

Published in final edited form as:

J Am Acad Child Adolesc Psychiatry. 2016 October ; 55(10): 896–905.e6. doi:10.1016/j.jaac.2016.05.025.

Correspondence to Christel M. Middeldorp, MD, PhD, VU University Amsterdam, Biological psychology, Van der Boechorststraat 1, 1081BT, Amsterdam; c.m.middeldorp@vu.nl.

EARly Genetics & Lifecourse Epidemiology (EAGLE) Consortium (<http://www.wikigenes.org/art/e/348.html>): EAGLE Working Groups and Leaders: Antenatal Growth (Vincent Jaddoe, MD, PhD and Craig Pennell, MBBS, PhD); Asthma, Allergy, and Atopy (Hans Bisgaard, MD, Klaus Bønnelykke, MD, PhD, and Joachim Heinrich, PhD); Behaviour and Cognition (Christel Middeldorp, MD, PhD, Camilla Stoltenberg, MD, PhD, and Henning Tiemeier, MD, PhD); Birth Biometry (Inga Prokopenko, PhD, MSc and Mark McCarthy, MD); Bone Health (Fernando Rivadeneira, MD, PhD and Nic Timpson, PhD); Cardiovascular Risk Factors (Lyle Palmer, PhD and Vincent W.V. Jaddoe, MD, PhD); Insulin and Metabolic Syndrome (Inga Prokopenko, PhD, MSc, Mark McCarthy, MD, and Tim Frayling, PhD); Postnatal Growth (Mark McCarthy, MD, Tim Frayling, PhD, and Marjo-Riitta Jarvelin, MD, PhD); Puberty (Elisabeth Widen, MD, PhD and Marjo-Riitta Jarvelin, MD, PhD).

Principal Investigator or Primary Contacts: Vincent W.V. Jaddoe, MD, PhD, Craig E. Pennell, MBBS, PhD, Mark McCarthy, MD, Hans Bisgaard, MD, Joachim Heinrich, PhD, Christel Middeldorp, MD, PhD, Camilla Stoltenberg, MD, PhD, Henning Tiemeier, MD, PhD, Inga Prokopenko, PhD, MSc, Nicholas J. Timpson, PhD, Tim Frayling, PhD, Elisabeth Widen, MD, PhD, Marjo-Riitta Jarvelin, MD, PhD, Struan Grant, PhD, Cock van Duijn, PhD, and Dorret I. Boomsma, PhD.

Psychiatric Genomics Consortium ADHD Working Group Members: Richard J.L. Anney, PhD, Alejandro Arias Vasquez, PhD, Philip Asherson, MD, Tobias J. Banaschewski, MD, PhD, Mònica Bayés, PhD, Joseph Biederman, MD, Jan K. Buitelaar, MD, PhD, Miguel Casas, MD, PhD, Alice Charach, MD, MSc, Bru Cormand, PhD, Jennifer Crosbie, PhD, Mark J. Daly, PhD, Alysa E. Doyle, PhD, Richard P. Ebstein, PhD, Josephine Elia, MD, Stephen V. Faraone, PhD, Barbara Franke, PhD, Christine Freitag, MD, MA, Michael Gill, MbBCh BAO, MD, MRCPsych, FTCD, Hakon Hakonarson, MD, PhD, Peter Holmans, PhD, Lindsey Kent, MD, Jonna Kuntsi, PhD, Nanda Lambregts-Rommelse, PhD, Kate Langley, PhD, Klaus-Peter Lesch, MD, Sandra K. Loo, PhD, James J. McGough, MD, Sarah E. Medland, PhD, Jobst Meyer, PhD, Eric Mick, ScD, Ana Miranda, MD, Fernando Mulas, MD, PhD, Benjamin M. Neale, PhD, Stan F. Nelson, MD, Michael C. O'Donovan, FRCPsych, PhD, Robert D. Oades, PhD, Michael J. Owen, PhD, Haukur Palmason, PhD, Josep Antoni Ramos-Quiroga, MD, PhD, Andreas Reif, MD, Tobias J. Renner, MD, Marta Ribasés, PhD, Stephan Ripke, MD, Olga Rivero, PhD, Herbert Roeyers, MD, PhD, Jasmin Romanos, MD, Marcel Romanos, MD, Aribert Rothenberger, MD, Cristina Sánchez-Mora, PhD, Russell Schachar, MD, Joseph Sergeant, PhD, Susan L. Smalley, PhD, Edmund J. S. Sonuga-Barke, PhD, Hans-Christoph Steinhausen, MD, PhD, DMSc, Anita Thapar, MBBS, FRCPsych, PhD, FmedSci, Alexandre Todorov, PhD, Susanne Walitza, MD, Yufeng Wang, MD, PhD, Andreas Warnke, MD, PhD, Nigel Williams, PhD, Yanli Zhang-James, PhD.

ALSPAC: The authors are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. GINI/LISA: GINIplus Study: The study team wishes to acknowledge the following: Helmholtz Zentrum Muenchen - German Research Center for Environmental Health, Institute of Epidemiology I, Munich (Heinrich J, Wichmann HE, Sausenthaler S, Chen C-M, Thiering E, Tiesler C, Standl M, Schnappinger M, Rzehak P); Department of Pediatrics, Marien-Hospital, Wesel (Berdel D, von Berg A, Beckmann C, Groß I); Department of Pediatrics, Ludwig Maximilians University, Munich (Koletzko S, Reinhardt D, KraussEtschmann S); Department of Pediatrics, Technical University, Munich (Bauer CP, Brockow I, Grübl A, Hoffmann U); IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (Krämer U, Link E, Cramer C); Centre for Allergy and Environment, Technical University, Munich (Behrendt H). LISAPlus Study: The study team wishes to acknowledge the following: Helmholtz Zentrum Muenchen - German Research Center for Environment and Health, Institute of Epidemiology I, Neuherberg (Heinrich J, Wichmann HE, Sausenthaler S, Chen C-M); University of Leipzig, Department of Pediatrics (Borte M), Department of Environmental Medicine and Hygiene (Herbarth O); Department of Pediatrics, MarienHospital, Wesel (von Berg A); Bad Honnef (Schaaf B); UFZ-Centre for Environmental Research LeipzigHalle, Department of Environmental Immunology (Lehmann I); IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (Krämer U); Department of Pediatrics, Technical University, Munich (Bauer CP, Hoffmann U). INMA: The authors are grateful to Silvia Fochs, Anna Sánchez, Maribel López, Nuria Pey, Muriel Ferrer, Amparo Quiles, Sandra Pérez, Gemma León, Elena Romero, Maria Andreu, Nati Galiana, Maria Dolores Climent, Amparo Cases, and Cristina Capo for their assistance in contacting the families and administering the questionnaires. The authors would particularly like to thank all the participants for their generous collaboration. A full roster of the INMA Project Investigators can be found at http://www.proyectoINMA.org/presentacion-inma/listado-investigadores/en_listadoinvestigadores.html. MoBa (Mother and Child Cohort of NIPH): The authors are grateful to all the participating families in Norway who take part in this ongoing cohort study. Raine: The authors are grateful to the Raine Foundation, the Raine Study Families, and the Raine Study research staff. TEDS: The authors gratefully acknowledge the ongoing contribution of the participants in the Twins Early Development Study (TEDS) and their families. TRAILS: The authors are grateful to everyone who participated in this research or worked on this project to make it possible.

Disclosure: Dr. Hudziak has received grant or research support from the National Institutes of Health, the National Institute of Mental Health, the National Institute of Diabetes and Digestive and Kidney Disease, and the state of Vermont. His primary appointment is with the University of Vermont. He has additional appointments with Erasmus University in Rotterdam, Netherlands, Washington University School of Medicine in St. Louis, Missouri, and the Geisel School of Medicine at Dartmouth in Hanover, New Hampshire. Drs. Middeldorp, Groen-Blokhuis, St Pourcain, Greven, Pappa, Tiesler, Nolte, Ebejer, Zhao, Davies, Ehli, Evans, Guxens, Hottenga, Jugessur, Kemp, Martin, Myhre, Ormel, Ring, Standl, Stergiakouli, Stoltenberg, Thiering, Timpson, Trzaskowski, van der Most, Nyholt, Medland, Neale, Jacobsson, Sunyer, Hartman, Whitehouse, Pennell, Heinrich, Plomin, Smith, Tiemeier, Posthuma, Boomsma, Ms. Hammerslag, Mr. Ouwens, Mr. Ang, Ms. Vilor-Tejedor, Mr. Bacelis, Ms. Fedko, Ms. Krapohl, Mr. Murcia, and Ms. Wang report no biomedical financial interests or potential conflicts of interest.

A Genome-Wide Association Meta-Analysis of Attention-Deficit/Hyperactivity Disorder Symptoms in Population-Based Paediatric Cohorts

A full list of authors and affiliations appears at the end of the article.

These authors contributed equally to this work.

Abstract

Objective—To elucidate the influence of common genetic variants on childhood attention-deficit/hyperactivity disorder (ADHD) symptoms, to identify genetic variants that explain its high heritability, and to investigate the genetic overlap of ADHD symptom scores with ADHD diagnosis.

Method—Within the EARly Genetics and Lifecourse Epidemiology (EAGLE) consortium, genome-wide single nucleotide polymorphisms (SNPs) and ADHD symptom scores were available for 17,666 children (< 13 years) from nine population-based cohorts. SNP-based heritability was estimated in data from the three largest cohorts. Meta-analysis based on genome-wide association (GWA) analyses with SNPs was followed by gene-based association tests, and the overlap in results with a meta-analysis in the Psychiatric Genomics Consortium (PGC) case-control ADHD study was investigated.

Results—SNP-based heritability ranged from 5% to 34%, indicating that variation in common genetic variants influences ADHD symptom scores. The meta-analysis did not detect genome-wide significant SNPs, but three genes, lying close to each other with SNPs in high linkage disequilibrium (LD), showed a gene-wide significant association (p values between 1.46×10^{-6} and 2.66×10^{-6}). One gene, *WASL*, is involved in neuronal development. Both SNP- and gene-based analyses indicated overlap with the PGC meta-analysis results with the genetic correlation estimated at 0.96.

Conclusion—The SNP-based heritability for ADHD symptom scores indicates a polygenic architecture and genes involved in neurite outgrowth are possibly involved. Continuous and dichotomous measures of ADHD appear to assess a genetically common phenotype. A next step is to combine data from population-based and case-control cohorts in genetic association studies to increase sample size and improve statistical power for identifying genetic variants.

Keywords

GWA; SNP heritability; attention problems; ADHD symptoms; meta-analysis

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common psychiatric condition in childhood with a prevalence of around five percent across countries worldwide.¹ As an objective diagnostic test is lacking, diagnoses are based on the occurrence of age-

inappropriate impulsive, hyperactive, and inattentive behaviors that occur in multiple settings and cause significant impairment.^{2,3} It is well established that genetic factors explain a large part of the individual differences in the vulnerability for ADHD. The heritability of childhood ADHD and related traits, such as continuous measures of attention problems and hyperactivity, has been estimated at around 75% (see e.g. Kan et al. 2013⁴ and Lichtenstein et al. 2010⁴). Consequently, several studies now aim to identify genetic variants for ADHD. Ten candidate genes show replicated evidence for association according to a recent review.⁵ In genome-wide association (GWA) studies, the test of the effects of single genetic variants has not yet yielded genome-wide significant hits, but a gene-enrichment analysis, including single nucleotide polymorphisms (SNPs) showing genome-wide suggestive signals, pointed to several biological pathways involved in neural processes, such as neurodevelopment.⁵ Additional evidence for the role of common SNPs (SNPs with a frequency above 5%) in ADHD comes from polygenic analyses in which the joint effect of a large number of SNPs or all SNPs is estimated. All studies but one found that the SNPs explained a significant proportion of the variance,^{6–13} suggesting that associated genetic variants are likely to be detected in larger GWA meta-analyses. This is confirmed by the results from the latest meta-analysis of the Psychiatric Genomics Consortium (PGC) ADHD subgroup that yielded several genome-wide significant hits (D Demontis for the PGC ADHD subgroup: presentation 23rd World Congress of Psychiatric Genetics, October 2015, Toronto, CA).

Another recommendation for future gene-finding studies has been to incorporate dimensional approaches of ADHD, such as continuous measures of ADHD symptoms.⁵ This is supported by polygenic risk score analyses showing that individuals' polygenic risk scores based on the effects of SNPs in a GWA analysis in ADHD case-control studies significantly predicted continuous ADHD symptom scores, and vice versa.^{13–15} Other studies have suggested that a diagnosis of ADHD can be regarded as the extreme end of a continuous distribution of inattentive and hyperactive behaviors,^{16–18} and twin studies also showed a substantial overlap between the genetic factors for a clinical diagnosis of ADHD and continuous measures of ADHD symptoms in the general population.^{17,18}

Many population-based paediatric cohorts have collected genome-wide SNP data and continuous ADHD symptom scores, providing an underused opportunity for gene-finding studies for ADHD. Case-control studies benefit from oversampling the high-scoring end of the distribution, but analyzing the full information on symptom severity in the population can also be a powerful approach, especially for a relatively common disorder as ADHD.^{13,19} The current report describes the first GWA meta-analysis of continuous measures of ADHD in 17,666 children from nine population-based cohorts. To investigate the polygenic nature of the phenotype, we estimated the variance in attention problems that was explained by all SNPs. Next, SNP and gene-based association analyses were performed. We specifically looked at whether the ten candidate genes identified in the recent review showed evidence for association.⁵ Finally, to examine the overlap in genetic influences on ADHD diagnosis and ADHD symptom scores, we investigated the concordance in the effects of SNPs and genes as found in the current meta-analysis with the results of a meta-analysis based on case-control ADHD GWA studies, and estimated the genetic correlation.

Method

Cohorts

The EARly Genetics and Life course Epidemiology (EAGLE) consortium is a collaboration among several population-based birth cohorts from Europe, Australia, and the United States (<http://www.wikigenes.org/e/art/e/348.html>). The consortium focuses on a wide range of phenotypes in childhood.^{20,21} EAGLE cohorts with ADHD symptom scores in childhood (age at measurement <13 years) were invited to participate in the meta-analysis. An overview of the nine cohorts included in the meta-analysis is provided in Table 1. Information on the individual cohorts can be found on their websites and in the publications listed in Table 1.

Phenotype

Different instruments were used across cohorts (Table 1), including the Attention Problems scale of the Child Behavior Checklist (CBCL) and the Teacher Report Form (TRF), the Hyperactivity scale of the Strengths and Difficulties Questionnaire (SDQ), and the *DSM-IV* ADHD items as, for example, included in the Conners' Rating Scale (see Table S1, available online, for the items included in each scale).^{3,22–25} For the meta-analysis, one phenotype was selected from each cohort. Based on the phenotype that was most available, school-age ratings were chosen over preschool-age ratings, parent ratings over teacher ratings, and the measurement instrument with the largest information density was preferred over the other instruments (Conners' *DSM-IV* > CBCL > SDQ).

SNP-Based Heritability

The variance in ADHD symptom scores accounted for by the SNPs was estimated using Genomic-Relationship-Matrix Restricted Maximum Likelihood (GREML) as implemented in the Genomic Complex Trait Analysis (GCTA) software.^{26,27} GREML is a linear mixed model that includes a genetic relatedness matrix (GRM) that contains a measure of genetic similarity between all possible pairs of (unrelated) individuals in a study. Genetic similarity is based on resemblance in SNP variants, hence the variance explained by the genetic relatedness matrix is often called the SNP heritability. Typically, only unrelated individuals (genetic relatedness < 0.025) are included in the construction of a GRM to prevent the estimate of the SNP heritability to be biased upwards.

GRM-based analyses were performed for the hyperactivity scale of the SDQ as measured in the Avon Longitudinal Study of Parents and Children (ALSPAC) at preschool (N=5,510) and at school-age (N=5,303), and for the Attention Problems scale of the CBCL 1.5-5 (N = 2,958) and the TRF (N=1,901) measured in the Netherlands Twin Register (NTR) and Generation R cohorts. These analyses were not performed in the other cohorts because of the smaller sample sizes.

In ALSPAC, the GRM was constructed based on observed genotypes. Sex, age at measurement, and two principal components were included as fixed effects in the model. In NTR, the CBCL 1.5-5 was assessed when the children were three years and in Generation R when they were six years of age. The NTR and Generation R samples were combined to

estimate the GRM. Individual-level genotype data from the NTR and Generation R were imputed together based on the Genome of the Netherlands reference set.^{28,29} Sex, age at measurement, sample, and five principal components were included as fixed effects in the model.

Data Quality Control and SNP and Gene-Based Association Meta-Analyses

Cohorts performed quality control (QC) and imputed their SNP genotype data using the March 2012 release of the 1000 Genomes reference set that includes all ethnicities.³⁰ Each cohort ran their own optimal pre-imputation genotype QC. An overview of the pre-imputation QC metrics and imputation methods applied in each cohort is provided in Table S2, available online. Briefly, filtering on sample and SNP call rate was similar between cohorts, with the exception of the Twins Early Development Study (TEDS), which had a lower threshold. The thresholds for Hardy-Weinberg Equilibrium, heterozygosity filtering, and other QC steps varied somewhat more. This likely has slightly decreased the final imputation quality of each cohort, as prior SNP filtering does not improve the imputation quality, since losing genotyped SNPs only makes the imputation worse.³¹ Imputation with the Mach software has been shown to perform slightly better,³¹ but differences tend to be small. All filtering decisions will eventually result in fewer SNPs in the meta-analysis, bringing the risk of a loss of potential signal but not leading to false-positive results.

A linear regression of the phenotype on sex, age at measurement, genotype dose, and principal components was performed in all cohorts. All cohorts analyzed data from unrelated individuals, except for the Netherlands Twin Register (NTR), which included both twins from a dizygotic twin pair and corrected standard errors in PLINK using the --family option (<http://pngu.mgh.harvard.edu/purcell/plink/>).³² Table S2, available online, lists the analytic tools applied by each cohort.

Results were checked and meta-analyzed by two independent analysts. QC included calculation of the inflation factor lambda (the ratio of the observed versus the expected median chi-square), format checking, visual inspection of QQ plots, Manhattan plots, histograms of minor allele frequency (MAF) and INFO scores, consistency of reported allele frequency with the reference set, consistency of reported *p* value with reported beta and standard error (SE), and consistency of reported SE with reported sample size, standard deviation (SD), and MAF. All files were filtered using the software EasyQC³³ (www.genepi-regensburg.de/easyqc) based on R^2 metric > 0.7 for MACH-based imputations and INFO metric > 0.8 for IMPUTE-based imputations. This filter was applied to all SNPs to ensure conservatism. In addition, SNPs were filtered for MAF > 0.03, Hardy Weinberg equilibrium p value < .0001, consistency of reported alleles and allele frequency with the reference set (maximum difference 0.2 with 1000G phase 1 v3), and duplicates (both occurrences removed).

As different phenotyping instruments were used across cohorts, the meta-analysis was based on *p* values and performed in METAL software (<http://www.sph.umich.edu/csg/abecasis/metal/>; option SCHEME SAMPLESIZE) including an application of genomic control to the results of the individual cohorts.³⁴ Meta-analysis results were filtered on a total sample size > 10,000. A *p* value < 5×10^{-8} was considered genome-wide significant.

Gene-based analyses were performed in MAGMA (Multi-marker Analysis of GenoMic Annotation).³⁵ In MAGMA, a gene test-statistic is calculated as the mean of the chi-square statistics for all the SNPs between the transcription start and stop sites of a gene. The gene p value is then obtained by using a known approximation of the sampling distribution. MAGMA corrects for gene size, number of SNPs in a gene, and linkage disequilibrium (LD) between SNPs in a gene using the SNP correlation matrix. We used the European ancestry samples from the 1000 Genomes project as reference data to estimate LD. Association was tested for 17,155 genes. The p value for gene-wide significance after Bonferroni correction was $0.05/17,155=2.91 \times 10^{-6}$.

Overlap in Results Between the EAGLE and a PGC ADHD Case-Control Meta-Analysis

We investigated the overlap in the results for the SNP and gene-based analyses obtained in EAGLE with the results of the PGC ADHD meta-analysis (P Holmans for the PGC: presentation 21st World Congress of Psychiatric Genetics, October 2013, Boston, USA, <http://2013.ispg.net/wp-content/uploads/2013/10/Oral-Presentations-Abstract-Book.pdf>). The PGC sample comprised 5,621 cases and 13,589 controls. The overlap in SNP effects was investigated with SNP effect Concordance Analysis (SECA)³⁶ and with Linkage Disequilibrium Score (LDSC) Regression analysis.^{37,38} Both methods only require the summary statistics of the association (meta)-analyses.

SECA takes the overlapping SNPs of both datasets and selects from each set of results SNPs with p values .01, .05, .1, .2, .3, .4, .5, .6, .7, .8, .9, and 1.0. This leads to a 12x12 matrix indicating the overlap in SNP effects for each combination of SNP sets in the two analyses. SECA performs several tests based on these 144 cells. We report the results of the Fisher exact tests analyzing for each combination of the SNP sets whether the number of SNPs that are concordant in the direction of effects for the two phenotypes is above chance. Next, an empirical p value is calculated that indicates whether the overlap is higher than expected by chance given the multiple testing (144 tests).

After testing for SNP concordance by SECA, we also tested for overlap at the gene level, following the procedure described in Zhao et al,³⁹ where gene-based associations were tested in GATES.⁴⁰ Independent genes were then identified by examining the LD between the most associated SNPs within each gene in the Genetic type I Error Calculator (GEC).⁴¹ Exact binomial statistical tests then determined whether the number of genes with p values $<.01$, $<.05$ or $<.1$ that were observed in both sets of results was significantly higher than expected.

The LDSC regression analysis was performed in the LDSC package (<https://github.com/bulik/ldsc>).^{37,38} This analysis yields SNP heritability estimates of the traits and a genetic correlation between the traits.

Results

SNP-Based Heritability

The estimates of the SNP-based heritability are shown in Table 2. The estimates for the maternal ratings were 5% (non-significant [ns]) for preschool SDQ and 13% (ns) and 14%

($p < .05$) for preschool CBCL and school-age SDQ, respectively. For teacher ratings, an SNP-based heritability of 34% ($p < .05$) was observed. These results indicated that SNPs tag variants associated with various ADHD symptom scores.

SNP and Gene-Based Meta-Analyses

The numbers of SNPs from each cohort that were included in the meta-analysis after QC are displayed in Table S3, available online. The QQ plot in Figure 1 shows the distribution of SNP p values from the meta-analysis filtered on SNPs that were present in at least 10,000 individuals. The lambda statistic of the meta-analysis was 0.98. Individual cohorts had lambdas ≤ 1.08 (Table S3, available online), implying absence of population stratification. The Manhattan plot in Figure 2 shows that none of the SNPs reached genome-wide significance. However, the QQ plot shows some departure from the expected line for the smallest p values, which may reflect the polygenic nature of the trait, i.e., many variants of small effects influencing ADHD symptoms. The strongest association was with rs56159542 on chromosome 19 ($p = 1.48 \times 10^{-7}$). A summary of the top-signals that crossed the threshold of suggestive association at $p < 1 \times 10^{-5}$ is included in Table 3a. Eight out of the top nine variants were located in genes. As shown in the locus zoom plots of the top SNPs (Figure S1, available online), the signals of rs79846815, rs61227778, rs77216358 were restricted to one to three SNPs, suggesting that they might not be genuine signals.

Table 3b shows the top 10 genes observed in the gene-based analysis as performed in MAGMA. Three genes, *WASL*, *LMOD2*, and *ASB15*, yielded gene-wide associations. An SNP in *LMOD2* also yielded a suggestive association with ADHD symptom scores (Table 3a). The locus zoom plot (Figure S2, available online) shows that several SNPs show similar signals in the three genes as they are in a region with SNPs in high LD. Thus, the signals of the three genes are not independent of each other. The previously identified ADHD candidate genes did not show evidence for association. P values ranged from 0.11 to 0.91 (Table S4, available online).

Overlap in Results Between the EAGLE and PGC ADHD Case-Control Meta-Analysis

The SECA software tested for 144 combinations of SNP subsets obtained from the EAGLE and the PGC ADHD meta-analyses, whether there were more SNPs showing concordance in the direction of effects than expected by chance (Fisher's Tests with OR = 1 and p value $\leq .05$). These analyses resulted in 111 SNP subsets with $p < .05$. This is significantly higher than expected by chance (empirical p value = .001), clearly indicating an overlap between the SNPs associated with ADHD symptom scores and ADHD diagnoses. This was confirmed by the analysis investigating the overlap in genes rather than SNPs.³⁹ The overlap in genes with a p value $< .05$ or $.1$ in both cohorts was larger than expected by chance, with binomial p values of $.05$ and 1.3×10^{-3} , respectively.

In the LDSC regression analysis, the genetic correlation between the EAGLE ADHD symptom scores and the PGC ADHD case-control phenotype was estimated at 0.96 ($se = 0.28$, $p < .001$), with the SNP heritability for the EAGLE ADHD symptom scores estimated at 0.08 ($se = 0.03$, $p < .01$).

Discussion

The current study comprised the largest GWA analysis of continuous ADHD symptom scores in children to date. We found that common variants included in GWA studies explained variation in ADHD symptom scores assessed in the general population. The SNP heritability estimates for the various measures from the participating cohorts ranged from 0.05 to 0.34. The SNP heritability based on the results of the meta-analysis in all cohorts with a total of 17,666 children was estimated at 8%. We did not detect SNPs at genome-wide significance levels, but detected three gene-wide significant results in the gene-based analyses. The analyses investigating the overlap in genetic influences on ADHD symptoms scores and ADHD diagnosis provided evidence for a considerable common genetic background with an estimate of the genetic correlation of 0.96.

The three associated genes, *LMOD2* (7q31.32), *ASB15* (7q31.32), and *WASL* (7q31.32), lie in a region with high LD, thereby making it difficult to decide on statistical grounds which gene contributes to this signal. Leiomodin (*LMOD*) is an actin-binding protein that acts as a filament nucleator in muscle cells.⁴³ Ankyrin repeat and SOCS box containing 15 (*ASB15*) gene product belongs to the ASB family of proteins that are part of a ubiquitination-mediated pathway.⁴⁴ The ubiquitin proteasome pathway has also been suggested to play a role in adult ADHD.⁴⁵ The protein encoded by Wiskott-Aldrich syndrome like (*WASL*) is involved in cytoskeletal organization during neuronal development, including long spine formation and neurite extension.⁴⁶ Given the enrichment of genes involved in directed neurite outgrowth in the analysis of the combined results of published GWA studies on ADHD, *WASL* seems the most likely candidate to drive the signal.

A power analysis in Quanto⁴⁸ suggested that the current sample has 80% power to detect a genome-wide significant effect explaining 0.21% of the variance, assuming an additive genetic effect, considering the largest possible sample of N=17,666, and assuming that a meta-analysis has as much statistical power as a single analysis of a similar sample size. The lack of genome-wide significant effects in combination with the observed SNP-based heritability estimates indicates that ADHD is likely to be highly polygenic, i.e., influenced by many common genetic variants of small effect sizes. These results are in line with earlier studies on ADHD based on case-control samples. Following Yang et al.,¹³ we estimated that the PGC ADHD subgroup GWA meta-analysis of 5,621 cases and 13,589 controls had 8% more power than the current study, and yet no significant results were found despite the SNP heritability estimated at 28% in this sample.¹¹ Several other studies also found evidence for polygenicity of ADHD,^{6–9,49} with the exception of one study.¹⁰ The range of the SNP heritability estimates for the symptom scores in the current study (Table 2) seems quite large. However, these differences could well be due to chance judging by the large standard errors.

The provided evidence for the genetic overlap in continuous and dichotomous measures of ADHD agrees with findings from previous studies with smaller samples.^{13–15} One of these studies also reported that an aggregate polygenic risk score derived from a sample of clinical cases of ADHD predicted preschool parent and school-age parent and teacher ratings of attention problems in a population-based cohort.⁴⁹ This indicates common genetic variance

across these measures and ages, which is confirmed by the SNP-based heritability of 8% that we calculated based on the results of the meta-analysis. Despite the use of various measures across the cohorts and the accompanying heterogeneity, there remains a signal after combining the results. Overall, it can be concluded that the different instruments assess an underlying common liability for ADHD. Therefore, combining various continuous ADHD measures assessed in the general population with dichotomous diagnosis of ADHD assessed in clinical samples can be a successful way to increase sample size and statistical power for GWA studies. This is supported by preliminary results of the PGC ADHD subgroup (R Walters: presentation 23rd World Congress of Psychiatric Genetics, October 2015, Toronto, CA).

Efforts to decrease heterogeneity across studies by harmonizing phenotypes can result in a further increase in power to detect genetic effects. Behavioral genetic studies have reported that genetic factors are not entirely similar across instruments, raters, and ages.^{50–54} We ran additional SECA analyses to investigate the overlap in results of the current meta-analysis with the results from a GWAS in an independent sample of 727 Australian adolescents whose mothers provided retrospective ratings of their childhood attention skills and problems using the Strengths and Weaknesses of ADHD Symptoms and Normal Behavior Rating Scale (SWAN). These analyses did not show concordance in SNP effects, but we note this could be due to the small size of the Australian sample. Statistical methods like Item Response Theory (IRT) can be used to synchronize different measurement instruments in a sophisticated manner, and have already been successfully applied in a GWA meta-analysis of personality measures.^{55–57} Another way to refine the phenotype when longitudinal data are available is to test the effect of the SNP on a latent variable that reflects stability over time and is more heritable than a measure at a single time point.⁵⁸

To conclude, our results support the notion that ADHD is influenced by genes involved in neuronal development. By performing GWA meta-analyses in larger samples, we should be able to identify genetic variants for ADHD, further elucidating its biological foundation. The use of continuous ADHD symptom scores available in population-based cohorts is an exciting possibility to achieve this goal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Christel M. Middeldorp, MD, PhD[#], Anke R. Hammerschlag, MSc[#], Klaasjan G. Ouwens, MSc[#], Maria M. Groen-Blokhuis, MD, PhD, Beate St. Pourcain, PhD, Corina U. Greven, PhD, Irene Pappa, MD, PhD, Carla M.T. Tiesler, PhD, Wei Ang, MSc, Ilja M. Nolte, PhD, Natalia Vilor-Tejedor, MSc, Jonas Bacelis, MSc, Jane L. Ebejer, PhD, Huiying Zhao, PhD, Gareth E. Davies, PhD, Erik A. Ehli, PhD, David M. Evans, PhD, Iryna O. Fedko, MSc, Mònica Guxens, PhD, Jouke-Jan Hottenga, PhD, James J. Hudziak, MD, Astanand Jugessur, PhD, John P. Kemp, PhD, Eva Krapohl, MSc, Nicholas G. Martin, PhD, Mario Murcia, MSc, Ronny Myhre, PhD,

Johan Ormel, PhD, Susan M. Ring, PhD, Marie Standl, PhD, Evie Stergiakouli, PhD, Camilla Stoltenberg, MD, PhD, Elisabeth Thiering, PhD, Nicholas J. Timpson, PhD, Maciej Trzaskowski, PhD, Peter J. van der Most, PhD, Carol Wang, BSc, EARly Genetics and Lifecourse Epidemiology (EAGLE) Consortium, Psychiatric Genomics Consortium ADHD Working Group, Dale R. Nyholt, PhD, Sarah E. Medland, PhD, Benjamin Neale, PhD, Bo Jacobsson, MD, PhD, Jordi Sunyer, PhD, Catharina A. Hartman, PhD, Andrew J.O. Whitehouse, PhD, Craig E. Pennell, MBBS, PhD, Joachim Heinrich, PhD, Robert Plomin, PhD, George Davey Smith, PhD, Henning Tiemeier, MD, PhD, Danielle Posthuma, PhD, and Dorret I. Boomsma, PhD

Affiliations

Dr. Middeldorp is with Biological Psychology, Neuroscience Campus Amsterdam, VU University Amsterdam, and GGZinGeest/ VU University Medical Center, Amsterdam. Ms. Hammerschlag is with the Generation R Study Group, Erasmus MC Rotterdam, the Netherlands, and Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University Amsterdam. Mr. Ouwens and Dr. Groen-Blokhuis are with Biological Psychology, VU University Amsterdam, and the EMGO+ Institute for Health and Care Research, VU University Medical Center. Dr. St. Pourcain is with MRC Integrative Epidemiology Unit (MRC IEU), University of Bristol, UK, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands, and School of Experimental Psychology, University of Bristol. Dr. Greven is with Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Karakter, Child and Adolescent Psychiatry University Center, Radboud University Nijmegen, and MRC Social Genetic and Developmental Psychiatry Centre, King's College London. Dr. Pappa is with Generation R Study Group, and Pedagogical and Education Science, Erasmus University Rotterdam, The Netherlands. Drs. Tiesler and Thiering are with Institute of Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany and the Division of Metabolic and Nutritional Medicine, Munich, and Dr. von Hauner Children's Hospital, University of Munich Medical Center, Germany. Mr. Ang, Ms. Wang, and Dr. Pennell are with School of Women's and Infants' Health, University of Western Australia, Perth. Dr. Nolte is with University of Groningen, University Medical Center Groningen, The Netherlands. Ms. Vilor-Tejedor is with Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Universitat Pompeu Fabra (UPF), Barcelona, and CIBER Epidemiology and Public Health (CIBERESP), Madrid. Mr. Bacelis is with Gothenburg University, Sweden. Drs. Ebejer, Martin, and Medland are with QIMR Berghofer Medical Research Institute, Brisbane, Australia. Drs. Zhao and Nyholt are with Institute of Health and Biomedical Innovation, Queensland University of Technology, Queensland, Australia. Drs. Davies and Ehli are with Avera Institute for Human Genetics, SD. Drs. Evans, Kemp, and Ring are with MRC IEU, School of Social and Community Medicine, and School of Social and Community Medicine, University of Bristol, and Diamantina Institute, Translational Research Institute, University of Queensland, Brisbane. Ms. Fedko is with Biological Psychology, VU University Amsterdam. Dr. Guxens is with CREAL, UPF,

CIBERESP, and Child and Adolescent Psychiatry/Psychology, Erasmus University Medical Center-Sophia Children's Hospital, The Netherlands. Dr. Hottenga is with Biological Psychology, VU University, and EMGO+ Institute for Health and Care Research, VU University Medical Center. Dr. Hudziak is with Vermont Center for Children, Youth and Families and College of Medicine, University of Vermont, Burlington, and Child and Adolescent Psychiatry, Erasmus Medical Center. Drs. Jugessur, Myhre, and Stoltenberg are with the Norwegian Institute of Public Health, Oslo. Ms. Krapohl and Drs. Trzaskowski and Plomin are with MRC Social, Genetic and Developmental Psychiatry Centre, King's College London. Mr. Murcia is with CIBERESP, and FISABIO–Universitat Jaume I–Universitat de València Joint Research Unit of Epidemiology and Environmental Health, Valencia, Spain. Drs. Ormel and Hartman are with the Interdisciplinary Center Psychopathology and Emotion regulation (ICPE), University Medical Center Groningen. Drs. Standl and Heinrich are with Institute of Epidemiology I, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany. Drs. Stergiakouli and Timpson are with MRC IEU; Dr. Timpson is also with School of Social and Community Medicine, University of Bristol. Dr. van der Most is with University of Groningen and University Medical Center Groningen. Dr. Neale is with Program in Medical and Population Genetics and Stanley Center for Psychiatric Genetics, Broad Institute of Massachusetts Institute of Technology, Boston, Analytic and Translation Genetics Unit, Massachusetts General Hospital and Harvard Medical School, Boston, and Harvard University, Cambridge, MA. Dr. Jacobsson is with Obstetrics and Gynecology, Gothenburg University, and the Norwegian Institute of Public Health. Dr. Sunyer is with CREAL, IMIM (Hospital del Mar Medical Research Institute), Barcelona, UPF, and CIBERESP. Dr. Whitehouse is with Telethon Kids Institute, University of Western Australia, Perth. Dr. Davey Smith is with MRC IEU, and School of Social and Community Medicine. Dr. Tiemeier is with Epidemiology, Child and Adolescent Psychiatry, and Psychiatry, Erasmus Medical Center. Dr. Posthuma is with the Generation R Study Group, Erasmus MC Rotterdam, the Netherlands, Child and Adolescent Psychiatry, Erasmus Medical Center, Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, VU University, and Clinical Genetics, VU University Medical Center. Dr. Boomsma is with Biological Psychology, VU University, Neuroscience Campus Amsterdam, VU University, and EMGO+ Institute for Health and Care Research, VU University Medical Center.

Acknowledgments

ALSPAC: The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and they will serve as guarantors for the contents of this paper. GWAS data was generated by Sample Logistics and Genotyping Facilities at the Wellcome Trust Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe.

Generation R: The Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), and the Ministry of Health, Welfare and Sport. H.T. received additional grants from the Netherlands Organization for Health Research and Development

(ZonMw VIDI 017.106.370). The work of A.H. and D.P. was supported by a grant from the Dutch Scientific Organisation for Scientific Research (NWO 433-09-228 and 453-14-005).

GINI/LISA: Personal and financial support by the Munich Center of Health Sciences (MCHEALTH) as part of the Ludwig-Maximilians University Munich LMU innovative is gratefully acknowledged.

INMA: This study was funded by grants from the Spanish Instituto de Salud Carlos III (CB06/02/0041, G03/176, FIS PI041436, PI081151, PI041705, PI061756, PI091958, and PS09/00432, FIS-FEDER 03/1615, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/1213, 07/0314, 09/02647, 11/01007, 11/02591, 11/02038, 13/1944, 13/2032, CP11/0178 and MS13/00054), Spanish Ministry of Science and Innovation (SAF2008-00357), European Commission (ENGAGE project and grant agreement HEALTH-F4-2007-201413, HEALTH.2010.2.4.5-1, FP7-ENV-2011 cod 282957), Fundació La Marató de TV3, Generalitat de Catalunya-CIRIT 1999SGR 00241, and Conselleria de Sanitat Generalitat Valenciana. Part of the DNA extractions and genotyping was performed at the Spanish National Genotyping Centre (CEGENBarcelona). N. Vilor-Tejedor thanks the Agència de Gestió d'Ajuts Universitaris i de Recerca - Generalitat de Catalunya for her pre-doctoral grant (2015 FI_B 00636).

MoBa (Mother and Child Cohort of NIPH): This work was supported by grants from the Norwegian Research Council (FUGE 183220/S10, FRIMEDKLI-05 ES236011), Swedish Medical Society (SLS 2008-21198), Jane and Dan Olsson Foundations, and Swedish government grants to researchers in the public health service (ALFGBG-2863, ALFGBG-11522, ALFGBG-426411), and the European Community's Seventh Framework Programme (FP7/2007-2013), ENGAGE Consortium, grant agreement HEALTH-F4-2007-201413. The Norwegian Mother and Child Cohort Study was also supported by the Norwegian Ministry of Health and the Ministry of Education and Research, NIH/NIEHS (contract no N01-ES-75558), NIH/NINDS (grant no.1 UO1 NS 047537-01 and grant no.2 UO1 NS 047537-06A1), and the Norwegian Research Council/FUGE (grant no. 151918/S10). Researchers interested in using MoBa data must obtain approval from the Scientific Management Committee of MoBa and from the Regional Committee for Medical and Health Research Ethics for access to data and biological material. Researchers are required to follow the terms of an Assistance Agreement containing a number of clauses designed to ensure protection of privacy and compliance with relevant laws. For further information, contact the principal investigator of MoBa, Per Magnus (per.magnus@fhi.no).

The Netherlands Twin Register: Genetics of Mental Illness (European Research Council ERC-230374); Genetic influences on stability and change in psychopathology from childhood to young adulthood (ZonMW 91210020); Biobanking and Biomolecular Resources Research Infrastructure (BBMRI -NL, 184.021.007); VU University's Institute for Health and Care Research (EMGO+) and Neuroscience Campus Amsterdam (NCA); Community's Seventh Framework Program (FP7/2007-2013); ENGAGE (HEALTH-F4-2007-201413); the Avera Institute, Sioux Falls, South Dakota, USA, and Grand Opportunity (grants 1RC2 MH089951 and 1RC2 MH089995).

Raine: We are grateful to the Raine Foundation, to the Raine Study Families and to the Raine Study research staff. The authors gratefully acknowledge the NH&MRC for their long term contribution to funding the study over the last 25 years and also the following Institutions for providing funding for Core Management of the Raine Study: The University of Western Australia (UWA), Raine Medical Research Foundation, UWA Faculty of Medicine, Dentistry and Health Sciences, Telethon Kids Institute and Women and Infants Research Foundation (King Edward Memorial Hospital), Curtin University, and Edith Cowan University. The authors gratefully acknowledge the assistance of the Western Australian Genetic Epidemiology Resource and the Western Australian DNA Bank (both National Health and Medical Research Council of Australia National Enabling Facilities). The authors also acknowledge the support of the Healthway Western Australia, the National Health and Medical Research Council of Australia (Grant 572613), and the Canadian Institutes of Health Research (Grant MOP 82893). The authors gratefully acknowledge the assistance of the Wind Over Water Foundation, the Telethon Institute for Child Health Research, and the Raine Medical Research Foundation of the University of Western Australia. A.J.O.W. was supported by a Senior Research Fellowship from the NHMRC (Grant number 1077966). This work was supported by resources provided by the Pawsey Supercomputing Centre with funding from the Australian Government and the Government of Western Australia.

TEDS: The Twins Early Development Study (TEDS) is supported by a programme grant to R.P. from the UK Medical Research Council [G0901245; and previously G0500079], with additional support from the US National Institutes of Health [HD044454; HD059215]. R.P. is supported by a Medical Research Council Research Professorship award [G19/2] and a European Research Council Advanced Investigator award [295366]; M.T. is supported by British Academy Post-doctoral Fellowship [pf140018]. E.K. is supported by an Institute of Psychiatry Excellence/Medical Research Council postgraduate Studentship.

TRAILS: This research is part of the TRacking Adolescents' Individual Lives Survey (TRAILS). Participating centers of TRAILS include 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 various 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 grants 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 and 481-11-001), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), Biobanking and Biomolecular Resources Research Infrastructure BBMRI-NL (CP 32), and the participating universities.

References

- Willcutt EG. The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. *Neurotherapeutics*. 2012; 9:490–499. [PubMed: 22976615]
- American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.
- American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. 4th ed., text rev ed. Washington, DC: Author; 2000.
- Lichtenstein P, Carlstrom E, Rastam M, Gillberg C, Anckarsater H. The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. *Am J Psychiatry*. 2010; 167:1357–1363. [PubMed: 20686188]
- Hawi Z, Cummins TD, Tong J, et al. The molecular genetic architecture of attention deficit hyperactivity disorder. *Mol Psychiatry*. 2015; 20:289–297. [PubMed: 25600112]
- Cross-Disorder Group of the Psychiatric Genomics C, Genetic Risk Outcome of Psychosis C. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*. 2013; 381:1371–1379. [PubMed: 23453885]
- Hamshere ML, Langley K, Martin J, et al. High loading of polygenic risk for ADHD in children with comorbid aggression. *Am J Psychiatry*. 2013; 170:909–916. [PubMed: 23599091]
- Hamshere ML, Stergiakouli E, Langley K, et al. Shared polygenic contribution between childhood attention-deficit hyperactivity disorder and adult schizophrenia. *Br J Psychiatry*. 2013; 203:107–111. [PubMed: 23703318]
- Pappa I, Fedko IO, Mileva-Seitz VR, et al. Single Nucleotide Polymorphism Heritability of Behavior Problems in Childhood: Genome-Wide Complex Trait Analysis. *J Am Acad Child Adolesc Psychiatry*. 2015; 54:737–744. [PubMed: 26299295]
- Trzaskowski M, Dale PS, Plomin R. No genetic influence for childhood behavior problems from DNA analysis. *J Am Acad Child Adolesc Psychiatry*. 2013; 52:1048–1056. [PubMed: 24074471]
- Cross-Disorder Group of the Psychiatric Genomics Consortium, Lee SH, Ripke S, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet*. 2013; 45:984–994. [PubMed: 23933821]
- Yang L, Neale BM, Liu L, et al. Polygenic transmission and complex neuro developmental network for attention deficit hyperactivity disorder: genome-wide association study of both common and rare variants. *Am J Med Genet B Neuropsychiatr Genet*. 2013; 162b:419–430. [PubMed: 23728934]
- Groen-Blokhuis MM, Middeldorp CM, Kan KJ, et al. Attention-deficit/hyperactivity disorder polygenic risk scores predict attention problems in a population-based sample of children. *J Am Acad Child Adolesc Psychiatry*. 2014; 53:1123–1129 e1126. [PubMed: 25245356]
- Martin J, Hamshere ML, Stergiakouli E, O'Donovan MC, Thapar A. Genetic risk for attention-deficit/hyperactivity disorder contributes to neurodevelopmental traits in the general population. *Biol Psychiatry*. 2014; 76:664–671. [PubMed: 24673882]
- Stergiakouli E, Martin J, Hamshere ML, et al. Shared genetic influences between attention-deficit/hyperactivity disorder (ADHD) traits in children and clinical ADHD. *J Am Acad Child Adolesc Psychiatry*. 2015; 54:322–327. [PubMed: 25791149]
- Lubke GH, Hudziak JJ, Derks EM, van Bijsterveldt TC, Boomsma DI. Maternal ratings of attention problems in ADHD: evidence for the existence of a continuum. *J Am Acad Child Adolesc Psychiatry*. 2009; 48:1085–1093. [PubMed: 19797980]
- Levy F, Hay DA, McStephen M, Wood C, Waldman I. Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large-scale twin study. *J Am Acad Child Adolesc Psychiatry*. 1997; 36:737–744. [PubMed: 9183127]

18. Larsson H, Anckarsater H, Rastam M, Chang Z, Lichtenstein P. Childhood attention-deficit hyperactivity disorder as an extreme of a continuous trait: a quantitative genetic study of 8,500 twin pairs. *J Child Psychol Psychiatry*. 2012; 53:73–80. [PubMed: 21923806]
19. Yang J, Wray NR, Visscher PM. Comparing apples and oranges: equating the power of case-control and quantitative trait association studies. *Genet Epidemiol*. 2010; 34:254–257. [PubMed: 19918758]
20. Benke KS, Nivard MG, Velders FP, et al. A genome-wide association meta-analysis of preschool internalizing problems. *J Am Acad Child Adolesc Psychiatry*. 2014; 53:667–676.e667. [PubMed: 24839885]
21. Paternoster L, Paternoster L, Standl M, et al. Meta-analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. *Nature Genetics*. 2011; 44:187–192. [PubMed: 22197932]
22. Achenbach, TM.; Rescorla, LA. Manual for the ASEBA Preschool forms & Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, and Families; 2000.
23. Achenbach, TM.; Rescorla, LA. Manual for the ASEBA School-Age Forms & Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, and Families; 2001.
24. Goodman R. The Strengths and Difficulties Questionnaire: a research note. *J Child Psychol Psychiatry*. 1997; 38:581–586. [PubMed: 9255702]
25. Conners, CK. Conners' Rating Scales-Revised. North-Tonawanda, NY: Multi-Health systems; 1997.
26. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011; 88:76–82. [PubMed: 21167468]
27. Yang J, Manolio TA, Pasquale LR, et al. Genome partitioning of genetic variation for complex traits using common SNPs. *Nat Genet*. 2011; 43:519–525. [PubMed: 21552263]
28. Fedko IO, Hottenga JJ, Medina-Gomez C, et al. Estimation of Genetic Relationships Between Individuals Across Cohorts and Platforms: Application to Childhood Height. *Behav Genet*. 2015; 45:514–528. [PubMed: 26036992]
29. Genome of the Netherlands Consortium. Whole-genome sequence variation, population structure and demographic history of the Dutch population. *Nat Genet*. 2014; 46:818–825. [PubMed: 24974849]
30. Consortium GP, Abecasis GR, Auton A, et al. An integrated map of genetic variation of 1,092 human genomes. *Nature*. 2012; 491:56–65. [PubMed: 23128226]
31. Roshyara NR, Kirsten H, Horn K, Ahnert P, Scholz M. Impact of pre-imputation SNP-filtering on genotype imputation results. *BMC Genet*. 2014; 15:88. [PubMed: 25112433]
32. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a toolset for whole-genome association and population-based analysis. *Am J Hum Genet*. 2007; 81:559–575. [PubMed: 17701901]
33. Winkler TW, Day FR. Quality control and conduct of genome-wide association meta-analyses. 2014; 9:1192–1212.
34. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010; 26:2190–1. [PubMed: 20616382]
35. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. *PLoS Comput Biol*. 2015; 11:e1004219. [PubMed: 25885710]
36. Nyholt DR. SECA: SNP effect concordance analysis using genome-wide association summary results. *Bioinformatics*. 2014; 30:2086–2088. [PubMed: 24695403]
37. Bulik-Sullivan B, Finucane HK, Anttila V, et al. An atlas of genetic correlations across human diseases and traits. *Nat Genet*. 2015; 47:1236–1241. [PubMed: 26414676]
38. Bulik-Sullivan BK, Loh PR, Finucane HK, et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet*. 2015; 47:291–295. [PubMed: 25642630]
39. Zhao H, Eising E, de Vries B, et al. Gene-based pleiotropy across migraine with aura and migraine without aura patient groups. *Cephalalgia*. 2016; 36:648–57. [PubMed: 26660531]
40. Li MX, Gui HS, Kwan JS, Sham PC. GATES: a rapid and powerful gene-based association test using extended Simes procedure. *Am J Hum Genet*. 2011; 88:283–293. [PubMed: 21397060]

41. Li MX, Yeung JM, Cherny SS, Sham PC. Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. *Hum Genet.* 2012; 131:747–756. [PubMed: 22143225]
42. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics.* 2010; 26:2336–2337. [PubMed: 20634204]
43. Chereau D, Boczkowska M, Skwarek-Maruszewska A, et al. Leiomodin is an actin filament nucleator in muscle cells. *Science.* 2008; 320:239–243. [PubMed: 18403713]
44. Kohroki J, Nishiyama T, Nakamura T, Masuho Y. ASB proteins interact with Cullin5 and Rbx2 to form E3 ubiquitin ligase complexes. *FEBS Lett.* 2005; 579:6796–6802. [PubMed: 16325183]
45. Alemany S, Ribases M, Vilor-Tejedor N, et al. New suggestive genetic loci and biological pathways for attention function in adult attention-deficit/hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet.* [published online ahead of print July 2015].
46. Takenawa T, Suetsugu S. The WASP-WAVE protein network: connecting the membrane to the cytoskeleton. *Nat Rev Mol Cell Biol.* 2007; 8:37–48. [PubMed: 17183359]
47. Poelmans G, Pauls DL, Buitelaar JK, Franke B. Integrated genome-wide association study findings: identification of a neurodevelopmental network for Attention Deficit Hyperactivity Disorder. *Am J Psychiatry.* 2011; 168:365–377. [PubMed: 21324949]
48. *Quanto 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies* [computer program]2006
49. Groen-Blokhuis MM, Middeldorp CM, Kan KJ, et al. Attention-Deficit/Hyperactivity Disorder polygenic risk scores predict Attention Problems in a population-based sample of children. *J Am Acad Child Adolesc Psychiatry.* 2014; 53:1123–1129. [PubMed: 25245356]
50. Derks EM, Hudziak JJ, Beijsterveldt CE, Dolan CV, Boomsma DI. Genetic analyses of maternal and teacher ratings on attention problems in 7-year-old Dutch twins. *Behav genet.* 2006; 36:833–844. [PubMed: 16773450]
51. Derks EM, Hudziak JJ, Dolan CV, Beijsterveldt TC, Verhulst FC, Boomsma DI. Genetic and environmental influences on the relation between attention problems and attention deficit hyperactivity disorder. *Behavior genetics.* 2008; 38:11–23. [PubMed: 18074222]
52. Kan K, Dolan CV, Nivard MG, et al. Genetic and environmental stability in attention problems across the lifespan: evidence from the Netherlands twin register. *J Am Acad Child Adolesc Psychiatry.* 2013; 52:12–25. [PubMed: 23265630]
53. Kuntsi J, Rijdsdijk F, Ronald A, Asherson P, Plomin R. Genetic influences on the stability of attention-deficit/hyperactivity disorder symptoms from early to middle childhood. *Biol Psychiatry.* 2005; 57:647–654. [PubMed: 15780852]
54. Thapar A, Harrington R, Ross K, McGuffin P. Does the definition of ADHD affect heritability? *J Am Acad Child Adolesc Psychiatry.* 2000; 39:1528–1536. [PubMed: 11128330]
55. Van den Berg SM, De Moor MH, Consortium GoP, Boomsma DI. Harmonization of Neuroticism and Extraversion phenotypes across inventories and cohorts in the Genetics of Personality Consortium: an application of Item Response Theory. *Behav Genet.* 2014; 44:295–313. [PubMed: 24828478]
56. Genetics of Personality C, de Moor MH, van den Berg SM, et al. Meta-analysis of Genome-wide Association Studies for Neuroticism, and the Polygenic Association With Major Depressive Disorder. *JAMA Psychiatry.* 2015; 72:642–650. [PubMed: 25993607]
57. van den Berg SM, de Moor MH, Verweij KJ, et al. Meta-analysis of Genome-Wide Association Studies for Extraversion: Findings from the Genetics of Personality Consortium. *Behav Genet.* 2016; 46:170–182. [PubMed: 26362575]
58. Lubke GH, Miller PJ, Verhulst B, et al. A powerful phenotype for gene-finding studies derived from trajectory analyses of symptoms of anxiety and depression between age seven and 18. *Am J Med Genet B Neuropsychiatr Genet.* [published online ahead of print Sept 2015].
59. Boyd A, Golding J, Macleod J, et al. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol.* 2013; 42:111–127. [PubMed: 22507743]

60. Fraser A, Macdonald-Wallis C, Tilling K, et al. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol.* 2013; 42:97–110. [PubMed: 22507742]
61. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol.* 2010; 25:823–841. [PubMed: 20967563]
62. Heinrich J, Bolte G, Holscher B, et al. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J.* 2002; 20:617–623. [PubMed: 12358337]
63. von Berg A, Filipiak-Pittroff B, Kramer U, et al. Allergies in high-risk schoolchildren after early intervention with cow's milk protein hydrolysates: 10-year results from the German Infant Nutritional Intervention (GINI) study. *J Allergy Clin Immunol.* 2013; 131:1565–1573. [PubMed: 23506844]
64. Guxens M, Ballester F, Espada M, et al. Cohort Profile: the INMA--Infancia y Medio Ambiente-- (Environment and Childhood) Project. *Int J Epidemiol.* 2012; 41:930–940. [PubMed: 21471022]
65. Magnus P, Irgens LM, Haug K, et al. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol.* 2006; 35:1146–1150. [PubMed: 16926217]
66. van Beijsterveldt CE, Groen-Blokhuis M, Hottenga JJ, et al. The Young Netherlands Twin Register (YNTR): longitudinal twin and family studies in over 70,000 children. *Twin Res Hum Genet.* 2013; 16:252–267. [PubMed: 23186620]
67. Evans S, Newnham J, MacDonald W, Hall C. Characterisation of the possible effect on birthweight following frequent prenatal ultrasound examinations. *Early Hum Dev.* 1996; 45:203–14. [PubMed: 8855394]
68. Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet.* 1993; 342:887–891. [PubMed: 8105165]
69. Williams LA, Evans SF, Newnham JP. Prospective cohort study of factors influencing the relative weights of the placenta and the newborn infant. *BMJ.* 1997; 314:1864–1868. [PubMed: 9224128]
70. Haworth CM, Davis OS, Plomin R. Twins Early Development Study (TEDS): a genetically sensitive investigation of cognitive and behavioral development from childhood to young adulthood. *Twin Res Hum Genet.* 2013; 16:117–125. [PubMed: 23110994]
71. Huisman M, Oldehinkel AJ, de Winter A, et al. Cohort profile: the Dutch 'TRacking Adolescents' Individual Lives' Survey'; TRAILS. *Int J Epidemiol.* 2008; 37:1227–1235. [PubMed: 18263649]

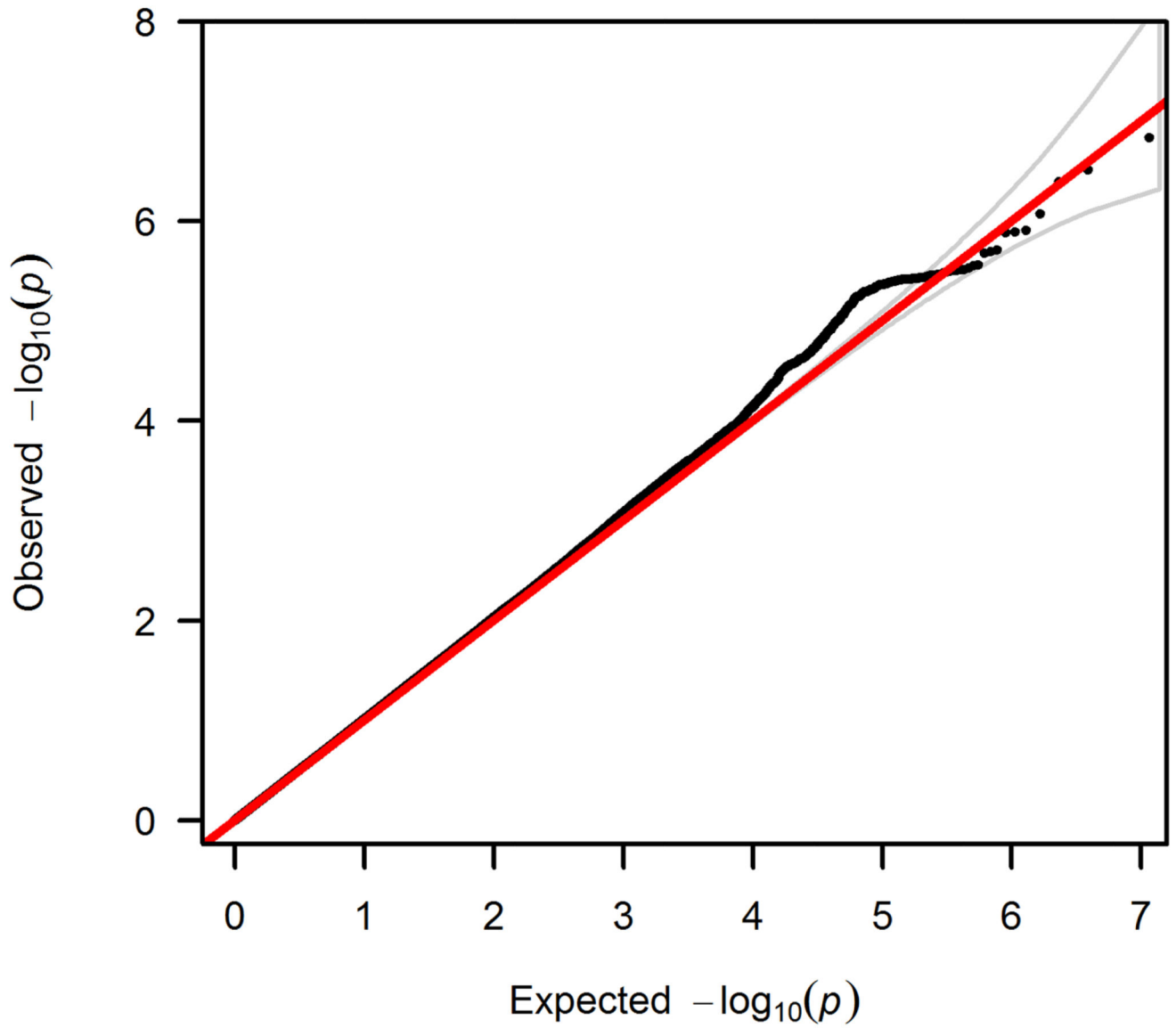


Figure 1.
QQ-plot of all meta-analysis results based on at least 10,000 individuals.

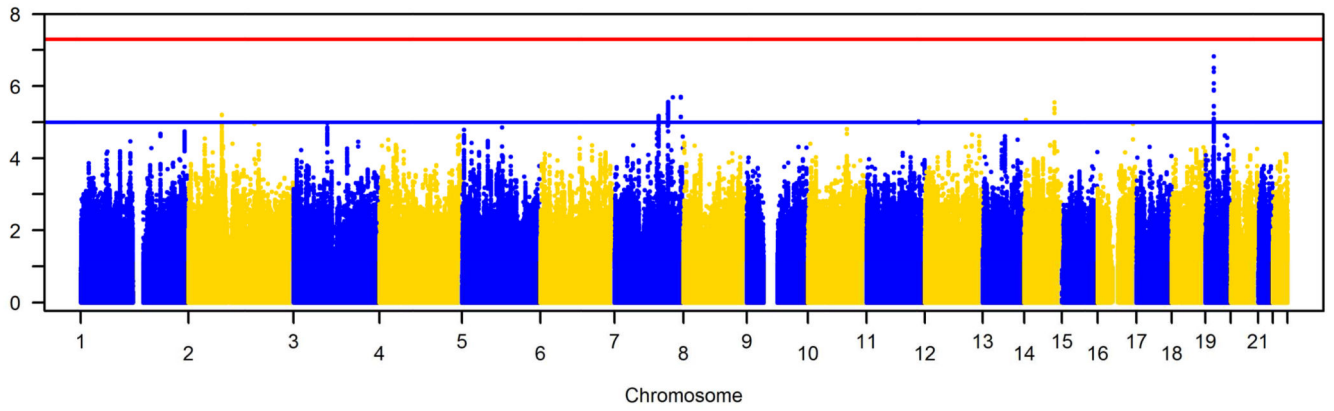


Figure 2.
Manhattan plot of the meta-analysis results based on at least 10,000 individuals.

Table 1
Description of the Cohorts and Attention-Deficit/Hyperactivity Disorder (ADHD) Instruments Included in the Meta-Analysis

Cohort	N	Phenotype instrument	Rater	Age in years mean (SD)	Sum score mean (SD)	Website	Reference papers
ALSPAC	5,757	SDQ	Parent	9.65 (0.12)	2.91 (2.24)	www.bristol.ac.uk/alspac/	59,60
Generation R	2,211	CBCL/1.5-5	Parent	6.01 (0.38)	1.38 (1.69)	www.generationr.nl	61
GINI / LISA	1,389	SDQ	Parent	10.04 (0.20)	2.71 (2.36)	http://www.helmholtz-muenchen.de/epi/arbeitsgruppen/umweltepidemiologie/projects-projekte/lisa-plus/index.html	62,63
INMA	804	DSM-based scale	Teacher	4.91 (0.69)	5.38 (6.83)	www.proyectoinma.org/	64
MoBa	665	CBCL/1.5-5	Parent	3.05 (0.10)	2.05 (1.67)	www.fhi.no/morogbarn	65
NTR	1,605	CBCL/6-12	Parent	9.95 (0.85)	3.25 (3.39)	www.tweelingenregister.org	66
Raine	1,344	CBCL/6-12	Parent	10.58 (0.20)	2.60 (3.17)	www.rainestudy.org.au	67-69
TEDS	2,606	Conners'	Parent	7.88 (0.52)	10.51 (8.62)	www.teds.ac.uk	70
TRAILS	1,285	CBCL/6-12	Parent	11.08 (0.56)	4.27 (3.40)	www.trails.nl	71

Note: ALSPAC = Avon Longitudinal Study of Parents and Children; CBCL = Child Behavior Check List; GINI = German Infant Nutritional Intervention; INMA = Infancia y Medio Ambiente; LISA = Influence of Life-style factors on Development of the Immune System and Allergies in East and West Germany plus Air Pollution and Genetics on Allergy Development; MoBa = Norwegian Mother and Child Cohort Study; NTR = Netherlands Twin Register; SDQ = Strengths and Difficulties Questionnaire; TEDS = Twins Early Development Study; TRAILS = 'Tracking Adolescents' Individual Lives' Survey.

Table 2

Genomic Complex Trait Analysis Single Nucleotide Polymorphisms Heritabilities for Mother^a- and Teacher^b-Rated Attention-Deficit/Hyperactivity Disorder (ADHD) Symptoms in the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Combined Generation R and Netherlands Twin Register (NTR) Data

	Generation R/NTR		ALSPAC	
	CBCL (3 and 6 yrs)	TRF (7yrs)	SDQ 4 yrs	SDQ 9 yrs
n	2958	1901	5510	5303
Variance explained	0.13	0.34	0.05	0.14
SE	0.11	0.17	0.06	0.07
<i>p</i> -value	0.11	0.02	0.20	0.02

Note: CBCL = Child Behavior Checklist; SDQ = Strengths and Difficulties Questionnaire; TRF = Teacher Report Form.

^aPreschool CBCL, preschool and school-age SDQ.

^bTRF.

Table 3a
Top Signals From the Single Nucleotide Polymorphism (SNP) and Gene-Based Meta-Analyses

3a. List of Independent Signals With $p < 1 \times 10^{-5}$

SNP	Chr	Position (GRCh37)	Effect/Other Allele	Freq (freq in refset)	Total N	Direction of Effect	z-Score	p-value	Location in/to Nearest Gene
rs56159542	19	19682971	T/C	0.21 (0.19)	17666	- (-----)	-5.26	1.48×10^{-7}	<i>PBX4</i> intronic
rs4629772	7	152823816	A/G	0.93 (0.93)	16322	- (----?--)	-4.76	1.97×10^{-6}	downstream <i>ACTR3B</i>
rs79846815	7	134563570	A/T	0.97 (0.96)	11175	+ (+?+???)	4.75	2.03×10^{-6}	<i>CALDI</i> intronic
rs7809453	7	123301940	A/G	0.54 (0.56)	17666	- (-----)	-4.69	2.78×10^{-6}	<i>LMOD2</i> exonic, synonymous
rs79162905	14	89796072	A/G	0.11 (0.10)	17666	- (-----)	-4.68	2.81×10^{-6}	<i>FOXN3</i> intronic
rs146855089	2	77317636	A/G	0.26 (0.27)	17666	- (-----)	-4.52	6.18×10^{-6}	<i>LRRTM4</i> intronic
rs10808119	7	101840716	A/G	0.46 (0.45)	17666	+ (+++++)	4.50	6.72×10^{-6}	<i>CUX1</i> intronic
rs61227778	14	24578916	A/G	0.95 (0.96)	10826	+ (+++++???)	4.45	8.62×10^{-6}	<i>NRL</i> intronic
rs77216358	11	120311157	A/G	0.96 (0.97)	11042	- (-?+???)	-4.43	9.48×10^{-6}	<i>ARHGGEF12</i> intronic

Table 3b
Top Signals From the Single Nucleotide Polymorphism (SNP) and Gene-Based Meta-Analyses

3b. The Top 10 Genes From the Gene-Based Tests in Multi-Marker Analysis of GenoMic Annotation (MAGMA)

Gene	Chr	Start Position (GRCh37)	Stop Position (GRCh37)	N SNPs	p-value
<i>LMOD2</i>	7	123295861	123304147	13	1.46×10^{-6}
<i>WASL</i>	7	123321997	123389116	117	1.50×10^{-6}
<i>ASB15</i>	7	123249112	123277932	45	2.66×10^{-6}
<i>CUX1</i>	7	101459184	101927250	1003	6.03×10^{-5}
<i>HAPLN4</i>	19	19366450	19373596	13	6.10×10^{-5}
<i>CILP2</i>	19	19649074	19657468	16	6.35×10^{-5}
<i>LRRTM4</i>	2	76974849	77749502	1858	8.50×10^{-5}
<i>ZNF234</i>	19	44645710	44664462	19	8.73×10^{-5}
<i>NDUFA13</i>	19	19627019	19639013	19	1.09×10^{-4}
<i>RWDD4</i>	4	184560789	184580331	41	1.52×10^{-4}

Note: Boldface text is gene-wide significant ($p < 2.91 \times 10^{-6}$).