.mp OR “GWASs”.mp OR “epigenome wide association”.mp OR ((“genome wide”.mp OR “genomic wide”.mp) AND “association”) OR “genetic association”.mp OR “Genetic Association Study”) AND (“Aggression” OR “aggression” OR aggression OR “aggressive behavior” OR “aggressive behaviour” OR “aggressive behaviors” OR “aggressive behaviours” OR aggressive behavi OR “Anger” OR “anger” OR “Rage” OR “angry” OR “Hostility” OR “hostility” OR “hostile” OR “Violence” OR “violence” OR “violent” OR (“violen*” AND (“crime*” OR “crimin*”)) OR (“aggress*” AND (“crime*” OR “crimin*”)) OR “aggressive trait*” OR “hyperaggression” OR “hyperaggress*” OR “oppositional defiant disorder” OR “oppositional defiant disorder*” OR “oppositional defiant*” OR “conduct disorder*” OR “Antisocial Personalit*” OR “anti-social personalit*” OR (aggressi* AND (“proactive” OR “reactive” OR “impulsive” OR “physical”)))) OR MJ((“Genome-Wide Association Study” OR “genome wide association”.mp OR “genomic wide association”.mp OR “GWA Study”.mp OR “GWA Studies”.mp OR “GWAS”.mp OR “GWASs”.mp OR “epigenome wide association”.mp OR ((“genome wide”.mp OR “genomic wide”.mp) AND “association”) OR “genetic association”.mp OR “Genetic Association Study”) AND (“Aggression” OR “aggression” OR aggression OR “aggressive behavior” OR “aggressive behaviour” OR “aggressive behaviors” OR “aggressive behaviours” OR aggressive behavi OR “Anger” OR “anger” OR “Rage” OR “angry” OR “Hostility” OR “hostility” OR “hostile” OR “Violence” OR “violence” OR “violent” OR (“violen*” AND (“crime*” OR “crimin*”)) OR (“aggress*” AND (“crime*” OR “crimin*”)) OR “aggressive trait*” OR “hyperaggression” OR “hyperaggress*” OR “oppositional defiant disorder” OR “oppositional defiant disorder*” OR “oppositional defiant*” OR “conduct disorder*” OR “Antisocial Personalit*” OR “anti-social personalit*” OR (aggressi* AND (“proactive” OR “reactive” OR “impulsive” OR “physical”)))) OR AB((“Genome-Wide Association Study” OR “genome wide association”.mp OR “genomic wide association”.mp OR “GWA Study”.mp OR “GWA Studies”.mp OR “GWAS”.mp OR “GWASs”.mp OR “epigenome wide association”.mp OR ((“genome wide”.mp OR “genomic wide”.mp) AND “association”) OR “genetic association”.mp OR “Genetic Association Study”) AND (“Aggression” OR “aggression” OR aggression OR “aggressive behavior” OR “aggressive

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Academic Search Premier

Searchterms for reviews on genetics of aggression:
TI(("genetics" OR "genetics" OR "Genetic Procedure" OR "Heredity" OR "molecular genetic phenomena and functions" OR "Gene" OR "genes" OR "gene" OR "heredity" OR "hereditary" OR "Epigenetics" OR epigenetic* OR "Genetic Polymorphism" OR polymorphism* OR "Genotype" OR genotype* OR "Genome" OR genome* OR "systems genetics approach" OR "systems genetics" OR "Genome-Wide Association Study" OR "genome wide association" OR "genomic wide association" OR "GWA Study" OR "GWA Studies" OR "GWAS" OR "GWASs" OR "epigenome wide association" OR (("genome wide" OR "genomic wide") AND "association") OR "genetic association" OR "Genetic Association Study" OR "candidate genes" OR "candidate gene" OR "candidates genes" OR "SNP" OR "SNPS" OR "Single Nucleotide Polymorphism" OR "single nucleotide polymorphisms" OR "single nucleotide polymorphism" OR "DNA Polymorphism" OR "Genetic Polymorphisms" OR "Genetic Polymorphism" OR "Genomic Structural Variation" OR "DNA Copy Number Variations" OR "Pharmacogenomic Variants" OR "Restriction Fragment Length Polymorphism " OR "Single-Stranded Conformational Polymorphism " OR "Genomic Structural Variations" OR "DNA Copy Number Variation" OR "Pharmacogenomic Variant" OR "Restriction Fragment Length Polymorphisms" OR "Single-Stranded Conformational Polymorphisms") AND ("Aggression" OR "aggression" OR aggression* OR "aggressive behavior" OR "aggressive behaviour" OR "aggressive behaviors" OR "aggressive behaviours" OR "aggressive behavi*" OR "Anger" OR "anger" OR "Rage" OR "angry" OR "Hostility" OR "hostility" OR "hostile" OR "Violence" OR "violence" OR

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AND TX(“Review” OR “review” OR review* OR overview* OR “systematic review”)

NOT ti(“veterinary” OR “rabbit” OR “rabbits” OR “animal” OR “animals” OR “mouse” OR “mice” OR “rodent” OR “rodents” OR “rat” OR “rats” OR “pig” OR “pigs” OR “porcine” OR “horse” OR “horses” OR “equine” OR “cow” OR “cows” OR “bovine” OR “goat” OR “goats” OR “sheep” OR “ovine” OR “canine” OR “dog” OR “dogs” OR “feline” OR “cat” OR “cats”)

Searchterms for genome-wide studies:
TI((“Genome-Wide Association Study” OR “genome wide association”.mp OR “genomic wide association”.mp OR “GWA Study”.mp OR “GWA Studies”.mp OR “GWAS”.mp OR “GWASs”.mp OR “epigenome wide association”.mp OR ((“genome wide”.mp OR “genomic wide”.mp) AND “association”) OR “genetic association”.mp OR “Genetic Association Study”) AND (“Aggression” OR “aggression” OR aggression OR “aggressive behavior” OR “aggressive behaviour” OR “aggressive behaviors” OR “aggressive behaviours” OR aggressive behavi OR “Anger” OR “anger” OR “Rage” OR “angry” OR “Hostility” OR “hostility” OR “hostile” OR “Violence” OR “violence” OR “violent” OR (“violen*” AND (“crime*” OR “crimin*”)) OR (“aggress*” AND (“crime*” OR “crimin*”)) OR “aggressive trait*” OR “hyperaggression” OR “hyperaggress*” OR “oppositional defiant disorder” OR “oppositional defiant disorder*” OR “oppositional defiant*” OR “conduct disorder*” OR “Antisocial

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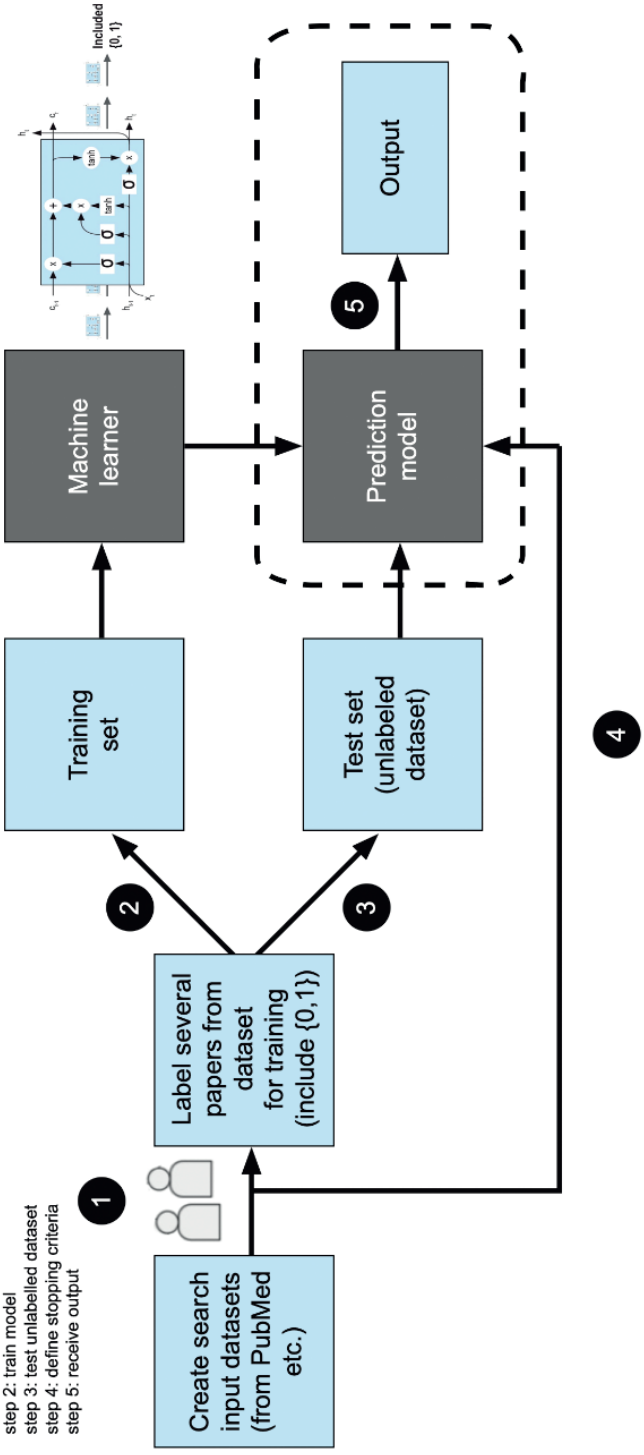
Chapter 5, Supplement S3. Additional materials on automated screening

Automated screening of titles and abstracts was performed with use of Automated Systematic Review Software (ASR) developed by researchers from Utrecht University, the Netherlands (PI A.G.J. van de Schoot) for screening abstracts and titles. The software is hosted at https://github.com (*Automated systematic reviews by using Deep Learning and Active Learning*, 2019). ASR is based on supervised machine learning approach with classification approach (the papers are classified in categories—i.e., 1=included or 0=not-included). The oracle modus is used to perform a systematic review with interaction by the reviewer.

During the training phase, the model is created, and in the prediction phase, the model is used to predict the future results of a literature search (see Figure S3.1).

Chapter 5, Figure S3.1 Process scheme of training and testing sets using ASR

step 0: create search input datasets (from PubMed etc.)
step 1: label several papers from dataset for training (include {0,1})
step 2: train model
step 3: test unlabelled dataset
step 4: define stopping criteria
step 5: receive output



We had two objectives in applying ASR:

1) To analyze screening parameters of ASR (time of screening, inclusion and exclusion rates, false positive rates (FPR), false negative rates (FNR), true positive rates (TPR), true negative rates (TNR), and receiver operating characteristics (ROC)) and compare it with parameters of manual screening (time of screening, inclusion and exclusion rates as workload characteristics);
2) To contribute to the current systematic review by predicting inclusion/exclusion in a large data set of records based on generated ASR models. To make automated screening of ASR on large dataset of records to make a new contribution to the current systematic review.

The following steps were done in our systematic review:

0. several literature searches were done in PubMed to create a training dataset with key words "human aggression GWAS", "human aggression genetic association studies", "human aggression epigenetics" (2,955 records)
1. the training dataset was labelled by reviewers to create training sets (0=not-included, 1=included) and comprised 152 positives and 2803 negatives labels
2. ASR models were trained with training sets from the labelled training dataset (500 records)
3. models with different parameters were used for screening
4. the ROC analyses were performed to define FNR and thresholds of positive and negative results

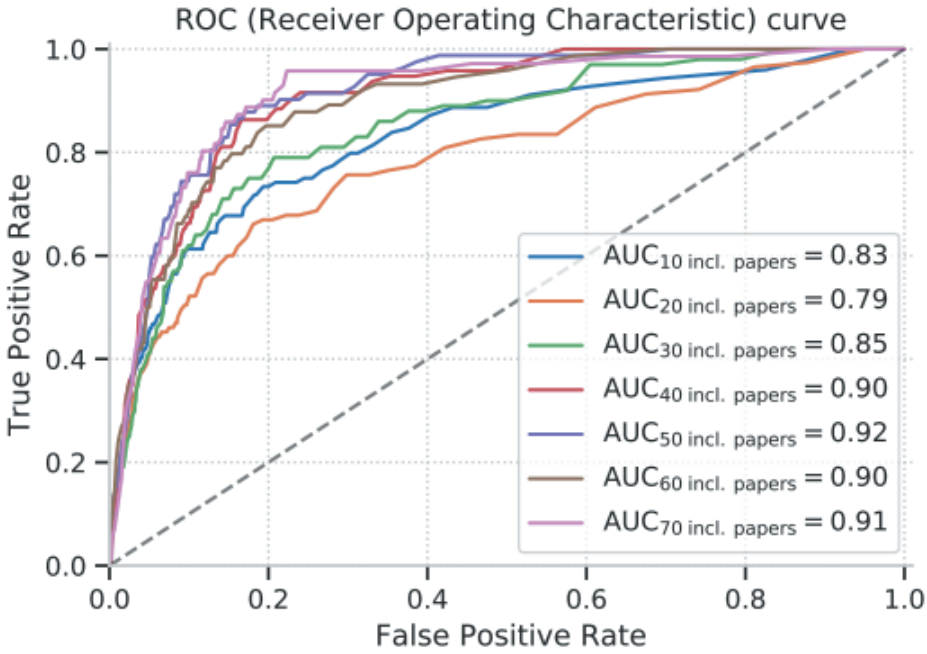
Receiver operating characteristics (ROC) analyses were performed on the models including different number of records labelled as "included": $N_{\text{label=1}}$ = [10, 20, 30, 40, 50, 60, 70] from the randomly selected training set of size $N_{\text{training dataset}}$ = 500 from the prelabeled list of N = 2,955 records. All models perform considerably better than random, since $\text{AUC} \in [0.79, 0.92$ (see Figure S3.2). We selected the model where we used $N_{\text{label=1}}$ = 50, since it resulted in the minimal FPR=0.39 at FNR ≤ 0.03 with optimal threshold of prediction.

Chapter 5, Table S3.1 ROC parameters used for model selection.

$N_{label=1}$	Minimal false positive rate at FNR≤ 0.03	Maximum threshold of prediction at FNR≤ 0.03
10	0.934363	0.01
20	0.878205	0.03
30	0.604671	0.09
40	0.571186	0.03
50*	0.386431	0.12
60	0.583788	0.05
70	0.455537	0.06

*The model using $N_{label=1}$ = 50 exhibits the lowest minimal FPR at FNR ≤ 0.03

Chapter 5, Figure S3.2 ROC curves for the trained models



AUC=area under the curve

Once the optimal model was defined, screenings were repeated on different datasets:

a. 1,713 records of potential reviews on genetics of human aggression (see Supplement S2);
b. 356 records of potential GWASs on genetics of human aggression (see Supplement S2);
c. 2,069 records that join together (1) and (2) datasets;
d. a new dataset of 14,400 records done with a wide search "humanANDaggressionANDgenes" in the same databases as previous datasets.

Screenings (1)-(3) were used to compare the parameters of automated screening with manual screening (see Table S3.2).
By screening dataset (3) with $N = 2{,}069$ ASR predicted relevant records and recovered 50 of the 51 expert-labelled true positives, yielding TPR = 0.980. The ASR model mislabeled 1 record as not-relevant from expert labeled true positive, yielding FNR = 0.020. The performance of the model applied to the above search is high. FPR was 0.600, meaning that a reduction in reading time of ~40% is expected.

It is worth noting that model generation and using it for predicting takes ~ 1 hour on a regular computer.

Chapter 5, Table S3.2 Comparison of titles and abstracts screening performed manually and automated

Step	Dataset	Screening type	Input Sample	Inclusion*	Inclusion rate	Exclusion	Exclusion rate
Training set	Training dataset	ASR	2,955	152	5,1%	2,803	94,9%
Titles and abstracts screening	Reviews	Manual	1,713	26	1,5%	1,687	98,5%
		ASR	1,713	1,018	59,4%	695	40,6%
	GWASs	Manual	356	25	7,0%	331	93,0%
		ASR	356	243	68,3%	113	31,7%
	"Human aggression genes"	ASR	14,400	7,297	50,7%	7,103	49,3%

Note * The inclusion numbers done on the base of titles and abstracts screening (not the final number of articles included in the review)
ASR=Automated Systematic Review

False-negative result

Sonuga- Barke EJ, Lasky-Su J, Neale BM, Oades R, Chen W, Franke B, et al. Does parental expressed emotion moderate genetic effects in ADHD? An exploration using a genome wide association scan. Am J Med Genet B Neuropsychiatr Genet. 2008;147B(8):1359-68.

Papers selected by researchers from automated selection in addition to traditional selection

Reviews

Baud P. Personality traits as intermediary phenotypes in suicidal behavior: genetic issues. Am J Med Genet C Semin Med Genet. 2005 Feb 15;133C(1):34-42. Review. PubMed PMID: 15648080.

Beaver K.M., Connolly E.J., Nedelec J.L., Schwartz J.A. On the genetic and genomic basis of aggression, violence, and antisocial behavior. Oxford Handbook of Evolution, Biology, and Society. 2018. p.1-18 DOI: 10.1093/oxfordhb/9780190299323.013.15

Davydova J.D., Litvinov S.S., Enikeeva R.F., Malykh S.B., Khusnutdino- va E.K. Recent advances in genetics of aggressive behavior. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2018;22(6):716-725. DOI 10.18699/VJ18.415

Tuvblad C, Beaver KM. Genetic and environmental influences on antisocial behavior. J Crim Justice. 2013;41(5):273–276. doi:10.1016/j.jcrimjus.2013.07.007

Empirical genetic studies

Neumann, A., Pappa, I., Lahey, B. B., Verhulst, F. C., Medina-Gomez, C., Jaddoe, V. W., . . . Tiemeier, H. (2016). Single nucleotide polymorphism heritability of a general psychopathology factor in children. *Journal of the American Academy of Child & Adolescent Psychiatry, 55*(12), 1038-1045. e1034.

Chapter 5, Supplement S4 Phenotypes in Genome-wide Association Studies on Aggression

Factor	Trait (subscale)	Measurement instrument	Study
Externalizing	Hostility (anger)	Irritability Scale of the Buss-Durkee Hostility Inventory (BDHI)	Merjonen 2011
	Anger temperament and anger reaction	Spielberger State-Trait Anger Scale (SSTAS)	Mick 2014; Salvatore 2015
	Physical aggression	Question in self-report "Did you ever get into physical fights while using marijuana?"	Montalvo-Ortiz 2018
	Destructiveness, aggression	Parental Account of Childhood Symptoms (PACS)	Sonuga-Barke 2008; Anney 2008
	Aggressive behaviour	Child Behavioural Checklist (CBCL)	Mick 2011; Pappa 2016; Tielbeek 2017
	Hyperactive-impulsive	Conners Parent Rating Scale (CPRSR)	Anney 2008; Aebi 2016; Brevik 2016
	Oppositionality and defiance	Conners Parent Rating Scale (CPRSR)	
	Conduct problems	CD based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)	Dick 2011; Tielbeek 2012
	Conduct problems	Strengths and Difficulties Questionnaire (SDQ)	Viding 2010; Pappa 2016
	Aggression and CD	composite of measures	McGue 2013
	Antisocial behaviour	Strengths and Difficulties Questionnaire (SDQ)	Viding 2010; Pappa 2016
	Antisocial behaviour	ASPD based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)	Tielbeek 2012; Salvatore 2015
	Violent behaviour	Crime characteristics	Tiihonen, 2014; Rautiainen 2016

Chapter 5, Supplement S5. Reported genetic variants in chromosomes in genome-wide association studies

$N_{studies}$=17, $N_{variants}$ =817

See Excel, Supplement S5, available online.

Chapter 5, Supplement S6 Overview of Reported Genetic Variants in Chromosomes in Genome-wide Association Studies on Aggression

Chromosome	N variants at suggestive significance ($p < 1E^{-05}$)	Number of SNPs at genome-wide significance ($p < 5.0E^{-08}$)	Genes with nearby or inside location of SNPS at genome-wide significance ($p < 5.0E^{-08}$)
1	53	1	
2	81	2	*HTR2B; PSMD1*
3	40		
4	35	2	*C1QTNF7*
5	52		
6	54	1	*LINC00915*
7	79		
8	25		
9	49		
10	56		
11	62	2	
12	34		
13	8	1	
14	15		
15	9		
16	27		
17	19		
18	21		
19	6		
20	44		
21	26		
22	8		
X	4	1	
	817	10	4

Note. $N_{studies}$=17

SUPPLEMENT TO CHAPTER 6

Chapter 6, Supplement 1. Brief description of buccal sample collection for (epi)genetics in ACTION in the Netherlands Twin Register

Buccal cells for DNA isolation and genotyping were collected during two days and were also collected from parents and additional siblings. All parents provided written informed consents for their own and their children's participation. Genotyping was done on the Axiom (N = 861; Ehli et al., 2017) or the GSA array (N = 2,151; Beck et al., 2019). Genotyping data were analyzed to establish zygosity (Odintsova et al., 2019), of which parents received the results.

For epigenetics 108 extra twins with buccal-cell samples and longitudinal aggression data were included from the NTR database. Thus in total 1,475 twins (737 complete pairs), either with first-morning urine (N = 1,362) and/or buccal-cell swabs (N = 1,468), were included in the ACTION project (Table S1). In the twins, epigenetic markers were measured on the Illumina EPIC 850K array (Van Dongen et al., 2018).

Chapter 6, Supplement 2. Medication use and other covariates

In the sensitivity analyses we assessed the potential impact of preexisting chronic conditions, medication use, or vitamin use on differences in biomarker levels and neurotransmitter ratios between the MZ twins scoring high and low on aggression.

Medication use has been assessed in the twin cohort through parent report at the time of urine collection, in the clinical cohort medication use was extracted from the patient files. ATC codes (https://www.whocc.no/atc_ddd_index/) were assigned to the medications used at the time of urine collection in both cohorts. Based on the ATC codes medications could be classified. At time of urine collection children included in the current study used medications classified as: alimentary tract and metabolism (A), cardiovascular system (C), dermatological (D), genito-urinary system and sex hormone (G), systemic hormonal preparations (H), anti-infectives for systemic use (J), nervous system (N) and respiratory system (R) medications.

Children were most frequently using nervous system or respiratory system medications, which is consistent with reported incidences of asthma or allergies. The respiratory medications included nasal preparations (R01), drugs for

obstructive airway diseases (R03) and antihistamines for system use (R06). The nervous system medications included analgesics (N02, e.g., paracetamol use), antiepileptics (N03), psycholeptics (N05), psychoanaleptics (N06) and other nervous system drugs (N07; here chiefly antivertigo medications). Medications belonging to the N05 and N06 classes (e.g., aripiprazole [N05AX12] or methylphenidate [N06BA04]) are also considered psychotropic medications and are prescribed for the treatment of psychiatric disorders, including for example attention-deficit/hyperactivity disorder. In **Table 1** we included an overview of the number of children on psychotropic medications in both cohorts.

Chapter 6, Supplement 3. Aggressive Behavior item-based biomarker discovery

Assessment of aggressive behavior

At or near the time of biological sample collection parents completed the CBCL. The CBCL Aggressive Behavior subscale consists of 18 items assessing multiple aspects of aggressive behavior (see Table S4). Parents were asked to indicate the applicability of each item to their child's behavior over the past 6 months. Answer categories ranged from "not true" (coded as "0"), to "somewhat or sometimes true" (coded as "1"), and "very true or often true" (codes as "2"). All items were dichotomized to reflect case/control status, with items scored as "not true" defining control status. The answer categories "somewhat or sometimes true" and "very true or often true" both reflected case status. Endorsement of the original answer categories as well as the dichotomized answer categories have been supplied in Table S4. In the NTR, items from mother-rated CBCL Aggressive Behavior subscale were analyzed, in the Curium-LUMC cohort the majority (90%) of ratings was also by the mother.

Statistical analyses

In the item-based discovery, replication and validation phases the same subjects as in the original discovery, replication and validation phases were classified as cases or controls based on each of the 18 CBCL Aggressive Behavior items (see section 3.1). GEE analyses, including sex and age as covariates, assessed the relationship of the biomarkers and neurotransmitter ratios with item case-control status. Analyses were corrected for relatedness using an 'exchangeable' correlation structure. The FDR of 5% at a threshold of $p \leq 0.05$ for 1602 tests

(biomarkers) or 126 tests (ratios) are provided. Note, that because of the large number of tests interpretation of the discovery phase in terms of significance is complex. The top 25% most associated biomarkers or ratios per item were tested in the replication phase. In the replication phase the FDR of 5% for 414 tests (biomarkers) and 54 tests (ratios) at $p \leq 0.05$ was used. Finally, in the validation phase the biomarkers or ratios with congruent directions of effect in the discovery and validation phase and which were significantly associated with item case-control status in the validation phase were assessed. For those items without significantly associated biomarkers or ratios, the top 5 biomarkers or top ratio were assessed in the replication phase. The significance threshold was set at $p \leq 0.05$ with a 5% FDR for 88 tests (biomarkers) and 18 tests (ratios) to control multiple testing.

Results

Participant descriptives

Both the original and dichotomized responses for each of the 18 items have been included in Table S4. Case and control status on an item-to-item basis vary considerably across children (Table S4). It must be noted that for some items, particularly the more extreme items such as "Threatens other people", item endorsement is low across all groups (Table S4). As a consequence, meaningful interpretation of associated metabolites, other biomarkers and neurotransmitters is not always feasible.

Association of urinary metabolites and other biomarkers with Aggressive Behavior items

Discovery

The discovery analyses showed significant metabolites or other biomarkers for each of the 18 Aggressive Behavior items, overall 3.8% of the tests were significant, however, after correcting for multiple testing none of the item-specific metabolites or other biomarkers remained significant (Table S13). Comparing the top 25% metabolites and other biomarkers for overall aggression, we observe that of the 23 metabolites or other biomarkers in the top 25% between 2 and 12 overlap per item (Table S14). Of the overlapping metabolites or other biomarkers

approximately 78% have congruent directions of effect among the overall aggression and item-specific analyses (Table S14).

Replication

The top 25% most associated metabolites and other biomarkers per item were assessed for replication in a sample of twin pairs discordant for aggression. In the replication analyses 29 metabolites or other biomarkers were significantly associated with aggression items, here only 12 of the 18 aggression items had significantly associated metabolites or other biomarkers (Table S15). In total 8.5% of the total number of conducted tests were significant. Five of the significantly associated metabolites or other biomarkers were also included in the top 25% for overall aggression. In the replication analyses isocitrate was associated with 'Disobedient at home' ($\beta = 0.26$; $SE = 0.10$; $p = 0.008$), for overall aggression this metabolite was not significant and showed an opposite direction of effect in the replication analysis. 'Disobedient at home' was also significantly associated with norepinephrine levels ($\beta = 0.22$; $SE = 0.10$; $p = 0.03$), in the overall aggression replication analysis this metabolite was also significantly associated, before multiple testing correction; however the association was in the opposite direction (mean difference = -0.19; $p = 0.02$). The associations of ethanolamine with 'Disobedient at school', isocitrate with 'Threatens' and succinic acid with 'Temper' were in the same direction of effect as observed for overall aggression (Table S7 and S15). Only ethanolamine was significantly associated with both 'Disobedient at school' ($\beta = -0.31$; $SE = 0.14$; $p = 0.03$) and with overall aggression (mean difference = -0.20; $p = 0.03$). After correction for multiple testing 10 of the 15 (66.7%) metabolites or other biomarkers associated with 'Threatens' were still significant (Table S15). However only 3 children were cases for 'threaten other people' (Table S4). For the other 11 items none of the metabolites or other biomarkers remained significant after correction for multiple testing (Table S15). Overall, we observed congruent directions of effect in the discovery and validation analyses for 3-19 out of 23 (13.0-82.6%) top 25% amines, organic acids and biomarkers per item (Table S14).

Validation

For the validation analyses we selected the top 5 most associated metabolites or other biomarkers from the replication analyses with congruent directions of effects in the discovery analyses. For the 'Fights' item only 3 metabolites or other biomarkers showed congruent direction of effect between the discovery and the replication, therefore, only these 3 were included. In the validation analyses

neopterin is significantly associated with 'Argues' (β = -0.25; *SE* = 0.10; *p* = 0.01) and L-proline with 'Mean' (β = -0.28; *SE* = 0.12; *p* = 0.02). None of the other biomarker-item combinations were significant and after correction for multiple testing, the associations of neopterin with 'Argues' and L-proline with 'Mean' were no longer significant (Table S16). Overall, congruent directions of effect between the replication and validation were observed for 0-4 out of the top 5 (0%-80%) amines, organic acids and biomarkers per item (Table S14).

Association of urinary neurotransmitter pathways with aggressive behavior items

Discovery

To elucidate the role of serotonergic, dopaminergic and GABAergic neurotransmitter pathways we performed discovery analyses with gee analyses for each of the 18 items of the CBCL Aggressive Behavior subscale. The discovery analyses showed that the catabolic dopamine neurotransmitter ratio 3MT to HVA was significantly associated with the 'Stubborn' (β = -2.50; *SE* = 1.16; *p* = 0.03) and 'Sulks' (β = -3.17; *SE* = 1.59; *p* = 0.05) items. The catabolic GABA neurotransmitter ratio GABA to succinic acid was significantly associated with the 'Physically attacks people' (β = -2.49; *SE* = 0.90; *p* = 0.01), 'Suspicious' (β = -1.82; *SE* = 0.83; *p* = 0.03) and 'Teases' (β = -2.34; *SE* = 0.88; *p* = 0.01) items. The anabolic GABA neurotransmitter L-glutamic acid to GABA was significantly associated with 'Disobedient at School' (β = -3.34; *SE* = 1.62; *p* = 0.04). After correction for multiple testing none of the neurotransmitter ratio-item associations was significant and none of the neurotransmitter ratios involved in the anabolism or catabolism of serotonin, dopamine or GABA significantly associated with the other 12 aggressive behavior items (Table S17). None of the most associated neurotransmitter ratios per item were included in the top 25% most associated neurotransmitter items for overall aggression. Of the 7 neurotransmitter ratios congruent directions of effect between the overall aggression discovery results and the item specific results were observed for 6 ratios across 17 items, with no congruent directions of effect observed for the ratio of 5HTP to serotonin and for the 'Sudden changes in mood or feelings' item (Table S18).

Replication

Replication of the top 25% most associated neurotransmitter ratios from the item-specific discovery analyses were performed in the sample of twins discordant for

overall aggression. The anabolic dopamine ratio L-phenylalanine to L-tyrosine was significantly associated with the 'Fights' (β = -0.85; *SE* = 0.42; p = 0.04) and 'Threatens' (β = -1.19; *SE* = 0.39; p = 0.002) items, though these associations did not survive multiple testing (Table S19). The direction of effect of L-phenylalanine to L-tyrosine for the 'Fights' and 'Threatens' items were congruent with the direction of effect as observed in the discovery analyses (Table S18). None of the other neurotransmitter ratio aggression item combinations reached significance in the replication analyses (Table S19) and the congruence of effect directions ranged from none ('Mean') to all (3; 'Threatens'), with an average of 1.5 (Table S18).

Validation

The top neurotransmitter ratio for each item was assessed in a sample of clinical cases and twin controls. Before correction for multiple testing the anabolic dopamine neurotransmitter ratio L-phenylalanine to L-tyrosine was significantly associated with the 'Disobedient at school' (β = 4.64; *SE* =1.96; p = 0.02) and 'Loud' (β = 4.18; *SE* = 2.00; p = 0.04) items (Table S20). For 'Disobedient at school' the direction of effect has flipped as compared to the replication analysis, for 'Loud' the direction of effect was congruent across the replication and validation phases (Table S18). Neurotransmitter ratios were not significantly associated with any of the other 16 aggression items and after correction for multiple testing the ratio of L-phenylalanine to L-tyrosine was not significantly associated with 'Disobedient at school' or 'Loud' (Table S20). In addition to the congruent direction of effect for 'Loud' we also observed congruent directions of effect of 'MoodSwings', 'Suspicious' and 'Teases' (Table S18).

Chapter 6, Supplementary Text 4. Description of aggression measures

In Table S5 we present the mean scores of the twins included in this project for aggression as obtained by different raters and instruments at different ages. The following questionnaires have been included in this overview:

The Aggressive Behavior scale of the ASEBA Child Behavior Checklist (CBCL) for preschool children (1.5-5 years; Achenbach et al., 2017) as rated by mothers and fathers of the twins at age 3.

The Aggressive Behavior scale of the Devereux Child Behavior (DCB) rating scale (Molenaar, Middeldorp, van Beijsterveldt, & Boomsma, 2015; Van Beijsterveldt, Verhulst, Molenaar, & Boomsma, 2004) as rated by mothers and fathers of the twins at age 5.

The Aggressive Behavior scale of the ASEBA CBCL for school-aged children (6-18 years; Achenbach et al., 2017) as rated by mothers and fathers of the twins at ages 7 and 10.

The Aggressive Behavior scale of the ASEBA Teacher Rating Form (TRF; Achenbach et al., 2017) as rated by teachers of the twins at ages 7, 10 and 12.

The Conduct Problems scale of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997, 2001) as rated by mothers and fathers of the twins at age 10.

The Conduct Disorder (CD) and Oppositional Defiant Disorder (ODD) scales from the Autism - Tics, ADHD and other Comorbidities inventory (A-TAC; Hansson et al., 2005; Kerekes et al., 2014) as rated by mothers and fathers of the twins at age 10.

The Aggressive Behavior scale of the ASEBA Brief Problem Monitor (BPM; Chorpita et al., 2010) as rated by mothers and father of the twins at age 12.

Chapter 6, Table S1 Descriptives for all twin pairs with urine (N = 1,362) and/or DNA (N = 1,468).

	Concordant Low	Discordant Low (n = 196)	Discordant High (n =196)	Concordant High
N (N complete twin pairs)	676 (337)	392 (196)		406 (203)
Mean (SD) age sample collection	9.4 (1.9)	10.1 (1.7)		9.5 (1.8)
Range age sample collection	5.6 - 12.6	6.1 - 12.7		5.8 - 12.9
N (%) females	354 (52.4%)	88 (44.9.6%)	82 (41.8%)	177 (43.6%)
N (%) MZ twins	540 (79.9%)	160 (81.6%)	160 (81.6%)	370 (91.1%)
Mean CBCL (SD) aggression score	2.7 (3.8)	4.5 (4.4)	6.3 (5.8)	7.5 (6.0)

Chapter 6, Table S2 Primary DSM-IV classification of the clinical cases (*N* = 183)

DSM classification	N (% of total sample)
ADHD combined type	45 (24.6%)
Pervasive developmental disorder not otherwise specified	40 (21.9%)
Autistic disorder	33 (18.0%)
ADHD inattentive type	13 (7.1%)
Learning disorder not otherwise specified	12 (6.6%)
Adjustment disorder	9 (4.9%)
Generalized anxiety disorder	3 (1.6%)
Obsessive compulsive disorder	2 (1.1%)
Undifferentiated somatoform Disorder	2 (1.1%)
Separation anxiety disorder	2 (1.1%)
Posttraumatic stress disorder	2 (1.1%)
Reactive attachment disorder	2 (1.1%)
Other	14 (7.7%)
Total classifications	179

Note. Not all clinical cases have received classifications because data were collected before the diagnostic process was ended. Classifications with a prevalence smaller than two have been grouped under "Other". ADHD = Attention Deficit Hyperactivity Disorder; DSM = Diagnostic and Statistical Manual of Mental Disorders.

Supplementary Tables 3 through 8 are available online

Chapter 6, Table S9 Sensitivity analyses for the 5 amines, organic acids and other biomarkers included in the validation phase. Sensitivity analyses were performed with paired t-tests in MZ twins discordant for aggression, after removal of the MZ twin (and co-twin) with a preexisting chronic condition or was on medication or vitamin supplements during urine collection. All p-values have been adjusted for multiple testing using the FDR of 5% for 15 tests.

			Low aggression		High aggression						
Analysis	Platform	Metabolite	mean	sd	mean	sd	Mean difference	df	t	p-value	FDR p-value
No chronic condition	Amines	Gamma. Glutamylglutamine	-0.09	0.91	-0.21	0.90	-0.14	132	-1.94	0.05	0.65
No chronic condition	Organic acids	Glyceric.acid	-0.02	0.85	0.13	0.96	0.12	132	1.33	0.19	0.65
No chronic condition	Amines	L.Arginine	0.16	1.00	0.10	1.06	-0.08	132	-0.78	0.44	0.75
No chronic condition	Organic acids	Succinic.acid	0.13	0.92	0.11	0.96	-0.03	132	-0.46	0.65	0.75
No chronic condition	Other	creatinine	0.15	0.95	0.21	0.95	0.02	132	0.28	0.78	0.78
No medication	Amines	Gamma. Glutamylglutamine	-0.09	0.91	-0.21	0.90	-0.11	116	-1.42	0.16	0.65
No medication	Organic acids	Glyceric.acid	-0.02	0.85	0.13	0.96	0.12	116	1.24	0.22	0.65
No medication	Amines	L.Arginine	0.16	1.00	0.10	1.06	-0.07	116	-0.56	0.57	0.75
No medication	Organic acids	Succinic.acid	0.13	0.92	0.11	0.96	-0.04	116	-0.53	0.60	0.75
No medication	Other	creatinine	0.15	0.95	0.21	0.95	0.03	116	0.33	0.74	0.78
No vitamines	Amines	Glyceric.acid	-0.02	0.85	0.13	0.96	0.15	115	1.56	0.12	0.65
No vitamines	Organic acids	Gamma. Glutamylglutamine	-0.09	0.91	-0.21	0.90	-0.08	115	-1.03	0.30	0.75
No vitamines	Amines	creatinine	0.15	0.95	0.21	0.95	0.06	115	0.70	0.48	0.75
No vitamines	Organic acids	L.Arginine	0.16	1.00	0.10	1.06	-0.08	115	-0.65	0.52	0.75
No vitamines	Other	Succinic.acid	0.13	0.92	0.11	0.96	0.04	115	0.53	0.60	0.75

Supplementary Table 10 is available online

Chapter 6, Table S11 Replication of the top 25% most strongly associated neurotransmitter ratios. Replication analyses were performed with paired t-tests for 189 twin pairs discordant (high-low)on aggression status. Analyzes were performed on the residuals as obtained with linear regression after regressing out sex and age at urine collection. The p-values have been adjusted for multiple testing using the FDR of 5% for 3 tests. * 3MT = 3-methoxytyramine; 5HTP = 5-hydroxy-L-tryptophan; HVA = homovanillic acid

Neurotransmitter pathways	Ratio*	Low aggression mean	Low aggression sd	High aggression mean	High aggression sd	Mean difference	df	t	p-value	FDR p-value
Dopamine	3MT:HVA	-0.38	24.80	2.72	26.53	3.00	188	1.28	0.20	0.61
Dopamine	L-phenylalanine: L-tyrosine	1.33	6.20	1.01	4.30	-0.32	188	-0.58	0.56	0.84
Serotonine	5HTP:serotonin	1.52	15.83	1.94	29.14	0.36	188	0.15	0.88	0.88

Supplementary Tables 12 through 20 are available online

APPENDICES

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NEDERLANDSE SAMENVATTING

Over dit proefschrift

Dit proefschrift verschaft inzicht in de etiologie, voorspellers en uitkomsten van agressie en andere vormen van antisociaal gedrag. Het eerste deel richt zich op meer conventionele voorspellers van agressie en agressie-gerelateerde uitkomsten, namelijk: psychiatrische stoornissen bij de ouder (**Hoofdstuk 2**), angst en depressie (**Hoofdstuk 3**) en symptomen van Oppositioneel Opstandige Gedragsstoornis (**Hoofdstuk 4**). Het tweede deel van dit proefschrift bespreekt biologische determinanten van agressie, namelijk: een review over genetica en agressie (**Hoofdstuk 5**) en een empirische studie naar metabolomische markers van agressie (**Hoofdstuk 6**).

Met deze doelen voor ogen zijn data uit verschillende steekproeven benut. In hoofdstuk twee en drie staan Zweedse tweelingen uit The Child and Adolescent Twin Study in Sweden (CATSS) centraal. Hoofdstuk vier focust op Nederlandse kinderen en jongeren die zijn aangemeld bij Curium-LUMC, een academisch centrum voor kinder- en jeugdpsychiatrie in Oegstgeest. De literatuurreview in hoofdstuk vijf bevat verschillende soorten populaties, variërend van volwassenen tot kinderen, van student tot delinquent. Hoofdstuk zes is gericht op zes- tot en met twaalfjarigen: een steekproef van Nederlandse tweelingen geworven door het Nederlands Tweelingen Register (NTR) en een klinische steekproef van kinderen die verwezen zijn naar Curium-LUMC vanwege psychiatrische problematiek.

Resultaten

In **hoofdstuk twee** wordt onderzocht of de combinatie van een psychiatrische stoornis bij een ouder en gedragsproblematiek bij hun kind extra risico oplevert voor een problematische adolescentie. Uit de wetenschappelijke literatuur volgt duidelijk dat zowel kinderen met gedragsproblematiek als kinderen van ouders met een psychiatrische stoornis slechter functioneren in de adolescentie. Kinderen met gedragsproblematiek hebben daarnaast vaker een ouder met een psychiatrische stoornis dan kinderen zonder gedragsproblematiek. Toch is het nog onduidelijk of de aanwezigheid van beide genoemde risicofactoren (kind zijn van een ouder met een psychiatrische stoornis én gedragsproblemen als kind) voorspellend kan zijn voor extra risico op slechte langetermijnuitkomsten in de adolescentie. In hoofdstuk twee wordt deze vraag beantwoord: kinderen met gedragsproblematiek die vaders hebben met een psychiatrische stoornis, gemiddeld slechter af zijn dan

kinderen waarbij alleen sprake is van gedragsproblematiek: als adolescent plegen ze vaker een strafbaar feit, spijbelen meer, halen lagere cijfers, ondervinden vaker negatieve gevolgen van hun agressieve gedrag en scoren hoger op een cumulatieve risico-index. De combinatie van een psychiatrische stoornis bij de moeder en gedragsproblematiek bij het kind blijkt daarentegen geen extra risico op te leveren.

In **hoofdstuk drie** worden angst en depressie in de kindertijd en hun relaties met gedragsproblematiek in de kindertijd en adolescentie onderzocht. In dit hoofdstuk wordt ook het feit benut dat het de steekproef uit tweelingen bestaat. Tweelingen stellen ons namelijk in staat om in grote mate te corrigeren voor "confounding" door genen en omgeving, omdat tweelingen substantiële genetische overlap hebben (volledige overlap in het geval van monozygote tweelingen, de helft in het geval van dizygote tweelingen) en ook nog eens hun opvoedingsomgeving delen. Aan de hand van een discordant tweelingenmodel wordt aangetoond dat confounding de associatie tussen angst en gedragsproblematiek compleet verklaart. Daarentegen blijft de relatie tussen depressie en gedragsproblemen in de kindertijd wel intact na controle voor genetische en omgevingsinvloeden. Longitudinale verbanden worden echter niet gevonden: angst en depressie in de kindertijd voorspellen geen gedragsproblematiek in de adolescentie boven de reeds bestaande voorspellende waarde van gedragsproblematiek in de kindertijd. Oftewel, de associatie tussen angst en gedragsproblemen is niet causaal, terwijl de associatie tussen depressie en gedragsproblemen hoogstens een klein effect is en beperkt is tot de kindertijd.

Hoofdstuk vier is gericht op de classificatie van jeugdige patiënten op de basis van twee vormen van probleemgedrag die deel uitmaken van de psychiatrische classificatie "Oppositioneel Opstandige Gedragsstoornis" (ODD), namelijk: geïrriteerd gedrag en oppositioneel gedrag. Geïrriteerd en oppositioneel gedrag zijn elk gecorreleerd aan verschillende soorten problemen. In deze studie wordt onderzocht of kinderen en jongeren uit een klinische steekproef ingedeeld kunnen worden in aparte groepen op de basis van hun ODD-kenmerken en of deze klinische relevant zijn. Latenteklasseanalyse op basis van ODD symptomen die ouders en leerkrachten melden bij de intake wordt gebruikt om kinderen en jongeren in te delen in verschillende ODD-groepen. De beste oplossing bestaat uit drie groepen: een groep hoog in irritatie en oppositionaliteit (Hoge ODD), één met lage irritatie en oppositionaliteit (Lage ODD) en een derde groep met gemiddelde waarden (Gemiddelde ODD). Kortom, de algehele aanwezigheid van

ODD-gedrag resulteert in de beste groepsverdeling, terwijl differentiatie op de basis van irritatie en oppositionaliteit geen valide optie is. De Hoge ODD-groep ondervindt de meeste psychische problemen bij de intake en in deze groep worden aan het einde van het diagnostische traject de meeste classificaties van ODD en van de normoverschrijdende-gedragsstoornis vastgesteld. Ook functioneren deze kinderen en jongeren slechter, zowel voor als na een eventuele behandeling. Dit alles impliceert dat het indelen van patiënten in aparte groepen aan de hand van geïrriteerd en oppositioneel gedrag beperkte waarde heeft voor de klinische besluitvorming. De algehele aanwezigheid van ODD-gedrag is daarentegen een betere indicator van de klinische prognose.

Hoofdstuk vijf geeft een overzicht van reviews over genetica en agressie en bevat een systematisch overzicht van alle genoombrede associatiestudies (genome-wide association studies, GWAS) naar agressie en antisociaal gedrag. Uit de reviews blijkt dat de erfelijkheid van agressie en antisociaal gedrag op basis van gedragsgenetisch onderzoek, met name bij tweelingonderzoek, rond de 50% ligt met variatie rondom dit gemiddelde. Deze substantiële erfelijkheid wordt echter niet teruggevonden in moleculair genetisch onderzoek. De 17 besproken GWAS-studies bevatten slechts 10 genetische varianten met genoombrede significantie (i.e., $p \leq 5.0E{-}08$) en 817 varianten bereiken marginale significantie (i.e., $5.0E{-}08 \leq p \leq 1.0E{-}05$). Gen-gebaseerde tests (gene-based tests) geven marginale associaties aan voor genen die betrokken zijn bij de regulatie van het immuunsysteem, het endocriene stelsel en zenuwstelsel. Er is geen overeenstemming tussen significante en marginaal significante genetische varianten tussen de verschillende studies. Kortom, deze review geeft aan dat er een substantieel deel van agressie en antisociaal gedrag wordt verklaard door erfelijke factoren, maar dat de daadwerkelijke biologische basis van deze erfelijkheid (i.e., specifieke genen) nog moet worden aangetoond.

Hoofdstuk zes presenteert de eerste metabolomische studie naar agressie bij kinderen op de basis van ochtendurine. De analyses bestaan uit drie fases: een ontdekkingsfase waarbij tweelingen hoog in agressie worden vergeleken met tweelingen laag in agressie; een replicatiefase waarbij tweelingenparen worden vergeleken die discordant zijn op agressie: en een validatiefase waarbij patiënten hoog in agressie worden vergeleken met tweelingen controles laag in agressie. Zes biomarkers voor agressie volgen uit de ontdekkingsfase, hiervan blijven O-fosfoserine en gamma-L-glutamyl-L-alanine significant na correctie voor meervoudig testen. In de replicatiefase worden geen significante biomarkers

gerepliceerd, zes van de biomarkers en twee neurotransmitter-ratio's hebben echter wel dezelfde effectrichting als in de replicatiefase. In de validatie-analyse worden naast congruente effectrichtingen, geen significante associaties met agressie gevonden. Hogere O-fosfoserine-waarden kunnen een indicatie zijn van disregulatie van het serotonerge en dopaminerge systeem en kan wijzen op verminderde conversie van L-tryptofaan naar serotonine en van L-tyrosine naar dopamine. Hogere Gamma-L-glutamyl-L-alanine-waarden kunnen een indicatie zijn voor de rol van oxidatieve stress in de ontwikkeling van agressie in kinderen.

Hoofdbevindingen

1. Agressie en antisociaal gedrag zijn de beste voorspellers voor latere agressie en later antisociaal gedrag (Hoofdstukken 2, 3 en 4). De aanwezigheid van een psychiatrische stoornis bij de vader, niet de moeder, verhoogt het risico op slechtere langetermijnuitkomsten in de adolescentie.
2. Het creëren van subtypes, zoals op basis van psychiatrische stoornissen bij de ouders, kan soms waardevolle informatie verschaffen (Hoofdstuk 2). Naarmate de ernst van de problematiek toeneemt (bijv. in een klinische setting) neemt de complexiteit en diversiteit van de problematiek ook toe. In deze gevallen is een focus op de algehele ernst van problematiek een betere indicatie van de klinische prognose dan subtyperingen op basis van de aard van symptomen.
3. Dit proefschrift benadrukt het belang van zowel het in ogenschouw nemen van karakteristieken van de onderzochte steekproef (bijv. klinische, algemene, of "at risk" populatie) als het uiteindelijke doel van de gemaakte voorspellingen (bijv. identificatie van "at risk" individuen, diagnostiek, inzicht in de ontwikkeling van agressie; Hoofdstukken 2, 4 en 6).
4. Gedragsgenetisch onderzoek, waarbij onderlinge verschillen tussen tweelingen in genetische en omgevingscomponenten worden verdeeld, geeft aan dat agressie en antisociaal gedrag voor een substantieel deel erfelijk zijn (Hoofdstuk 5). Deze verschillen worden echter niet gevonden in directere maten van biologische processen, zoals metabolomische en genoombrede associatiestudies (GWAS; Hoofdstukken 5 en 6).

Discussie

Agressief gedrag is geassocieerd met een veelvoud van andere problemen. Deze problemen variëren echter sterk in de mate waarin zij agressie veroorzaken

of verergeren. In dit proefschrift wordt de stelling dat eerder gedrag de beste voorspeller is voor toekomstig gedrag duidelijk bevestigd; agressie en ander antisociaal gedrag waren veruit de beste voorspellers voor ditzelfde gedrag in de toekomst (Hoofdstukken 2, 3, en 4). Alhoewel dit een waardevolle bevinding is voor risico-taxatie, wordt maar deels verklaard waarom kinderen en jongeren agressief blijven (omdat ze dit toch eerder al waren), noch geeft het algeheel niveau van agressie duidelijke aanwijzingen voor behandeling of preventie.

Een risicofactor die concrete aanwijzingen biedt voor mogelijke interventie werd gevonden in hoofdstuk twee; het hebben van een vader met een psychiatrische stoornis voorspelde extra risico in de adolescentie voor kinderen met gedragsproblematiek in vergelijking met kinderen die alleen gedragsproblematiek hadden. Deze bevinding is waardevol, omdat er inzicht wordt verschaft in de ontwikkeling van agressie: suboptimale opvoedingsvaardigheden en erfelijke belasting spelen hoogstwaarschijnlijk een rol. Ook wordt, terwijl de meeste literatuur zich op moeders richt, het belang van vaders in deze specifieke context onderstreept.

Verder rijst uit dit proefschrift de vraag of de nadruk moet komen te liggen op het vinden van subtypes van antisociaal gedrag enerzijds of dat er een focus moet liggen op heterogeniteit anderzijds. In het geval van ODD levert een onderscheid tussen irritatie en oppositionaliteit bijvoorbeeld interessante correlaties op: irritatie heeft een grotere associatie met affectieve symptomen en oppositionaliteit met ADHD-symptomen en andere gedragsproblemen (Hipwell et al., 2011; Vidal-Ribas et al., 2016). In de algemene bevolking kunnen zelfs kinderen en jongeren in groepen worden verdeeld op basis van deze symptomen (Althoff et al., 2014; Herzhoff & Tackett, 2016; Kuny et al., 2013; Wesselhoeft et al., 2019). Deze groepen hebben over het algemeen unieke correlaten. Hoofdstuk vier laat echter zien dat classificatie op basis van irritatie of oppositionaliteit in een klinische groep niet zinnig is. Latenteklasseanalyse geeft aan dat classificatie op de basis van het algehele niveau van ODD-symptomen (i.e., hoog, gemiddeld en laag) de beste oplossing is. Deze overlap van symptomen komt overeen met de klinische realiteit waarin comorbiditeit regel is, niet uitzondering. Mensen met psychische problemen zijn vaak relatief stabiel in hun probleemniveau, maar wisselen regelmatig van psychiatrische classificatie. Indicatoren van algemene symptoomernst, zoals een psychopathologie-factor (p-factor; Caspi et al., 2014), lijken meer recht te doen aan deze patiëntenpopulatie dan classificatie op basis van subtyperingen. In plaats van een focus op subtypes zou een nadruk op een

algeheel probleemniveau waarschijnlijk betere indicaties opleveren van klinische prognose en zorgbehoefte. Tenslotte is het belangrijk om de setting waarin een voorspeller wordt gebruikt in ogenschouw te nemen. Kind zijn van een ouder met een psychiatrische stoornis kan in de algemene bevolking, waar relatief weinig kinderen een ouder met een psychiatrische stoornis hebben, een goede voorspeller zijn van toekomstige problemen (Hoofdstuk 2). Daarentegen hebben in de kinder- en jeugdpsychiatrie een substantieel deel van de jongeren een ouder met een psychiatrische stoornis. Hierdoor verschaft de aanwezigheid van een ouderstoornis niet veel extra informatie. In dit geval zou een onderzoek naar de soort en ernst van de aandoening bij de ouder eerder relevante informatie kunnen opleveren.

Gedragsgenetisch onderzoek, met name tweelingonderzoek, wijdt een substantieel proportie van agressie en antisociaal gedrag aan erfelijke factoren (Hoofdstuk 4). Deze erfelijke component zien we niet terug in meer directe maten van biologie, zoals de GWAS-studies in hoofdstuk vier en de metabolomische studie in hoofdstuk vijf; er zijn weinig associaties die bovendien een klein deel van de variantie verklaren. Een mogelijke verklaring is dat individuen worden blootgesteld aan wisselende "push-" en "pull-factoren" die ervoor zorgen dat ze tot bepaald gedrag, bijvoorbeeld agressie, overgaan. Onder pushfactoren worden grotere politieke, culturele en sociaaleconomische factoren verstaan die invloed uitoefenen op menselijk gedrag. Pullfactoren zijn meer individueel van aard, hierbij kan men bijvoorbeeld denken aan het verlangen om bij een groep te horen, persoonlijk economisch gewin, neurobiologische kenmerken (bijv. impulsiviteit) en individuele ervaringen (bijv. trauma's). Deze twee factoren interacteren met elkaar en hebben ook invloed op de uiting en erfelijkheid van antisociaal gedrag. In goede buurten zijn bijvoorbeeld erfelijkheidpercentages relatief hoger dan in slechte buurten (Tuvblad et al., 2006; Burt et al., 2016, Hendriks et al., 2020). Daarentegen wordt in slechte buurten een grotere proportie van de variantie in antisociaal gedrag bepaald door omgevingsinvloeden. Ook draagt de heterogeniteit van antisociaal gedrag mogelijk bij aan het moeilijk kunnen vinden van directe biologische correlaten. Fysieke agressie heeft bijvoorbeeld een hogere erfelijkheid dan andere soorten van antisociaal gedrag (Waltes, et al., 2016).

Een ander belangrijk punt van kritiek is de manier waarop gedrag wordt gemeten in tweelingonderzoek; meestal vult een ouder vragenlijsten in over het gedrag van zijn/haar beide tweelingkinderen. Erfelijkheidspercentages worden opvallend genoeg substantieel lager als er meerdere informanten (bijv. leraren) of

observaties worden gebruikt (Tuvblad & Baker, 2011). Er zijn slechts twee studies waarbij agressief gedrag in een gecontroleerde setting werd bestudeerd. Deze lieten een ander beeld zien dan de meeste tweelingliteratuur. Eén studie toonde grote unieke omgevingsinvloeden (74% CI: 0.63–0.90), een middelgroot erfelijke component (A= 20%, CI: 0–37), en een klein aandeel van gedeelde omgeving (C = 6%, CI: 0–34; Achterberg, van Duijvenvoorde, van der Meulen, Bakermans-Kranenburg, & Crone, 2018). De andere studie bij volwassenen toonde zelfs een 100% aandeel van unieke omgeving indien er sprake was van toenemende provocatie van agressief gedrag (Dinić et al., 2020). Deze studies benadrukken het belang om agressie op verschillende manieren te meten en behalve door ouders ook door andere informanten te laten scoren. Ook wordt geopperd dat omgevingsinvloeden belangrijk zijn in het opwekken van geprovoceerde agressie.

Een andere kritiek is de medische lens waardoor menselijk gedrag wordt bekeken in ons onderzoeksveld. Of het nu een psychiater is die een gedragsstoornis vaststelt of een leraar die een vragenlijst over zijn leerling invult, bij het definiëren van (problematisch) menselijk gedrag is er altijd tot bepaalde hoogte sprake van een moreel oordeel, die bovendien wordt geveld in een specifieke culturele context. Dit maakt de psychiatrie anders dan andere medische disciplines waarbij er concreter kan worden vastgesteld of een biologische functie naar behoren functioneert. In veel gevallen zijn bij het vaststellen van een (somatische) ziekte laboratoriumtests een essentieel deel van de anamnese, in sommige gevallen zijn de indrukken van de arts zelfs van secundair belang en de laboratoriumuitslagen leidend. In de psychiatrie zijn daarentegen observaties en culturele kennis duidelijk het primaire diagnostisch instrument. Psychose is een duidelijk voorbeeld waarbij zowel de expressie als de beleving als ziektebeeld cultuurgebonden is (Kendler, Zachar, & Craver, 2019). Extreme schuldgevoelens zijn bijvoorbeeld zeer prevalent in Westerse landen en wellicht door de christelijke cultuur ingegeven, denk hierbij bijvoorbeeld aan de erfzonde (Bhavsar & Bhugra, 2008). In pre-industriële samenlevingen ziet men bijvoorbeeld eerder wanen waarbij mensen geloven in een dier te zijn veranderd. Dit is waarschijnlijk te wijten aan animisme en een uitgebreidere flora en fauna (Garlipp, Gödecke-Koch, Dietrich, & Haltenhof, 2004). Dat er culturele verschillen zijn in de expressie van psychiatrische symptomen betekent echter niet dat er geen onderliggende biologische oorzaak aanwezig is. Het ervaren van wanen en hallucinaties zijn namelijk gemeenschappelijk elementen die we tot zekere hoogte terugzien in psychose over heel de wereld. Dit voorbeeld

geeft eerder aan dat het vinden van een biologische oorzaak van agressie nog moeilijker is, agressie is namelijk een veel fundamenteler menselijk gedrag dan een psychose en bovendien veel contextgevoeliger.

Aanbevelingen voor de klinische praktijk

De context waarin risicotaxaties of voorspellingen worden gemaakt is belangrijk om in ogenschouw te nemen. Antisociaal gedrag en agressie zijn uitgebreid onderzocht in verschillende omgevingen. Dit proefschrift beschrijft dat bij kinderen met gedragsproblemen de aanwezigheid van psychiatrische stoornissen bij de ouders, met name die van vaders, een krachtige risico-indicator zijn voor slechter psychosociaal functioneren in de adolescentie. Hoewel deze bevinding waardevol kan zijn voor clinici, moeten zij zich bewust zijn dat bevindingen uit onderzoek, dat is uitgevoerd in relatief hoog-functionerende populaties, zich soms een weg baant naar de klinische praktijk en vice versa. In andere woorden, kennis die vergaard is in een specifieke omgeving wordt soms onterecht gegeneraliseerd naar andere omgevingen. Om een voorbeeld te noemen: tot mijn verbazing was sporten niet geassocieerd met een afname in angst en depressie in de algemene populatie als er door middel van een tweelingenmodel gecorrigeerd werd voor confounding (De Moor, Boomsma, Stubbe, Willemsen, & de Geus, 2008). Gerandomiseerd onderzoek met controlegroepen (RCT's) in patiëntengroepen met depressie laat echter zien, dat bewegen wel degelijk effectief is in het verminderen van symptomen en laat bovendien effectgroottes zien die vergelijkbaar zijn met die van psychotherapie en antidepressiva (Kvam, Kleppe, Nordhus, & Hovland, 2016). Dit voorbeeld toont aan dat clinici in hun behandelrelatie wel degelijk substantiële verschillen kunnen maken. Alhoewel onderzoek in de algemene populatie veronderstelt dat mensen met depressie en angst niet uit zichzelf zouden zijn gaan sporten, begonnen diezelfde mensen met bewegen wanneer deze optie werd aangeboden in een therapeutische setting en bleken hier baat bij te hebben.

In dit proefschrift heb ik tevens aan de orde gesteld dat in klinische populaties het algehele probleemniveau een leidend principe moet zijn van diagnostiek en behandeling, niet specifieke gedragssubtypes. Indien er gericht wordt op specifieke gedragssubtypes dan moeten deze beïnvloed kunnen worden door interventie, dan wel gerelateerd zijn aan factoren die beïnvloed kunnen worden door interventie.

Aanbevelingen voor toekomstig onderzoek

Een groter begrip van onderliggende biologische processen van agressie en antisociaal gedrag kan worden verworven door de onderlinge samenhang van meerdere biologische systemen tegelijkertijd te bestuderen in plaats van apart van elkaar. Een dergelijke integratieve aanpak maakt het mogelijk om de effecten en interacties van meerdere biologische processen te aggregeren. Daarom worden momenteel genetische, epigenetische en metabomische data die in het kader van ACTION zijn verzameld, geïntegreerd in een overkoepelende "cross-omics approach".

Een andere suggestie voor toekomstig (tweeling)onderzoek is de nadruk op experimenteel en gerandomiseerd onderzoek met controlegroepen (RCT's). Het gebruik van tweelingen in onderzoek stelt wetenschappers in staat om in grote mate worden te controleren voor genetische en omgevingsinvloeden. Tot nu toe bestaat de literatuur bijna uitsluitend uit observationele tweelingstudies. Meer nadruk op experimenteel onderzoek en RCT's maakt het mogelijk om causale verbanden te vinden; welke karakteristieken zijn bepalend voor het ontwikkelen van antisociaal gedrag en wat zijn effectieve behandelingen? Uitgebreide werving zou een essentieel deel van dit type onderzoek moeten zijn. Tweelingen zijn namelijk relatief zeldzaam (15.9 twin births per 1000 births; Glasner, Van Beijsterveldt, Willemsen, & Boomsma, 2013), daarnaast is in eerste instantie de kans kleiner dat jongeren met agressie (en hun families) deelnemen aan onderzoek én is de kans op uitval groter in vergelijking met hun minder agressieve leeftijdsgenoten.

De toenemende aandacht voor agressie als universeel menselijk gedrag is zeker van toegevoegde waarde en kan verklaringen bieden waarom in extreme situaties (bijv. oorlog) veel mensen tot geweld kunnen overgaan. Onder normale levensomstandigheden is er echter een klein percentage van de populatie die verantwoordelijk is voor een relatief groot deel van de delicten: 1% van de Zweedse bevolking pleegt 63% van alle geweldsdelicten (Falk et al., 2014). Het is treffend dat bij de verdeling van "output" er geen wezenlijke verschillen zijn in antisociale en academische carrières (Laherrere & Sornette, 1998). Een minderheid van de mensen is verantwoordelijk voor de meerderheid van het werk, denk hierbij in de wetenschap aan veel geciteerde wetenschappers en op antisociaal gebied delinquenten met levenslange, ernstige, antisociale carrières. Daarentegen krijgt een meerderheid van de mensen een minderheid van het werk gedaan, zoals promovendi die de wetenschap verlaten na hun promoties en

kinderen en adolescenten waarbij hun agressie en antisociaal gedrag gebonden zijn aan een ontwikkelingsfase. Gezien dit gegeven zou de grootste winst kunnen worden geboekt door in te zetten op deze hoog presterende antisociale “elite”, niet het gemiddelde individu.

CURRICULUM VITAE

Peter Roetman was born April 1st 1989 in Leiden. He followed his secondary education (Gymnasium) at Bonaventuracollege. Between 2007-2013, he completed the bachelor Education and Child Studies (specialization: orthopedagogiek) and the (Research) Master Developmental Psychopathology in Education and Child Studies at Leiden University. During his studies he worked as a researcher at the Department of Education and Child Studies. In 2014 he completed his clinical internship (basisaantekening psychodiagnostiek) at the Leo Kannerschool in Oegstgeest, a school for children with autism spectrum disorders, and worked as a teacher at Utrecht University. In 2015 Peter started his PhD at Curium-LUMC (Department of Child and Adolescent Psychiatry, Leiden University Medical Centre). He is currently working as a researcher at a health insurance company.

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*** =** shared first authorship

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