A Potential Role for the STXBP5-AS1 Gene in Adult ADHD Symptoms


Received: 17 March 2018 / Accepted: 26 December 2018 / Published online: 18 January 2019
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Abstract
We aimed to detect Attention-deficit/hyperactivity (ADHD) risk-conferring genes in adults. In children, ADHD is characterized by age-inappropriate levels of inattention and/or hyperactivity-impulsivity and may persist into adulthood. Childhood and adulthood ADHD are heritable, and are thought to represent the clinical extreme of a continuous distribution of ADHD symptoms in the general population. We aimed to leverage the power of studies of quantitative ADHD symptoms in adults who were genotyped. Within the SAGA (Study of ADHD trait genetics in adults) consortium, we estimated the single nucleotide polymorphism (SNP)-based heritability of quantitative self-reported ADHD symptoms and carried out a genome-wide association meta-analysis in nine adult population-based and case-only cohorts of adults. A total of n = 14,689 individuals were included. In two of the SAGA cohorts we found a significant SNP-based heritability for self-rated ADHD symptom scores of respectively 15% (n = 3656) and 30% (n = 1841). The top hit of the genome-wide meta-analysis (SNP rs12661753; p-value = 3.02 × 10−7) was present in the long non-coding RNA gene STXBP5-AS1. This association was also observed in a meta-analysis of childhood ADHD symptom scores in eight population-based pediatric cohorts from the Early Genetics and Lifecourse Epidemiology (EAGLE) ADHD consortium (n = 14,776). Genome-wide meta-analysis of the SAGA and EAGLE data (n = 29,465) increased the strength of the association with the SNP rs12661753. In human HEK293 cells, expression of STXBP5-AS1 enhanced the expression of a reporter construct of STXBP5, a gene known to be involved in “SNAP” (Soluble NSF attachment protein) Receptor” (SNARE) complex formation. In mouse strains featuring different levels of impulsivity, transcript levels in the prefrontal cortex of the mouse ortholog Gm28905 strongly correlated negatively with motor impulsivity as measured in the five choice serial reaction time task (r² = −0.61; p = 0.004). Our results are consistent with an effect of the STXBP5-AS1 gene on ADHD symptom scores distribution and point to a possible biological mechanism, other than antisense RNA inhibition, involved in ADHD-related impulsivity levels.

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Electronic supplementary material. The online version of this article (https://doi.org/10.1007/s10519-018-09947-2) contains supplementary material, which is available to authorized users.
Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder affecting 2–5% of children (Polanczyk and Rohde 2007) and 2.5–4.9% of adults (Franke et al. 2018). In children, ADHD is characterized by age-inappropriate, sustained symptoms of inattention and/or hyperactivity-impulsivity. In children and adults (Faraone et al. 2005) ADHD shows substantial heritability. Heritability (h²) estimates are largely independent of the phenotypic measurement scale (i.e., categorical or continuous) and estimates are lower when using self-report rating scales (Franke et al. 2012). For clinically diagnosed ADHD, the genetic contribution to variation in ADHD was estimated at 72% in adult twins from the Swedish Twin Registry (n = 37,714) (Larsson et al. 2014), whereas the estimate was around 30% for the self-assessed ADHD index scored from the Conners’ Adult ADHD Rating Scales in a large (n = 12,594 subjects) Dutch sample of twins and their relatives (Boomsma et al. 2008).

For Attention Problems assessed by ASEBA (Achenbach System of Empirically Based Assessment; Achenbach et al. 2003) in 44,607 young and adult Dutch twins (57% with longitudinal data), there was a downward trend with age. Age to age correlations were age dependent and ranged from 0.33 (age 50–60+) to 0.73 (age 10–12). The stability across ages was explained by both genetic and environmental factors. Heritability at age three was 70% and remained high before age 12 (72–74%). After age 12, h² became 50–56% in adolescents, and this declined further to 40–54% in adults (Kan et al. 2013). However, part of the decrease in h² may not be due to age but to differences in assessment (Kan et al. 2013, 2014).

A large Swedish study addressed the etiology of the association of the major dimensions of ADHD in adults and estimated that 52% of the correlation between inattentive and hyperactive-impulsive symptoms was accounted for by genetic influences, and 48% by non-shared environmental influences (n = 15,198 adult twin pairs) (Larsson et al. 2013). Shared genetic factors also underlie the increased risks for other psychiatric disorders adults with ADHD and their relatives, for example borderline personality disorder (Distel et al. 2011; Kuja-Halkola et al. 2018) autism spectrum disorder (Ghirarri et al. 2018) and problem drinking (Derks et al. 2014). However, not all comorbidities are due to genetic risk; for the liability of attention-deficit/hyperactivity and tics and obsessive–compulsive symptoms, the majority of genetic variance was specific rather than shared (Pinto et al. 2016).

The genetic contributions to ADHD in children and adults are complex, with multiple different genetic variants contributing to the disorder (Faraone et al. 2015), both common and rare (Franke et al. 2012). Recently, 16 genome-wide associations have been established in an ADHD Genom-Wide Association Studies meta-analysis (GWASMA) of childhood case-control studies from the psychiatric genomics consortium (PGC) and The Lundbeck foundation initiative for integrative psychiatric research (iPSYCH) (Demontis et al. 2018) and population-based samples from the early genetics and lifecourse epidemiology (EAGLE) consortium (Middeldorp et al. 2016). These studies estimated that the single nucleotide polymorphism (SNP)-based h² of ADHD symptom scores in children ranged from 5 to 34% in population-based samples (EAGLE consortium) and was 21.6% in the PGC + iPSYCH case-control samples.

Inattention and hyperactivity–impulsivity symptoms can be reliably assessed in population-based cohorts based on rating scales. Phenotypic and genetic correlations between symptom scores assessed with the different instruments are substantial: in a clinical sample of 120 adults with ADHD the phenotypic correlation between the CAARS ADHD-index (Conners 1999) and the ADHD-RS, which obtains ADHD DSM-IV symptoms self-report was 0.73 (p < 0.01) (Kooij et al. 2005). In 380 parents of children with ADHD, this correlation was of similar magnitude (r = 0.69; p < 0.001) (Thissen et al. 2012). As such, the disorder may be viewed being at the extreme end of normally distributed behavioral traits in the population (Larsson et al. 2013). This creates the possibility to collect large samples for gene-finding studies.

Here, we aimed to identify genes associated with ADHD symptoms in adults from population-based and case-control cohorts in order to detect disease-relevant genes. Within the study of ADHD trait genetics in adults (SAGA) consortium, we estimated the SNP-based h² of self-reported adult ADHD symptoms and subsequently carried out a GWASMA in nine cohorts of European-Caucasian origin (n = 14,689 individuals, age 18 years or older). These samples included six population-based cohorts, two clinical ADHD samples and one clinical cohort ascertained for depressive and anxiety disorders. We followed up the locus with the strongest statistical association in a replication analysis of quantitative childhood ADHD symptom scores (n = 14,776) from the EAGLE consortium (Middeldorp et al. 2016). Genetic correlations were estimated between the SAGA sample and the PGC + iPSYCH sample of children (Demontis et al. 2018) and between SAGA and the ADHD GWASMA results of the EAGLE consortium (Middeldorp et al. 2016). Finally, we conducted gene-based tests for genes with SNPs showing

**Keywords** ADHD symptoms · GWAS · Adults · STXBP5-ASI gene
a \( p \)-value < \( 1 \times 10^{-6} \) in the meta-analysis, making use of the common SNPs from SAGA and rare variant data from the Erasmus Rucphen Family (ERF) study (see Table 1), included in SAGA.

Functional follow-up studies downstream of gene finding in ADHD, e.g. in model systems, to determine the biological relevance of a genetic finding are scarce (Klein et al. 2017). Core features of ADHD, inattention, hyperactivity, and impulsivity are well defined in mouse models (Loos et al. 2014). Here we carried out functional follow-up studies for the gene with the top association result from the GWASMA in three mouse inbred strains with large differences in motor impulsivity derived from reaction time tasks, and in a human cell assay.

## Methods

### Study populations in the SAGA consortium

ADHD symptom scores in adults were available in nine cohorts of European descent (please see references in Table 1; Supplementary Table 1; Supplementary Fig. 4).

1. The Netherlands Twin Registry (NTR) is a population-based longitudinal cohort of twins and their family members, e.g. parents, siblings, spouses, which recruited (young) adult twins in the early 1990s through city council registrations. Continued recruitment of adult twins took place continuously via the NTR website and e.g. via media campaigns. Twin families are followed longitudinally through survey studies and biobank projects.

2. The Netherlands Study of Depression and Anxiety (NESDA) is an ongoing longitudinal naturalistic cohort study.

### Table 1 Information on cohorts and phenotype assessment in the SAGA consortium included in the GWASMA

<table>
<thead>
<tr>
<th>Cohort name</th>
<th>N (% F)</th>
<th>Age (SD)</th>
<th>Symptom list (N items)</th>
<th>Score rangea</th>
<th>Mean score (SD)a</th>
<th>Sample type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands twin registry (NTR)</td>
<td>5935 (63%)</td>
<td>43.7 (15.2)</td>
<td>CAARS ADHD-index (12)</td>
<td>0–30</td>
<td>7.9 (3.7)</td>
<td>Population-based</td>
<td>Willemsen et al. (2010)</td>
</tr>
<tr>
<td>Netherlands study of depression and anxiety (NESDA)</td>
<td>1977 (66%)</td>
<td>46.5 (13.0)</td>
<td>CAARS ADHD-index (12)</td>
<td>0–32</td>
<td>8.7 (5.4)</td>
<td>Clinical population of major depression disorder</td>
<td>Boomsma et al. (2008)</td>
</tr>
<tr>
<td>Erasmus rucphen family study (ERF)</td>
<td>1043 (53%)</td>
<td>45.6 (13.3)</td>
<td>CAARS ADHD-index (12)</td>
<td>0–25</td>
<td>7.8 (4.4)</td>
<td>Population-based</td>
<td>Aulchenko et al. (2004)</td>
</tr>
<tr>
<td>NeuroIMAGE</td>
<td>470 (51%)</td>
<td>42.3 (5.3)</td>
<td>ADHD-RS (23)</td>
<td>0–43</td>
<td>14.1 (8.9)</td>
<td>Parents of children of ADHD</td>
<td>von Rhein et al. (2014)</td>
</tr>
<tr>
<td>Brain imaging genetics (BIG)</td>
<td>448 (63%)</td>
<td>22.3 (3.2)</td>
<td>ADHD-RS (23)</td>
<td>0–40</td>
<td>14.0 (6.4)</td>
<td>Population-based</td>
<td>Hoogman et al. (2012)</td>
</tr>
<tr>
<td>Nijmegen biomedical study (NBS)</td>
<td>2925 (53%)</td>
<td>57.4 (16.3)</td>
<td>ADHD-RS (23)</td>
<td>0–15</td>
<td>1.4 (2.2)</td>
<td>Population-based</td>
<td>Galesloot et al. (2017)</td>
</tr>
<tr>
<td>(International multicentre persistent ADHD collaboration IMPACT)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>113 (63%)</td>
<td>37.7 (11.5)</td>
<td>ADHD-RS (23)</td>
<td>1–18</td>
<td>12.04 (3.3)</td>
<td>Clinical population of adults with ADHD</td>
<td>Mostert et al. (2015)</td>
</tr>
<tr>
<td>Vall d’Hebron Institute de recerca (VHIR)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>559 (32%)</td>
<td>33.3 (10.6)</td>
<td>ADHD-RS (18)</td>
<td>4–54</td>
<td>31.0 (9.7)</td>
<td>Clinical population of adult ADHD</td>
<td>Richarte et al. (2017)</td>
</tr>
<tr>
<td>TRacking adolescents’ individual lives survey (TRAILS)</td>
<td>1215 (48%)</td>
<td>19.0 (0.6)</td>
<td>ASR ADHD (13)</td>
<td>0–26</td>
<td>5.9 (4.4)</td>
<td>Population-based</td>
<td>Oldehinkel et al. (2015)</td>
</tr>
</tbody>
</table>

<sup>F</sup> female, <sup>CAARS ADHD-index</sup> Conners’ Adult ADHD Rating Scale, <sup>ADHD-RS DSM-IV</sup> ADHD Rating Scale, <sup>ASR ADHD</sup> Attentional Deficit/Hyperactivity Problems subscale from the Adult Self Rating

<sup>a</sup>Untransformed values observed per cohort

<sup>b</sup>Only affected individuals included
The Nijmegen Biomedical Study (NBS) is a population-based study of 2981 people, aged 18–65 years at baseline, with lifetime and/or current depressive and/or anxiety disorders (n = 2329, 78%) and healthy controls (n = 652, 22%). Participants were recruited from the community (n = 564, 19%), primary care (n = 1610, 54%) and specialized mental healthcare (n = 807, 27%) from September 2004 to February 2007 at three study sites (Amsterdam, Groningen, Leiden). Exclusion criteria were: (a) having a primary clinical diagnosis of psychotic disorder, obsessive–compulsive disorder, bipolar disorder or severe addiction disorder, and (b) not being fluent in Dutch.

The TRacking Adolescents’ Individual Lives Survey (TRAILS) is an ongoing, multidisciplinary research project on the psychological, social and physical development of adolescents and young adults. More than 2500 young people participate, since their tenth or eleventh year of age. These participants have been examined every 2–3 years for the past 15 years, through questionnaires, interviews, tests and/or physical measurements. Information is provided by youngsters, family members, teachers and partners.

Erasmus Rucphen Family (ERF) is a family-based cohort originating from 22 couples and spread over 23 generations. All descendants of those couples were invited to visit the clinical research center in the region, where they were examined in person and interviewed on a broad range of topics, including medication use and medical history.

International Multicenter ADHD Genetics (NeuroIMAGE) collected DNA and information on ADHD and relevant comorbidities from families with (at least) one child diagnosed with ADHD between 2003 and 2007. The Dutch site of the IMAGE project also collected other (cognitive and behavioral) measures on (unaffected) parents. The behavioral information on the parents was used in this study.

The Brain Imaging Genetics (BIG) study is a population-based study of healthy individuals (age range 18–45 years) who participated in imaging studies carried out in the Donders Institute, in Nijmegen, the Netherlands. The assessment of ADHD symptoms was performed through internet-based testing, as part of an electronic questionnaire and test battery.

The Nijmegen Biomedical Study (NBS) is a population-based study that was initiated in 2000 in Nijmegen in the eastern part of The Netherlands. Eligibility criteria were age 18 years or older, not living in an institution or rest home and the ability to fill out a questionnaire in Dutch. Participants were invited to fill out a postal questionnaire (NBS-1 QN) and to donate a blood sample. Psychological problems and symptoms for ADHD were collected in the second wave of the study (NBS-2), that was initiated in 2005.

The International Multicenter persistent ADHD CollaboraTion (IMpACT) included patients and healthy controls recruited at the department of psychiatry, Radboud University Medical Center, Nijmegen and through advertisements. Patients were included if they had previously been diagnosed with persistent ADHD, i.e. present since childhood, by a psychiatrist according to the DSM-IV-TR. Exclusion criteria were psychosis, addictions in the last 6 months, current Major Depression Disorder, full-scale IQ estimate < 70, neurological disorders, sensorimotor disabilities, medication use other than psychostimulants, atomoxetine or bupropion and failure to withhold stimulant medication 24 h prior to testing. These same criteria were applied to the NeuroIMAGE sample.

The Vall d’Hebron institute de recerca (VHIR) sample consisted of ADHD cases only. Participants were referred to the program from primary care centres, children’s neuropsychiatric surgeries or mental health hospitals in Barcelona, Spain because of a diagnostic suspicion of ADHD. The diagnosis of ADHD was evaluated with the structured clinical interview for DSM-IV (SCID-I) and the conners adult ADHD diagnostic Interview for DSM-IV (CAADID-II).

ADHD symptom scores in the SAGA consortium

ADHD symptom scores were assessed by three instruments (see Table 1). First, the ADHD-index of the Conners Adult ADHD Rating Scale (CAARS ADHD-index) (Conners 1999) which consists of 12 items, scored on a 4-point scale (0 = never, 3 = very often; see Supplementary Table 8 for details). The CAARS ADHD-index (used in NESDA, NTR, ERF) is an extensively tested psychometric instrument with high internal consistency and reliability.

Second, the total scores of the DSM-IV ADHD Rating Scale (ADHDRS) (Kooij et al. 2005). Five cohorts (NeuroIMAGE, BIG, IMpACT, VHIR, NBS) collected information using the ADHDRS (Kooij et al. 2005), which has high validity in population-based and case samples. Subjects were asked to complete the ADHDRS for current symptoms in adults. Symptoms were reported over the last 6 months. Participants had to answer 23 questions on a four-point scale (never, sometimes, often, very often). The 23 current item scores were recalculated to the original 18 DSM-IV-TR ADHD criteria.

Third, the Attentional Deficit/Hyperactivity Problems subscale from the adult self report (ASR ADHD; 13 items) (Achenbach et al. 2003) was used by one cohort, TRAILS. The ASR ADHD scale has 13 items; response format is 0, 1, and 2 per item. Possible range in scores 0–26.
GWAS analyses were done with the phenotype measured at the level of the item average rather than the item sum.

**Genetic variant calling and quality control in the SAGA consortium**

An overview of genome-wide SNP genotyping, quality control, and imputation is given in Supplementary Table 1. Exomes of 1336 individuals from the ERF population, which is a genetically isolated population in the Netherlands (Aulchenko et al. 2004), were sequenced (see Supplementary Methods), and ADHD index data were available for 587 of these individuals. Detection of rare variants in the ERF study was done for genes harboring SNPs with association \( p < 1 \times 10^{-5} \) in the GWASMA, and variants identified in these exomes were used to estimate the contribution of rare variants in the genes of interest to ADHD behavior (see Supplementary Methods).

**GCTA**

Genome-wide Complex Trait Analysis (GCTA) (Yang et al. 2011) was used to compute the variance in the ADHD symptom score explained by common SNPs in the two largest cohorts included in the SAGA meta-analysis, the NTR and NESDA (\( n > 1500 \) unrelated subjects, we selected one random sibling per twin pair across all pairs). A genetic relationship matrix (GRM) for all individuals in the dataset was estimated based on SNPs with high imputation quality (see Supplementary Methods). Bivariate GCTA (Yang et al. 2011) was additionally run on the CAARS ADHD-index and ASR-ADHD data also available in the NTR cohort, to assess the genetic correlation (\( r_g \)) between the two diagnostic instruments.

**Genome-wide association and meta-analysis in the SAGA consortium**

GWAS was conducted in each cohort by linear regression under an additive model. Age was included as a covariate, but not gender, which was not significantly associated with the ADHD scores in any study. Four principal components were added to account for possible population stratification effects. Information on software packages is provided in Supplementary Table 1. In all analyses, the uncertainty of the imputed genotypes was taken into account depending on the genotyping array and imputation software (threshold used: \( 0.8 < \text{INFO} < 1.1, \text{RSQR} \geq 0.6, \text{INFO} \geq 0.6; \) see Supplementary Table 1). Location of SNPs reported is from the build 37 (hg19) 1000G data. Meta-analysis was conducted in METAL (http://www.sph.umich.edu/csg/abecasis/metal/index.html) by the \( p \)-value-based method, given the intrinsic variability of the quantitative traits used that limits our ability to combine betas and standard errors freely. While the trait scores used in the different GWAS are correlated, we cannot be completely sure that the sampling distribution of these traits is exactly the same (see Supplementary Methods). The meta-analysis was performed in the full sample (nine cohorts) and restricted to the population-based samples (seven cohorts; “restricted sample”).

**Replication in the EAGLE consortium**

Within EAGLE, association of ADHD-related measures was assessed in nine population-based childhood cohorts with genotype data imputed against the 1000 Genomes reference panel (Middeldorp et al. 2016). Linear regression of the phenotype on sex, age, genotype dose, and principal components was performed in all cohorts, followed by meta-analysis based on \( p \)-values in METAL. The TRAILS cohort is part of both consortia, and was excluded from the EAGLE consortium for replication analysis, leaving a total of 14,776 children from eight cohorts.

**Lookup of significant GWAS Loci**

Evidence for an effect of the 12 independent ADHD-associated SNPs from the PGC + iPSYCH GWASMA on adult ADHD symptoms was studied through a look-up of results. Linkage disequilibrium (LD)-independent loci with corresponding index-SNPs were obtained from Table 1 of Demontis et al. (2018). If the index variant was not present in the SAGA data set, a proxy variant was selected using LDlink (https://analysis-tools.nci.nih.gov/LDlink/). The Bonferroni-corrected significance level was set at \( p = 0.05/12 = 0.00417 \).

**Linkage disequilibrium score regression (LDSR) analysis**

LDSR was used to estimate the genetic correlation between the PGC + iPSYCH sample of children (Demontis et al. 2018) the SAGA sample of adults and the ADHD GWASMA data of the EAGLE consortium. Each dataset underwent additional filtering for markers overlapping with HapMap Project Phase 3 SNPs, INFO score \( \geq 0.9 \) (where available), and MAF \( \geq 1\% \). Indels and strand-ambiguous SNPs were removed. LDSR analysis was performed using the LDSR package (https://github.com/bulik/lodc (Bulik-Sullivan et al. 2015), see Supplementary Methods).

**Gene-based analysis of common and rare variants**

Genes containing SNPs with \( p \)-values \( < 1 \times 10^{-6} \) in the meta-analysis of the nine cohorts were selected for gene-based tests using common and rare variants. The common variant
analysis was performed in MAGMA (de Leeuw et al. 2015). Flanking regions of 25 kb for each gene were included in the analyses. The rare variant analysis was performed with the sequence kernel association test (SKAT; only in the ERF study) library of the R-software (Wu et al. 2011). Only characterized genes plus the STXBP5-AS1 gene were analyzed.

**Functional analyses**

Follow-up functional analyses were performed on the STXBP5-AS1 locus containing the best association p-value in the SAGA full sample GWASMA. Given that the STXBP5-AS1 gene, which contains the top hits, is hitherto uncharacterized, we investigated its function. STXBP5-AS1 encodes a long noncoding RNA (lncRNA). Although human STXBP5-AS1 does not have any orthologues listed in the UniGene database, it is conserved in primates and shows a modest conservation in rodents (Supplementary Table 7 and alignment in Supplemental Fig. 2). In the hg19 genome release annotation STXBP5-AS1 is located next to STXBP5 in the opposite orientation, with antisense sequence overlap in exon one of STXBP5 (Fig. 1a). It may be hypothesized that STXBP5-AS1 affects STXBP5 expression. For such natural antisense RNAs, both repression and enhancement of the expression of cognate genes have been described (Kimura et al. 2013; Matsui et al. 2008). We tested this hypothesis by designing a reporter gene fusing exon 1 of STXBP5 to EGFP, and quantifying its expression in human HEK293 cells. Human STXBP5-AS1 encodes multiple splice variants, many of which lack a region that overlaps the STXBP5 gene. To test for regulatory effects of STXBP5-AS1 on the expression of STXBP5, a fluorescent reporter construct was designed to contain the region of antisense overlap (see Supplementary Methods).

**Mouse models**

According to current annotation in GRCm38.p6, the mouse lncRNA gene Gm28905 shares sequence similarity with human STXBP5-AS1 but lacks antisense overlap with mouse Stxbp5. Nonetheless, we use the annotation Stxbp5-AS1 next to the official gene name. Given the in vitro effects

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**Fig. 1** STXBP5-AS1 positively regulates the expression of its cognate mRNA. a **Top:** Design of two reporter constructs. Top: Exon 1 of human STXBP5, containing the natural 5′UTR and encoding the first 50 amino acids, was fused in-frame to EGFP. The STXBP5-AS1 transcript including the region showing perfect (100%) sequence overlap with the encoded STXBP5 transcript is depicted schematically in blue. **Bottom:** To control for transfection efficiency and differences in cellular metabolic rates, we co-expressed a non-target mRNA comprised of human HPRT1 exon 1 fused to mCherry. b Typical examples of HEK293 cells expressing both constructs with or without STXBP5-AS1. c Quantitation of EGFP and mCherry fluorescence in presence or absence of STXBP5-AS1 (947 and 974 cells respectively). d, e The ratio of STXBP5-EGFP and HPRT1-mCherry was calculated for each cell. Data are presented as a histogram d or as mean ± SEM e. ***p = 6 × 10^-51; t_{946} = 4.4412, Student’s t-test
on STXBP5-EGFP protein expression, we tested the relationship between gene expression of both mouse Stxbp5 and/or Gm28905 / Stxbp5-AS1 and measures of behavioral impulsivity. We analyzed gene expression in medial prefrontal cortex of three mouse inbred strains previously described to have large differences in motor impulsivity (Loos et al. 2014). RNA was derived from prefrontal cortex of adult male mice from the inbred strain C57BL/6J (n = 8) and recombinant inbred strains BXD29 (n = 8) and BXD68 (n = 7), and gene expression was quantitated (see Supplementary Methods). For one C57BL/6J animal the RNA isolation failed, and for one BXD68 animal we did not have behavioral data; hence, the n-numbers vary between analyses. Strains were bred in the facility of the Neuro-Bsik consortium of the VU University (Amsterdam, The Netherlands) and used for behavioral analyses (Loos et al. 2014; Spijker et al. 2004).

**Results**

**ADHD symptom scores phenotypic characterization**

Quantitative assessment instruments are listed in Table 1. The quantitative phenotypes showed a weak, negative correlation with age and no association with gender in any cohort. We estimated the phenotypic correlation between the CAARS ADHD-index and the ASR-ADHD in the NTR (n = 15,226; average age 40 years, SD = 16.1) to be 0.67 (p < 0.0001). In younger participants in the age range of the TRAILS cohort (18–22 years, n = 2687), the correlation was similar (r = 0.68, p < 0.0001).

**Genetic correlations of ADHD symptom scores**

A significant SNP-based heritability was estimated for the CAARS ADHD-index in a subsample of each of the two largest cohorts: 30% (SE = 16.7%, p = 0.035) in NESDA (n = 1841 unrelated subjects) and 15% (SE = 7.8%, p = 0.020) in NTR (n = 3881 unrelated participants). Both estimates are significant, but given the sample sizes in each cohort, they are not statistically different from each other. We also estimated the genetic correlation for the CAARS ADHD-index and the ASR-ADHD using bivariate GCTA. In all individuals from the NTR with genotype and phenotype data (n = 6036 related and unrelated subjects), the genetic correlation was 0.818 (SE = 0.0256). When analyzing the bivariate data in 2921 unrelated subjects, the point estimate of the genetic correlation was 0.813 (SE = 0.364).

We estimated the genetic correlation between PGC + iPSYCH and the complete SAGA sample to be 0.541 (SE = 0.447, p = 2.26 × 10⁻¹; the VHIR cohort present in both studies). The SNP-based heritability of the complete SAGA samples, assessed by LDSR, was 0.0183 (SE = 0.038). The genetic correlation between the complete SAGA sample and the childhood ADHD cohort of the EAGLE-ADHD consortium was modest and non-significant (rg = 0.2959, SE = 1.2906, p = 0.8187).

In NTR and NESDA, a subset of participants (n = 6678) had additional phenotype data on hyperactivity/impulsivity and inattention symptom subscales of the CAARS available. These scales of each nine items are non-overlapping with the 12 ADHD-index items (see Supplementary Table 8). For hyperactivity/impulsivity symptoms, the p-value for association with rs12661753 was 1.51 × 10⁻³, whereas for inattention it was 3.53 × 10⁻², suggesting a differential effect of the variant between hyperactivity and impulsivity but we cannot claim that this is a significant difference between the two traits.

**Genome-wide association, meta-analysis and replication**

For the nine separate GWAS (in the SAGA Consortium), the genomic control inflation factors (lambda) ranged between 0.960 and 1.027 (mean lambda 0.999, Supplementary Table 2). Meta-analysis (Fig. 2a) of the full sample revealed the lowest p-value (3.02 × 10⁻⁷) for the intronic SNP rs12661753 in STXBP5-AS1 (Supplementary Fig. 3E); for the meta-analysis of the restricted sample, p-value for this SNP was 1.48 × 10⁻⁶ (Fig. 2b). Replication was observed for rs12661753 (p = 3.07 × 10⁻²) for childhood ADHD symptoms in the EAGLE-ADHD consortium (Middeldorp et al. 2016). The subsequent GWASMA between SAGA and EAGLE revealed the best association p-value = 2.05 × 10⁻⁷ for SNP rs12661753 (n = 29,465; Supplementary Fig. 3F) located in the STXBP5-AS1 gene, and in high LD (D² = 1.0, r² = 0.98) with rs12661753 (PSAGA−EAGLE=3.55 × 10⁻⁷; Fig. 2c).

The index variant rs12661753 was not associated with ADHD risk in the recent case-control PGC + iPSYCH GWASMA of ADHD in a sample mainly consisting of children (p = 0.6316, n = 55,374). A look-up of genome-wide significant ADHD index SNPs from this PGC + iPSYCH GWASMA for association in the SAGA consortium also revealed no significant associations with adult ADHD symptoms (Supplementary Table 6).

As shown in Table 2 and Supplementary Table 3, eight independent clumped loci (including 50 common variants) showed p-values < 1 × 10⁻⁶. Of these, four were also amongst the top-associated loci from the restricted SAGA GWASMA (no patients; Supplementary Table 4). The genes closest to these SNPs were selected for gene-based analysis (Table 2). Analysis of common variants in seven genes (plus 25 kb flanking regions) in the SAGA GWASMA showed significant association with ADHD symptoms. Three significant
Fig. 2 Manhattan & QQ plot of the ADHD Symptom Total Score meta-analysis from the complete SAGA consortium (Panel a), without patients (Panel b) and meta-analysis from the SAGA & EAGLE consortia (Panel c). a Manhattan & QQ plot of the ADHD symptom total score meta-analysis from the complete SAGA consortium. Association $p$-values > 0.05 in the GWAS plot are not shown. All $p$-values are shown in the QQ-plot. b Manhattan & QQ plot of the ADHD Symptom Total Score meta-analysis from the SAGA consortium without patient cohorts. Association $p$-values > 0.05 in the GWAS plot are not shown. All $p$-values are shown in the QQ-plot. c Manhattan & QQ plot of the ADHD Symptom Total Score meta-analysis from the SAGA & EAGLE consortia

Table 2 Most strongly associated (clumped) SNPs ($p$-value < $1 \times 10^{-6}$) coming from the meta-analysis of nine cohorts from the SAGA consortium in physical position order (hg19)

<table>
<thead>
<tr>
<th>SNP name</th>
<th>Chr</th>
<th>Locus</th>
<th>Pos*</th>
<th>$p$-Value</th>
<th>Tested/Non-tested Allele</th>
<th>Frequency tested Allele</th>
<th>Gene(s) in locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12661753</td>
<td>6</td>
<td>6q24.3</td>
<td>147409235</td>
<td>3.02 × 10^{-7}</td>
<td>A/G</td>
<td>0.962</td>
<td>STXBPS-AS1</td>
</tr>
<tr>
<td>rs1564034</td>
<td>2</td>
<td>2p25.2</td>
<td>6510305</td>
<td>2.15 × 10^{-6}</td>
<td>T/G</td>
<td>0.670</td>
<td>LINC01247</td>
</tr>
<tr>
<td>rs1930272</td>
<td>1</td>
<td>1p31.1</td>
<td>83491910</td>
<td>4.75 × 10^{-6}</td>
<td>T/C</td>
<td>0.544</td>
<td>LOC107985037</td>
</tr>
<tr>
<td>rs28734069</td>
<td>4</td>
<td>4q26</td>
<td>120042409</td>
<td>5.77 × 10^{-6}</td>
<td>T/C</td>
<td>0.516</td>
<td>LOC102723967; LOC105377395</td>
</tr>
<tr>
<td>rs13274695</td>
<td>8</td>
<td>8p23.2</td>
<td>3723378</td>
<td>6.00 × 10^{-6}</td>
<td>A/G</td>
<td>0.077</td>
<td>CSMD1; LOC105377790</td>
</tr>
<tr>
<td>rs73204517</td>
<td>13</td>
<td>13q21.33</td>
<td>69920315</td>
<td>7.19 × 10^{-6}</td>
<td>C/G</td>
<td>0.126</td>
<td>Downstream LINC00383</td>
</tr>
<tr>
<td>rs11209188</td>
<td>1</td>
<td>1p31.3</td>
<td>68455306</td>
<td>7.88 × 10^{-6}</td>
<td>A/G</td>
<td>0.534</td>
<td>GNG12-AS1</td>
</tr>
<tr>
<td>rs2189255</td>
<td>8</td>
<td>8q21.3</td>
<td>91190297</td>
<td>9.61 × 10^{-6}</td>
<td>T/C</td>
<td>0.703</td>
<td>CALB1; LINC00534</td>
</tr>
</tbody>
</table>

SNPs organized on association $p$-values. *bp position based on the GRCh37.p13 build; Allele frequency of tested allele based on n = 14,689
findings \((p < 0.007)\) were for the \(STXBP5-AS1\), \(LINC01247\) and the \(LINC00534\) genes. Nominal significant associations \((p < 0.05\) gene-based) were seen for, \(CALB1\), \(GNG12-AS1\), \(STXBP5\) (Supplementary Table 5). It is important to note that \(STXBP5\) and \(STXBP5-AS1\) have no physical separation, thus their 25 kb flanking regions overlap \(STXBP5\) is located on the forward \([-+\)] and \(STXBP5-AS1\) on the reverse \([-\text{−}+]\) strand—see Supplementary Figs. 3E, F). The rare variant analysis also showed nominal association for \(STXBP5\). For four genes \((GNG12-AS1, LINC01247, STXBP5-AS1, LINC00534)\), rare variants were not observed/detected (Supplementary Table 5).

Functional analysis

Expression of the antisense lncRNA variant \(STXBP5-AS1-003\) (containing the overlap with \(STXBP5\)) caused an increase in the fluorescence ratio between \(STXBP5\)-EGFP and the control (Fig. 1b–e).

Mouse models

We confirmed the recombinant inbred (RI) strain difference in motor impulsivity between the BXD68, BXD29, and C57BL/6J strains (Kruskal–Wallis, \(p = 0.006\), measured as premature responses in the 5-choice serial reaction time task (Loos et al. 2014). In addition, these RI strains showed differences in the attention parameter of errors of omission \((F_{2,19} = 4.98, p = 0.018)\), but not the attention parameter of percentage correct responses \((F_{2,19} = 0.733)\) (Fig. 3a). In these mice, we detected expression of \(Gm28905\) / \(Stxbp5-AS1\) lncRNA in the prefrontal cortex by real-time quantitative PCR, which differed across strains \((F_{2,19} = 11.53; p = 0.001)\). This transcript showed lowest expression in the most highly impulsive strain, BXD68 \((BXD68: 4.58 \pm 0.11, C57BL/6J: 5.25 \pm 0.14, BXD29: 5.19 \pm 0.07)\), Bonferroni-corrected post-hoc \(P_{BXD68 vs C57BL/6J} = 0.001\); \(P_{BXD68 vs BXD29} = 0.002\) (Fig. 3b). Expression of \(Stxbp5\) mRNA was not different between the three strains \((BXD68: 9.89 \pm 0.09; C57BL/6J: 9.83 \pm 0.04; BXD29: 9.99 \pm 0.08; F_{2,19} = 1.23; p = 0.314)\). These results suggest that \(Gm28905\) / \(Stxbp5-AS1\) affects impulsivity independent of the \(Stxbp5\) transcript. Examining correlations between \(Gm28905\) / \(Stxbp5-AS1\) transcript level and impulsivity/(in)attention measures, we found a significant correlation with motor impulsivity (Spearman’s \(r = −0.61\); \(p = 0.004\); BCa −0.15 to −0.85) that withstood Bonferroni correction (threshold \(p\)-value <0.008). A nominally significant association was found for expression of \(Gm28905\)/\(Stxbp5-AS1\) with attention, when measured as errors of omission (Guillem et al. 2011) (Spearman’s \(r = 0.51\); \(p = 0.019\); BCa 0.01–0.86), but not when measured as percentage correct responses (Pearson \(r = 0.31\); \(p = 0.178\); BCa −0.08–0.61). Expression of \(Stxbp5\) did not correlate with any of these parameters (Fig. 3c).

Discussion

We report a genome-wide association meta-analysis with three different but correlated adult ADHD symptom lists in nine European adult population-based and case-only cohorts \((n = 14,689\) individuals). The \(STXBP5-AS1\) locus (best SNP \(p = 3.02 \times 10^{-7}\)) was the most strongly associated in this meta-analysis. This association was replicated in the EAGLE meta-analysis \((P_{EAGLE} = 2.89 \times 10^{-2}\)), and the top-hit from the full SAGA-EAGLE GWASMA was also located in the \(STXBP5-AS1\) gene and in almost perfect LD with the original finding (SNP rs12664716, \(P_{SAGA-EAGLE} = 2.05 \times 10^{-7}\); \(n = 29,465\)).

For the adult ADHD-index, an earlier large twin family study estimated total \(h^2\) at 30%. For clinically assessed adult ADHD as well as for total sum scores for Attention Problems the \(h^2\) estimates were even higher, as summarized in the introduction. Common SNPs thus are hypothesized to contain substantial information concerning the genetic variance underlying adult ADHD and Attention Problems. The SNP-based heritability analyses, which were ran prior to GWASMA, provided estimates of 15–30% explained variance of adult ADHD symptom scores in the general population. Such estimates are comparable with the estimates obtained for ADHD and four additional categorically defined psychiatric disorders (Psychiatric Genomics Consortium et al. 2013), providing rationale for a gene-finding enterprise for adult ADHD symptoms in the general population. The significant SNP-based heritability and the considerable phenotypic and genetic correlations between assessment instruments support the validity of our meta-analysis approach of GWA results obtained across contributing data sets.

The function of the \(STXBP5-AS1\)-encoded lncRNA is currently unknown. \(STXBP5-AS1\) has been proposed as a prognostic biomarker for survival of cancer patients (Guo et al. 2016), but no information is available for its role in ADHD, related traits, or other psychiatric diseases. In humans it overlaps in anti-sense with \(STXBP5\), encoding a protein involved in synaptic function by regulating neurotransmitter release through stimulating SNARE complex formation (Sakisaka et al. 2008; Yizhar et al. 2004). This complex plays a major role in intracellular vesicular trafficking in eukaryotic cells and is involved in the exocytic release of neurotransmitters during synaptic transmission (Antonacci et al. 2016). Genes related to the SNARE complex and its regulators have been investigated in ADHD (Bonvicini et al. 2016), and current results suggest that this complex may exert distinct roles throughout development, with age-specific effects of its genetic variants on ADHD
behavior (Cupertino et al. 2016). Specifically, deletions and mutations of \textit{STXBP5} occur in autism (Davis et al. 2009) and epilepsy (Dhillon et al. 2011). \textit{STXBP5} has a presynaptic role that negatively regulates neurotransmitter release by forming syntaxin-SNAP25-tomosyn complex (Sakisaka et al. 2004). However, the postsynaptic role of \textit{STXBP5} has not been elucidated well.

\textit{Post-hoc} analysis suggested that \textit{STXBP5-AS1} could significantly affect both hyperactivity-impulsivity and inattention. We were able to determine that both hyperactivity/
impulsivity and inattention symptoms were significantly associated with rs12661753 ($p = 1.51 \times 10^{-5}, p = 3.53 \times 10^{-2}$, respectively). Even though the effect size is larger for hyperactivity, we cannot claim that this is a significant difference (Supplemental Fig. 1). Our experiments in HEK293 cells showed that the lncRNA does not cause antisense inhibition of STXBP5, but may enhance STXBP5 expression. The role of STXBP5-AS1 with impulsivity was corroborated in behavioral studies in mice. The unchanged Stxbp5 mRNA levels in mouse strains expressing different Gm28905 levels is consistent with the current annotation that lack antisense overlap, and suggests that the lncRNA might contribute to impulsivity by a Stxbp5-independent mechanism. In line with this idea, Gm28905 expression (but not that of Stxbp5) correlated negatively with motor impulsivity in mice.

Our study should be viewed in the light of some strengths and limitations. A clear strength was the functional analyses that provided a likely candidate associated with adult and childhood ADHD symptoms. A limitation of our study was the limited sample size in the SAGA meta-analysis in combination with the use of different phenotyping instruments in the cohorts. Our results did not replicate the findings from the latest GWASMA on ADHD cases and controls in children (Demontis et al. 2018). Nevertheless, we were able to detect a strong association signal that (i) was also found associated in the EAGLE GWASMA (albeit less strong but in a pediatric population-based data) and (ii) shows a robust functional effect in two independent functional studies corrected for multiple testing, as indicated on page five of the Supplementary Methods, ($p$-value of $5 \times 10^{-2}/6 = 0.0083$; correlations of two transcripts were tested for three parameters).

We did see some heterogeneity in the meta analysis result for SNP rs12661753 (Supplementary Table 3). This is partly due to the combination of the between-study heterogeneity of the measures, even for the higher correlated phenotypes,
the inclusion of small studies that can skew the true variances of the investigated traits and a (somewhat) low(er) frequency of the tested allele of the best associated SNP (mean MAF = 0.038; range MAF = 0.013–0.049; for details on each cohort see Supplementary Table 9). The aim of our study was to identify ADHD related genes in adults by using the quantitative ADHD symptom scores provided by both population-based and case-control samples. This means that some compromise had to be achieved when combining this information. We took care of reducing the noise by (i) performing individual GWAS (per cohort, per trait) and then meta-analizing the results (which maximizes information per study including the smaller ones) and (ii) profiting from the strong phenotypic and genetic correlations between the instruments, which might have helped us maintain a better power to detect true association signals. Nevertheless, while our sample is not small (~ 15 k participants), in order to detect genome wide significant signals, sample sizes need to increase.

The genetic correlation of PCG + iPSYCH with SAGA should be interpreted carefully because the standard error was high. The fact that the PCG + iPSYCH/SAGA \( r_g \) (0.54; SE = 0.447) did not differ from the published \( r_g \) estimate between the PCG + iPSYCH GWASMA and a GWAS of the 23 andMe sample (0.65, SE = 0.114) (Demontis et al. 2018) is encouraging. These correlations would confirm the genetic stability of ADHD in childhood and adulthood, as was also suggested from longitudinal modeling of twin data (Kan et al. 2013). The estimated genetic correlation between the 23 andMe and PGC + iPSYCH analyses was significant but lower than the genetic correlation of the EAGLE and PCG + iPSYCH childhood cohorts (\( r_g = 0.943, \ SE = 0.204, p = 3.65 \times 10^{-6} \) (Demontis et al. 2018). The ADHD diagnosis (yes/no) in 23 andMe is based on the self-reported answer

![Fig. 3 (continued)](image)
to a single question about presence of a lifetime diagnosis of ADHD (Demontis et al. 2018) and we do not know if the 23 andMe participants were diagnosed in childhood or as adults. Also, the modest and non-significant genetic correlation between the SAGA and the EAGLE samples should be interpreted cautiously. For both samples, the SNP-based heritability as estimated by LDSC was low and both samples may suffer from their phenotypic heterogeneity as well as limited sample size. A further increase in GWAS sample size may suffer from their phenotypic heterogeneity as well as heritability as estimated by LDSC was low and both samples be interpreted cautiously. For both samples, the SNP-based

Our study shows that self-reported adult ADHD symptoms measured in the general population have a genetic component and that performing population-based GWAS of adult ADHD symptoms provides novel insights into the genetic underpinnings of hyperactivity/impulsivity symptoms that are a hallmark of ADHD. We were able to carry out functional follow-up studies which considerably strengthened our findings for a possible role of STXBP5-AS1 and its mouse ortholog Gm28905 in ADHD symptom etiology.

Acknowledgements This work was sponsored by the Stichting Nationale Computerfaciliteiten (National Computing Facilities Foundation, NCF) for the use of supercomputer facilities, with financial support from the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (Netherlands Organization for Scientific Research, NWO) This work made use of the BIG (Brain Imaging Genetics) database, first established in Nijmegen, The Netherlands, in 2007. This resource is now part of Cognomics (http://www.cognomics.nl), a joint initiative by researchers of the Donders Centre for Cognitive Neuroimaging, the Human Genetics and Cognitive Neuroscience departments of the Radboud University Medical Center and the Max Planck Institute for Psycholinguistics in Nijmegen. The Cognomics Initiative is supported by the participating departments and centres and by external grants, i.e. the Biobanking and Biomolecular Resources Research Infrastructure (Netherlands) (BBMRI-NL), the Hersenstichting Nederland, and the Netherlands Organisation for Scientific Research (NWO). We wish to thank all persons who kindly participated in the BIG research. The research leading to these results also receives funding from the European Community’s Seventh Framework Programme (Grant No. FP7/2007–2013) under grant agreements n° 602805 (Aggressotyope), n° 278948 (TACTICS), and n° 602450 (IMAGEMEND), and from the European Community’s Horizon 2020 Programme (H2020/2014–2020) under grant agreement n° 643051 (MiND) and n° 667302 (CoCA). For the Netherlands Study of Depression and Anxiety (NEDSA), funding was obtained from the Netherlands Organization for Scientific Research (Geestkracht program grant 10-000-1002); the Center for Medical Systems Biology (CSMB, NWO Genomics), Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL), VU University’s Institutes for Health and Care Research (EMGO+) and Neuroscience Campus Amsterdam, University Medical Center Groningen, Leiden University Medical Center, National Institutes of Health (NIH, RO1D0042157-01A, MH081802, Grand Opportunity grants 1RC2 MH089951 and 1RC2 MH089995). Part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health. Computing was supported by Big Grid, the Dutch e-Science Grid, which is financially supported by NWO. The NeuroIMAGE study was supported by grants from The Netherlands Organization for Health Research and Development (ZonMW 60-60600-97-193), the Netherlands Organization for Scientific Research (NWO, grants 1750102007010, 433-09-242 and 056-13-015), and by the European Community’s Seventh Framework Programme (FP7/2007–2013) under grant agreement number 278948 (TACTICS), 602450 (IMAGEMEND), 602805 (AGRESSOTYPE), 603016 (MATRICS), and Horizon 2020 (grant agreements 643051 (MiND) and 642996 (BRAINVIEW)) research programs. The Nijmegen Biomedical Study (NBS) is a population-based survey conducted at the Department for Health Evidence, and the Department of Laboratory Medicine of the Radboud University Medical Center. Principal investigators of the Nijmegen Biomedical Study are L.A.L.M. Kiemeney, A.L.M. Verbeek, D.W. Swinkels and B. Franke. NTR Research was funded by the Netherlands Organization for Scientific Research (NWO, grants 91210020, Addiction-31160008, Middelgroot-911-09-032, Spino-zaemrijke 56-464-14192 and NWO 480-15-001/674), Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL, 184.021.007), and the European Research Council (230374 and 284167). We thank the Avera Institute of Human Genetics for genotyping and technical support. DIB acknowledges the Royal Netherlands...
November 2013-January 2015, 2016-2017, Academy of Science Professor Award (PAH/6635) We thank all participants in the Netherlands Twin Register. The TRacking Adolescents’ Individual Lives Survey (TRAILS) is a collaborative project involving various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen, and the Parnassia Bavo group, all in the Netherlands. TRAILS has been financially supported by grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMW Risk Behavior and Dependence grant 60-60600-97-118; ZonMW Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 452-04-314 and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005; NWO Longitudinal Survey and Panel Funding 481-08-013); the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), Biobanking and Biomolecular Resources Research Infrastructure BBMRI-NL (CP 32), the participating universities, and Accare Center for Child and Adolescent Psychiatry. We are grateful to all adolescents, their parents and teachers who participated in this research and to everyone who worked on this project and made it possible. Statistical analyses were carried out on the Genetic Cluster Computer (http://www.geneticcluster.org), which is financially supported by the Netherlands Scientific Organization (Grant No. NWO 480-05-003) along with a supplement from the Dutch Brain Foundation. The Vall d’Hebron Research Institute (VHIR) received financial support from “Fundació La Marató de TV3” (ref. 092330/31), “Instituto de Salud Carlos III-FIS” (Grant Nos. PI11/00571, PI11/011269, PI12/01139, PI14/01700), “Agència de Gestió d’Ajuts Universitaris i de Recerca AGAUR, Generalitat de Catalunya” (Grant No. 2014SGR1357) and “Departamento de Salut”, Government of Catalonia, Spain. We wish to thank all persons who kindly participated in this research. Part of the DNA extractions and genotyping was performed at the Spanish National Genotyping Centre (CEGEN-Barcelona). Harmen H.M. Draisma is supported by an EMGO+ Fellowship as part of the Mental Health research program of the EMGO Institute for Health and Care Research. Sabine Spijker was partially funded by the Center for Medical Systems Biology (CSMB). Sander Gofton was financially supported by grant 91113022 from the Netherlands Organization for Health Research and Development (ZonMW). Marta Ribasés is a recipient of a Miguel de Servet contract from the “Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación”, Spain. Barbara Franke’s research is supported by grants from the Netherlands Organisation for Scientific Research (NWO): grants 433-09-229 and 016-130-669, from the European Union 7th Framework (grant agreements 278948 (TACTICS), 602450 (IMAGEMEND), and 602805 (Aggressotype)) and Horizon 2020 (grant agreements 643051 (MIND) and 667302 (CoCA)) research programmes. A.B. Smit was partially funded by the Dutch Neuro-Bisq Mouse Pharma Datasets (grant BSIK 03053 from SENTENNovem). This work was partly carried on the Dutch national e-infrastructure with the support of SURF Foundation.

**Compliance with ethical standards**

**Conflict of interest** J.K.B. has been in the past 3 years a consultant to/member of advisory board of/and/or speaker for Janssen Cilag BV, Eli Lilly, Shire, Lundbeck, Roche and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties. J.J.S.K. has been a speaker for Eli Lilly, Janssen and Shire until 2012, and received unrestricted research grants in 2010 from Janssen and Shire. J.A.R.Q. was on the speakers’ bureau and/or acted as consultant for Eli-Lilly, Janssen-Cilag, Novartis, Shire, Lundbeck, Ferrer and Rubió in the last 3 years. He also received travel awards (air tickets + hotel) for taking part in psychiatric meetings from Janssen-Cilag, Rubió, Shire and Eli-Lilly. The ADHD Program chaired by him received unrestricted educational and research support from the following pharmaceutical companies in the last 3 years: Eli-Lilly, Janssen-Cilag, Shire, Rovi and Rubió. B.F. received educational speaking fees from Shire and Medice. A.A.V., A.J.G., H.H.M.D., M.K., D.V., S.S., T.E.G., J.J.H., P.J.dM., V.M. K., R.P., I.M.N., B.W.H.P., I.O.F. A.dB., C.M.vD., P.J.H., L.A.K., M.H., M.K., C.M.M., K.G.O., S.H.V., C.S.M., M.R., C.A.H., N.A., A.B.S., D.I.B. report no conflict of interest.

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