**Genome-wide association meta-analysis of childhood and adolescent internalising symptoms**

Eshim S Jami1,2, Anke R Hammerschlag1,2,3, Hill F Ip1, Andrea G Allegrini4, Beben Benyamin5,6, Richard Border7, Elizabeth W Diemer8, Chang Jiang9,10, Ville Karhunen11, Yi Lu12, Qing Lu13, Travis T Mallard14, Pashupati P Mishra15, Ilja M Nolte16, Teemu Palviainen17, Roseann E Peterson18, Hannah M Sallis19,20,21, Ashley E Tate12, Elisabeth Thiering22,23, Natàlia Vilor-Tejedo24,25,26,27, Carol Wang28, Ang Zhou2, Daniel E Adkins29, Silvia Alemany30,27,31, Helga Ask32, Qi Chen12, Robin P Corley7, Gareth E Davies33,31,1, Erik A Ehli33, Luke M Evans34, Alexandra Havdahl32, Fiona A Hagenbeek1, Christian Hakulinen35, Anjali K Henders36, Jouke Jan Hottenga1, Tellervo Korhonen17, Abdullah Mamun37, Shelby Marrington38, Alexander Neumann39,40, Kaili Rimfeld4, Fernando Rivadeneira41,42,43, Judy L Silberg18, Catharina E van Beijsterveldt1, Eero Vuoksimaa17, Alyce M Whipp17, Tong Xiaoran13,10, Ole A Andreassen44,45, Dorret Boomsma1, Sandra A Brown46, S Alexandra Burt9, William Copeland47, Elizabeth J Costello48, Danielle M Dick49, Lindon J Eaves18, K Paige Harden14, Kathleen Mullan Harris50, Catharina A Hartman51, Joachim Heinrich22,52,53, John K Hewitt7, Christian Hopfer54, Elina Hypponen5,6, Marjo-Riitta Jarvelin11, Jaakko Kaprio17, Liisa Keltikangas-Järvinen35, Kelly L Klump9, Kenneth Krauter55, Ralf Kuja-Halkola12, Henrik Larsson56, Terho Lehtimäki35, Paul Lichtenstein12, Sebastian Lundstrom57, Hermine H Maes38, Per Magnus58, Marcus R Munafò19,20,59, Jake M Najman38, Pål R Njølstad60,61, Albertine J Oldehinkel61, Craig E Pennell28, Robert Plomin4, Ted Reichborn-Kjennerud32, Chandra Reynolds62, Richard J Rose63, Andrew Smolen7, Harold Snieder16, Michael Stallings7, Marie Standl22, Jordi Sunyer64,27,31,65, Henning Tiemeier39,66, Sally Wadsworth7, Tamara L Wall67, Andrew J O Whitehouse68, Gail M Williams38, Eivind Ystrom69, Michel G Nivard1, Meike Bartels*1,2, Christel M Middeldorp*1,3,70

*Both authors contributed equally to this work*

1Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands
2Amsterdam Public Health Research Institute, Amsterdam, the Netherlands
3Child Health Research Centre, University of Queensland, Brisbane, Australia
4Social, Genetic and Developmental Psychiatry Centre, King’s College London, London, UK
5Australian Centre for Precision Health, University of South Australia Cancer Research Institute, Adelaide, Australia
6South Australian Health and Medical Research Institute, Adelaide, Australia
7Institute for Behavioral Genetics, University of Colorado Boulder, Boulder, CO, USA

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
9Department of Psychology, Michigan State University, East Lansing, USA
10Department of Biostatistics, University of Florida, Gainesville, USA
11Department of Epidemiology and Biostatistics, Imperial College London, London, UK
12Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
13Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, USA
14Department of Psychology, University of Texas, Austin, Texas, USA
15Department of Clinical Chemistry, Fimlab Laboratories, and Finnish Cardiovascular Research Center, Tampere University, Tampere, Finland
16Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
17Institute for Molecular Medicine Finland - FIMM, University of Helsinki, Helsinki, Finland
18Virginia Institute for Psychiatric and Behavioral Genetics, Department of Human & Molecular Genetics, Virginia Commonwealth University, Richmond, VA, USA
19School of Psychological Science, University of Bristol, Bristol, UK
20MRC Integrative Unit, University of Bristol, Bristol, UK
21Centre for Academic Mental Health, Population Health Sciences, University of Bristol, Bristol, UK
22Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany
23LMU – Ludwig-Maximilians-Universität Munich, Dr. von Hauner Children's Hospital, University of Munich Medical Center, Munich, Germany
24Computational Biology of RNA Processing, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain
25BarcelonaBeta Brain Research Center, (BBRC). Pasqual Maragall Foundation, Barcelona, Spain
26Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands
27Universitat Pompeu Fabra (UPF), Barcelona, Spain
28School of Medicine and Public Health, Faculty of Medicine and Health, University of Newcastle, Newcastle, NSW, Australia
29Departments of Psychiatry and Sociology, University of Utah, Salt Lake City, UT, USA
30Child and Environment Programme, ISGlobal, Barcelona Institute of Global Health, Barcelona, Spain
31CIBER Epidemiología y Salud Pública (CIBERESP), Spain
32Department of Mental Disorders, Norwegian Institute of Public Health, Oslo, Norway
33Avera Institute for Human Genetics, Avera McKennan Hospital & University Health Center, Sioux Falls, USA
34Institute for Behavioral Genetics, Department of Ecology & Evolutionary Biology, University of Colorado Boulder, Boulder, CO, USA
35Department of Psychology and Logopedics, Faculty of Medicine, University of Helsinki, Helsinki, Finland
36Institute for Molecular Biosciences, University of Queensland, Brisbane, Australia
37Institute for Social Science Research, University of Queensland, Brisbane, Australia
38School of Public Health, Faculty of Medicine, University of Queensland, Brisbane, Australia
39Department of Child and Adolescent Psychiatry/Psychology, Erasmus University Medical Center, Rotterdam, the Netherlands
Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Canada
The Generation R Study Group, Erasmus University Medical Center, Rotterdam, the Netherlands
Department of Epidemiology, Erasmus University Medical Center, Rotterdam, the Netherlands
Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, the Netherlands
Departments of Psychiatry, University of Utah, Salt Lake City, UT, USA
NORMENT Centre, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway
Departments of Psychology and Psychiatry, University of California San Diego, La Jolla, CA, USA
Vermont Center for for Children, Youth and Families in the Department of Psychiatry, University of Vermont, Burlington, VT, USA
Department of Psychiatry, Duke University School of Medicine, Durham, NC, USA
Departments of Psychology and Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA, USA
Department of Sociology, Carolina Population Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
Department of Psychiatry, Interdisciplinary Center Psychopathology and Emotion Regulation, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University Hospital of Ludwig-Maximilians-Universität, Munich, Germany
Allergy and Lung Health Unit, Melbourne School of Population and Global Health, The University of Melbourne, Victoria, Australia
Department of Psychiatry, University of Colorado, Aurora, USA
Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO, USA
Department of Medical Epidemiology and Biostatics and School of medical sciences, Karolinska Institutet, Stockholm and Örebro, Sweden
Gillberg Neuropsychiatry Centre, Centre of Ethics, Law and Mental Health, Institute of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden
Centre for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway
NIHR Biomedical Research Centre at the University Hospitals Bristol NHS Foundation Trust and the University of Bristol, Bristol, UK
Center for Diabetes Research, Department of Clinical Science, University of Bergen, Bergen, Norway
Department of Pediatrics and Adolescents, Haukeland University Hospital, Bergen, Norway
Department of Psychology, University of California at Riverside, Riverside, CA, USA
Department of Psychological & Brain Sciences, Indiana University, Bloomington, Indiana, USA
Child and Environment Programme, Barcelona Institute of Global Health, Barcelona, Spain
MIM (Hospital del Mar Medical Research Institute), Barcelona, Spain
Department of Social and Behavioral Science, Harvard TH Chan School of Public Health, Boston, MA, USA
Department of Psychiatry, University of California, San Diego, La Jolla, CA, USA
Telethon Kids Institute, University of Western Australia, Perth, Australia
Department of Psychology, University of Oslo, Oslo, Norway
Child and Youth Mental Health Service, Children's Health Queensland Hospital and Health Service, Brisbane, Australia
Abstract

Internalising symptoms in childhood and adolescence are as heritable as adult depression and anxiety, yet little is known of their molecular basis. This genome-wide association meta-analysis of internalising symptoms included repeated observations from 64,641 individuals, aged between 3 and 18. The N-weighted meta-analysis of overall internalising symptoms (INT_{overall}) detected no genome-wide significant hits and showed low SNP heritability (1.66%, 95% confidence intervals 0.84-2.48%, N_{effective}=132,260). Stratified analyses showed rater-based heterogeneity in genetic effects, with self-reported internalising symptoms showing the highest heritability (5.63%, 95% confidence intervals 3.08-8.18%). Additive genetic effects on internalising symptoms appeared stable over age, with overlapping estimates of SNP heritability from early-childhood to adolescence. Gene-based analyses showed significant associations with three genes: WNT3 (p=1.13×10^{-06}), CCL26 (p=1.88×10^{-06}), and CENPO (p=2.54×10^{-06}). Of these, WNT3 was previously associated with neuroticism, with which INT_{overall} also shared a strong genetic correlation (r_g=0.76). Genetic correlations were also observed with adult anxiety, depression, and the wellbeing spectrum (|r_g| > 0.70), as well as with insomnia, loneliness, attention-deficit hyperactivity disorder, autism, and childhood aggression (range |r_g|=0.42-0.60), whereas there were no robust associations with schizophrenia, bipolar disorder, obsessive-compulsive disorder, or anorexia nervosa. Overall, childhood and adolescent internalising symptoms share substantial genetic vulnerabilities with adult internalising disorders and other childhood psychiatric traits, which could explain both the persistence of internalising symptoms over time, and the high comorbidity amongst childhood psychiatric traits. Reducing phenotypic heterogeneity in childhood samples will be key in paving the way to future GWAS success.
Introduction

Internalising disorders, including anxiety and depression, are substantial contributors to the global burden of disease (1, 2). Whilst the estimated 12-month prevalence of depression and anxiety disorders in adults is 15% (3), internalising disorders are also present in early life, with an estimated prevalence of 2-3% of depression and 6-7% of anxiety in childhood and adolescence (4). Prior to the development of internalising disorders, as many as one in five children self-report internalising symptoms (5). These early symptoms of anxiety and depression appear to pose a long-term risk, as longitudinal studies show that internalising symptoms in childhood are associated with mood disorders, anxiety, and suicidality in adulthood (6-8). Findings from twin research show that internalising symptoms have a moderately strong genetic component, as 40-50% of individual differences in internalising symptoms are explained by genetic factors (9-11). Moreover, research suggests that both stability and change in anxious and depressive symptoms from early childhood to adulthood are genetically influenced (10, 12-14).

Published in 2013 and 2014, the first genome-wide association studies (GWASs) on childhood internalising symptoms did not identify any genome-wide significant hits for maternal-reported anxiety-related behaviours in children aged seven (N=2,810) (15), or internalising problems in children aged three (N=4,596) (16). Estimates of SNP-based heritability (the proportion of phenotypic variance explained by single nucleotide polymorphisms (SNPs) included in the GWAS), using genome-wide complex trait analysis (GCTA), were not robust in both studies (15, 16). Other GCTA studies similarly show mostly inconsistent and broad estimates of SNP heritability, mainly due to small sample sizes (17-22). Large-scale GWASs have led to significant discoveries in adult samples, with now 102 variants identified for depression (23) and 5 variants for anxiety (24). Given the comparable heritability estimates of adult and childhood internalising phenotypes, the next step in this line of research is to increase childhood sample sizes in order to generate sufficient power to capture the small effects of common variants that have been observed in adult studies.

Here, we present a genome-wide association meta-analysis which aims to identify genetic loci associated with the development and course of internalising symptoms. The study combines repeated measurements from 22 independent cohorts, resulting in an overall sample of 64,641 individuals and 251,152 observations in children and adolescents aged between 3 and 18. All datasets were combined to produce a GWAS of overall internalising symptoms (INToverall), with an effective sample size of
132,260. Stratified analyses were used to investigate age, rater, and instrument-specific genetic effects. The overall GWAS of \( INT_{\text{overall}} \) was followed up with gene-based analyses. Genetic overlap with external traits was examined by computing genetic correlations, with a focus on psychiatric phenotypes. Non-psychiatric traits were also investigated if they were previously found to be genetically correlated with adult anxiety and depression (23-25). Finally, polygenic scores were computed to test prediction of internalising symptoms in independent samples. With this study, we aim to gain insight into the genetic underpinnings of internalising symptoms throughout childhood and adolescence in order to improve our understanding of the development and progression of internalising disorders.

**Methods**

This project was pre-registered at the Open Science Framework (https://osf.io/edas6). Minor deviations from the pre-registration are explained in the Supplementary Note.

**Sample and univariate analyses**

The sample includes cohorts that are part of the EARly Genetics and Lifecourse Epidemiology (EAGLE) consortium, behaviour and cognition working group (https://www.eagle-consortium.org/) (26), and additional cohorts with appropriate data. In total, 22 cohorts of European ancestry participated in the study. Ethical approval was provided by local committees at cohort level (Supplementary Note). Many cohorts were longitudinal birth or childhood cohort studies with long-term follow-up and multiple raters, i.e., mother, father, self, teacher. Repeated assessments of internalising symptoms within childhood and adolescence, from age 3 to age 18, were included. All cohorts performed univariate GWASs stratified by (i) age, (ii) rater, and (iii) instrument, with a minimum of 450 observations in each analysis. Internalising symptoms were positively scored on continuous scales, with higher scores indicating more internalising symptoms. Detailed descriptions of the cohorts, phenotypic measures, and genotyping and imputation procedures can be found in Supplementary Tables 1-6, and the Supplementary Note.

In total, 125 univariate GWASs were collated, with 251,152 observations based on 64,641 unique participants. The observations included ratings by mothers (40.7%), fathers (6.8%), teachers (18.3%), self (19.7%) and siblings (0.7%). An additional 13.8% of ratings were parental reports, where the informant was either the mother or the father. 15.1% of observations were in early-childhood (3 to 6 years), 36.0% in mid-childhood (7 to 10 years), 18.4% in late-childhood (11 to 12 years), and 30.0% in adolescence (13 to 18 years). Twelve instruments were used to measure internalising symptoms, of which the most commonly used were the Strengths and Difficulties Questionnaire (SDQ; 38.2%) (27), Achenbach System
of Empirically Based Assessment (ASEBA; 36.7%) (28) and Rutter Children’s Questionnaires (8.2%) (29, 30).

**Meta-analyses and the calculation of SNP heritabilities stratified by age, rater and instrument**

Quality control for each univariate GWAS was performed using EasyQC (Supplementary Text) (31). After QC, most cohorts retained between 3.4 and 7.1 million autosomal SNPs per GWAS (Supplementary Table 7). The exception was Philadelphia Neurodevelopmental Cohort which retained fewer SNPs after merging data from different genotyping platforms. To account for dependency of repeated measurements of internalising symptoms within cohorts, the N-weighed meta-analysis approach was applied (32, 33). In short, two N × N matrices, representing sample overlap and phenotypic covariance within cohorts, were created, where N was the total number of univariate GWASs. As there was no overlap across cohorts, sample overlap and phenotypic covariance between cohorts were set to zero. Using the observed sample overlap within cohorts and their phenotypic covariance matrices, expected pairwise cross-trait intercept (CTI) values between GWASs were calculated. The pairwise CTI is approximately equal to the covariance between the test statistics from univariate GWASs. N-weighted meta-analyses were performed to obtain a multivariate test statistic per SNP, which represents a weighted sum of test statistics, adjusted by the CTI in order to account for sample overlap between GWASs. Formulas for the calculation of the multivariate test statistic for each SNP in the meta-analyses, the CTI between GWASs, and estimation of effective sample size to account for repeated measurements (N_{\text{eff}}) are provided in Ip et al. supplementary text (33).

A meta-analysis was performed based on the results of all available GWASs on internalising symptoms (INT_{overall}). SNPs with minor allele frequency < 5% or N_{\text{eff}} < 15,000 were removed from further analyses. SNP-based heritabilities (h^2) were estimated using linkage disequilibrium score regression (LDSC) (34), first for INT_{overall}, and next based on results of meta-analyses stratified according to rater, age, rater-by-age, and instrument (Supplementary Table 8). To ensure that the stratified analyses had sufficient power, a sample size threshold was set so that the total number of observations (N_{\text{obs}}) for each meta-analysis was at least 15,000. Rater-specific SNP heritabilities were estimated using assessments from parents (mother and/or father), mothers, fathers, teachers, and self, respectively. Age-specific SNP heritabilities focused on internalising symptoms during early childhood (3 to 6 years), mid-childhood (7 to 10 years), late-childhood (11 to 12 years), and adolescence (13 to 18 years). Rater-by-age SNP heritabilities assessed age effects within and between raters, provided that the univariate N_{\text{obs}} exceeded 15,000. Lastly, instrument-specific SNP heritabilities were calculated for SDQ, ASEBA, and Rutter for which the N_{\text{obs}} exceeded 15,000.
If the z-score of the heritability estimate was ≥ 4, genetic correlations across stratified GWAMAs were calculated using LDSC(35).

**Gene-based analysis**

Using summary statistics for INToverall, a MAGMA (36) gene-based test (implemented in FUMA (37)) was performed to identify genes with a significant effect on internalising symptoms. The gene-based test applies a multiple regression model in which p-values from individual SNPs in a gene are combined into a test-statistic for each gene, while accounting for linkage disequilibrium between SNPs. European populations from the 1000 Genomes Phase III reference panel were used to estimate linkage disequilibrium. A total of 18,639 protein-coding genes were assessed for an association with internalising symptoms. A Bonferroni correction was applied to correct for multiple testing (α = 0.05 / 18,639; p < 2.68×10^{-06}). Significant results were looked up in the GWAS Catalog (ebi.ac.uk/gwas/) and OMIM (https://www.omim.org).

**Tissue expression and gene-set analyses**

Tissue enrichment and gene-set analyses were conducted in FUMA. The tissue enrichment analyses used two types of tissues from GTEx version 8: 30 general tissue types from multiple organs and 53 specific tissue types within these organs. A MAGMA gene-property test was performed to test one-sided relationships between cell type-specific gene expression and disease–gene associations. Bonferroni corrections were applied to correct for multiple testing for the general (α = 0.05 / 30; p < 1.7×10^{-04}) and specific (α = 0.05 / 53; p < 9.4×10^{-04}) tissue types.

The gene-set analysis was performed with default parameters in MAGMA v1.6. Gene-based P-values were converted to Z values and a between-gene correlation matrix was used as input to perform gene-set enrichment tests. Predefined gene sets from the molecular signature database MsigDB v6.1 were used. In total, 15,496 gene sets were tested. A Bonferroni correction was applied to correct for multiple testing (α = 0.05 / 15,496; p < 3.2×10^{-06}).

**Genetic correlations with external traits**

Genetic correlations between internalising symptoms and other phenotypes were investigated using publicly available summary statistics for a curated set of traits (N=27). These primarily included adult psychiatric traits, in addition to other phenotypes selected based on previously identified correlations with adult anxiety and depression (23-25). Additionally, we obtained summary statistics from the GWA
meta-analyses of overall and rater-specific childhood and adolescent aggression (33), that were based on overlapping cohorts and similar statistical methods, and calculated genetic correlations with these traits. The external traits and source studies are summarised in Supplementary Table 9. Summary statistics from INT_overall and INT_self (for which the z-score of the $h^2$ was $\geq 4$ (35)) were used. Genetic correlations were calculated using LDSC (34), which calculates genetic covariance between two traits based on all polygenic effects captured by included SNPs. Overlapping samples or population differences in GWAS summary statistics do not bias the computation of genetic correlations in LDSC. LDSC corrects for sample overlap by including a covariance matrix of the cross-trait LD score intercept, which is an estimate of sample overlap and phenotypic correlation. The genetic correlation estimate was based on the estimated slope from regressing the product of z-scores from two GWASs on the LD score. The LD scores used were computed using 1000 Genomes Phase III European data (35). Genetic correlations were considered significant at $p < 9.26 \times 10^{-04}$, after applying a Bonferroni correction for 54 independent tests.

**Sensitivity analysis: polygenic score prediction**

Polygenic score prediction of INT_overall was tested as a sensitivity analysis. The Netherlands Twin Register (NTR) was used as the target sample to examine prediction of internalising symptoms in childhood and adolescence. We considered maternal-reported internalising symptoms at age 7 (N=3,845), and self-reported internalising symptoms during adolescence (age 13 to 18, N=2,679), using the ASEBA Child Behaviour Checklist and the Youth Self Report scales (28), respectively. A leave-one-cohort-out meta-analysis omitting data from NTR was performed for INT_overall. The NTR target dataset was restricted to SNPs with minor allele frequency $> 5\%$ and imputation quality of $R^2 > 90\%$. Polygenic scores were constructed using LDpred (38), using a prior value of 0.5 to account for high polygenicity. Associations between polygenic scores of internalising symptoms and internalising problems were examined using Generalized Estimating Equations as implemented in the “gee” package in R (version 3.5.2). To account for relatedness in the target sample, the exchangeable working correlation matrix in gee was used, which applies a sandwich correction over the standard errors to account for clustering in the data. Age, sex, genotyping array, and the first 10 genetic principal components were included as covariates. Polygenic prediction was considered significant at $p < 0.025$, after applying a Bonferroni correction for 2 independent tests.
Results

Overall meta-analysis of childhood and adolescent internalising symptoms

The genome-wide association meta-analysis of INT\textsubscript{overall} found no genome-wide significant hits (Figure 1). Assuming a N\textsubscript{eff} of 132,260, SNP-based heritability of INT\textsubscript{overall} was estimated at 1.66% (95% confidence interval (CI) 0.84-2.48%). The mean chi-squared statistic was 1.086, with an LDSC-intercept of 1.043 (standard error (SE)= 0.0075), indicating that a small part of the inflation in test statistics might have been due to confounding biases, such as population stratification.

Stratified SNP heritabilities and within-trait genetic correlations

Estimates of SNP heritability from stratified meta-analyses are shown in Figure 2 and Supplementary Table 8. In rater-specific meta-analyses, self-reported internalising symptoms showed the highest heritability (5.63%; 95% CI 3.08–8.18%), followed by teacher, maternal, and parental report, which were all significant. Although father-reported internalising symptoms had the highest SNP heritability in rater-specific analyses (8.98%), the wide confidence intervals overlapped zero (-0.06–18.02%). In age-specific meta-analyses, SNP \( h^2 \) for internalising symptoms in adolescence was highest (1.97%, 95% CI 0.30–3.64%), whereas estimates for early childhood, mid-childhood, and late childhood were similar, but not robust to significance testing. In rater-by-age meta-analyses, self-reported internalising symptoms during adolescence showed the highest SNP \( h^2 \) (3.20%, 95% CI 0.34–6.06%). Instrument-specific meta-analyses showed that variance in internalising symptoms explained by ASEBA and SDQ scales were comparable, ~3%. The estimate for Rutter was smaller (.3%), but the difference was not substantial, based on the overlapping confidence intervals.

\( \text{INT}_{\text{overall}} \) and self-reported internalising symptoms were highly genetically correlated \((r_g = 0.84, \text{SE}= 0.12, p=2.08\times10^{-12})\). The other stratified meta-analyses were insufficiently powered to estimate genetic correlations \((z\text{-score of heritability estimates} < 4)\).

Gene-based analysis, tissue expression and gene-set analyses

The genome-wide gene-based analysis revealed three genes (Figure 1, Supplementary Table 10) that were significantly associated with \( \text{INT}_{\text{overall}} \) after correction for multiple testing: \( WNT3 \) \((p=1.13\times10^{-06})\), \( CCL26 \) \((p=1.88\times10^{-06})\), and \( CENPO \) \((p=2.54\times10^{-06})\). Regional plots for these three gene areas are shown in the Supplementary Text. \( WNT3 \) (WNT Family Member 3) encodes signalling proteins and has been associated with neuroticism and its measurement items, including depressed affect, feeling guilty,
irritability, and experiencing mood swings, in two prior GWASs with overlapping samples (39, 40). CCL26 (C-C motif chemokine ligand 26) encodes cytokines which are a family of secreted proteins involved in immuno-regulatory and inflammatory processes, and CENPO (Centromere Protein O) is involved in the cell cycle and encodes a component of the interphase centromere complex. CCL26 and CENPO have not been implicated in internalising-related phenotypes in previous GWASs, although both CENPO and WNT3 have been related to cognitive function and performance. Prior associations of these three genes are summarised in Supplementary Table 1.

MAGMA tissue expression analyses of 30 general and 53 specific tissue types did not show any statistically significant associations with internalising symptoms (Supplementary Table 12). The gene-set analysis also did not show any significant associations (Supplementary Table 13).

**Genetic correlations with external traits**

Genetic correlations between INT\textsubscript{overall} and INT\textsubscript{self} (for which the z-score of the $h^2$ was ≥ 4(35)), and a set of preselected external traits are shown in Figure 3 and Supplementary Table 14. INT\textsubscript{overall} held strong positive genetic correlations ($r_g > 0.7$) with Major Depressive Disorder, anxiety, and neuroticism, and a strong negative correlation ($r_g < -0.7$) with the well-being spectrum. High correlations ($|r_g| > 0.5$) with other adult and childhood psychiatric and psychological traits, including attention-deficit hyperactivity disorder, autism spectrum disorder, depressive symptoms, loneliness, and overall and maternal-reported aggression were found. Moderate genetic correlations ($|r_g| > 0.3$) with insomnia, age at first birth, cigarettes per day, educational attainment, and intelligence were also observed. INT\textsubscript{self} showed a similar pattern, but generally weaker genetic associations with external traits, with some exceptions. Autism spectrum disorder, overall and maternal-reported aggression, age at first birth, and intelligence were correlated with INT\textsubscript{overall}, but showed weaker correlations with INT\textsubscript{self}, whereas self-reported aggression, smoking initiation and body-mass index (BMI) were correlated with INT\textsubscript{self}, but showed weaker or no correlation with INT\textsubscript{overall}.

**Polygenic score prediction**

Prediction of internalising symptoms in childhood and adolescence by polygenic scores based on INT\textsubscript{overall} are shown in Supplementary Table 15. After correction for multiple testing, polygenic scores for INT\textsubscript{overall} ($N_{\text{eff}}=132,260$) were significantly associated with maternal-reported internalising problems in 7-year-olds, and explained up to 0.38% of the phenotypic variance. Polygenic scores for INT\textsubscript{overall} were not associated with self-reported internalising problems in adolescence.
Discussion

This genome-wide association meta-analysis of childhood and adolescent internalising symptoms included 64,641 individuals, 250,152 observations, and an effective sample size of 132,260. We identified three genes (WNT3, CCL26, and CENPO) significantly associated with childhood and adolescent internalising symptoms, although no genome-wide significant loci were identified at single SNP level. Most notably, the gene WNT3 was also associated with neuroticism, with which internalising symptoms also shared a strong genetic correlation. Previous research shows that neuroticism is a significant risk factor for depression and anxiety and is also strongly associated with an overall internalising factor that is common between the two disorders (39, 41-43). A secondary, albeit weaker, link to cognitive function was identified; intelligence and educational attainment shared moderate negative correlations with INToverall, and two of the identified genes (WNT3 and CENPO) were previously associated with cognitive function. High genetic correlations with adult internalising disorders and related traits, as well as with childhood-onset disorders and traits were of note. In stratified analyses, higher SNP heritability estimates in rater-specific analyses than in the overall meta-analysis (Figure 2) indicate that rater-based heterogeneity in internalising symptoms may have diluted the SNP effects in the overall GWAS. Finally, age-stratified analyses showed that variance explained by genetic effects remained similar across childhood and adolescence. Overall, our results suggest that childhood and adolescent internalising symptoms share substantial genetic vulnerabilities with adult internalising disorders and other childhood psychiatric traits, which could explain both the persistence of internalising symptoms over time, and the high comorbidity amongst childhood psychiatric traits.

Patterns of genetic correlations between internalising symptoms and external traits were insightful. Strong genetic correlations (|r_g| > 0.7) with adult depression, anxiety, neuroticism, and the wellbeing spectrum were of note, and suggest a substantial shared genetic etiology between childhood internalising symptoms and adult internalising disorders and related traits, that has also been observed in previous studies (44-46). However, the observed correlations with adult internalising disorders were partial rather than complete, indicating that from a developmental perspective, childhood and adolescent internalising symptoms are not genetically identical to adult depression or anxiety. Comparisons with other psychiatric disorders showed high genetic correlations (|r_g| > 0.5) with childhood-onset disorders attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), but no robust associations with bipolar disorder, obsessive-compulsive disorder, or anorexia nervosa. A small genetic correlation with schizophrenia was observed (r_g=0.20, p=.0025), which, albeit not significant due to the strict correction for multiple testing applied here, is in line with previous studies showing successful prediction of internalising symptoms in childhood using polygenic scores for
schizophrenia (46-49). The overall pattern of genetic correlations with other psychiatric traits is comparable to adult cross-disorder genetic correlations, where depression shows stronger associations with ADHD and ASD than with schizophrenia or bipolar disorder (50). It appears that like adult depression, internalising symptoms share fewer genetic similarities with less common disorders such as schizophrenia, but are more closely tied to childhood-onset disorders ADHD and ASD. This also resembles findings from the recent GWAS of total child psychiatric problems, which similarly found no robust genetic correlations with less common disorders (51). Correlations with other traits, including insomnia, loneliness, intelligence, educational attainment, cigarettes per day, and age at first birth, were observed, as also seen in GWASs of adult depression and anxiety (23, 24), but unlike adult depression, no robust associations with coronary artery disease, BMI, smoking initiation, or age at menarche were found. However, both BMI and smoking initiation held robust associations with INTself, for which ratings were only available during adolescence. This could indicate that genetic factors during adolescence are particularly important in these associations. Age-specific genetic effects that emerge in adulthood may also explain why coronary artery disease and age at menarche were not associated with INToverall in contrast to the robust genetic correlations these traits share with adult depression (23).

With regards to childhood traits, as well as sharing high genetic correlations with childhood-onset disorders ADHD and ASD, internalising symptoms were also highly correlated with childhood aggression. The high correlations observed across childhood traits indicate the presence of specific genetic effects that are common between childhood disorders within the neurodevelopmental spectrum. These shared genetic effects could partially explain the high comorbidity between psychiatric traits in childhood (52, 53). In investigating the association between childhood internalising symptoms and aggression in more detail, INToverall shared high genetic correlations with overall and maternal-reported aggression, but not with teacher or self-report. On the other hand, self-reported aggression and self-reported internalising symptoms were highly correlated, whereas INTself did not share robust associations with overall, teacher, or maternal reported aggression. These patterns of rater-stratified genetic correlations suggest that observed genetic effects on childhood phenotypes can vary substantially due to differences in the phenotype captured by different raters, with the same set of raters showing the highest correlation between traits.

The difficulty in identifying causal loci for early-life internalising symptoms is not novel, and resembles the trajectory of GWAS investigations of adult internalising disorders. GWAS studies of adult depression also made slow progress due to limited sample sizes and heterogeneity (54-56). As depression is a polygenic disorder influenced by many genetic variants of small effect and has several potential sources of heterogeneity, including a diverse presentation of symptoms, large sample sizes were required to
achieve success in identifying specific genomic loci (23, 25). GWAS studies of anxiety similarly saw increased success as sample sizes grew (24, 57). However, here we found that applying the brute force strategy of increased sample sizes was not an effective approach for childhood internalising symptoms. Our findings show that in addition to heterogeneity due to broad symptomology, GWAS investigations of childhood internalising symptoms are further disadvantaged by rater-based heterogeneous effects. Unlike adult samples where self or clinician reports are common, childhood samples, particularly those focusing on early childhood, rely heavily on parent and teacher report, which act as an additional source of heterogeneity. We observed rater-based differences in SNP heritability (Figure 2), which likely diluted the effects in the overall meta-analysis. The estimated genetic correlation between INToverall and self-reported internalising symptoms was high but not perfect, indicating the presence of rater-specific genetic effects. Additionally, polygenic scores based on INToverall did not predict self-reported internalising symptoms, which may also point to heterogeneity between the target and discovery traits (58). Rater-specific genetic effects on internalising symptoms are noted in previous research (59-61), and rater-based heterogeneity is also reported in the GWAS of childhood aggression (33).

Heterogeneous effects underlying childhood internalising symptoms can be accounted for in multivariate GWAS approaches, but our study shows that current childhood samples seem unable to meet the power requirements of these types of analyses. Instead, we expect that reducing heterogeneity at phenotypic level will be key in paving the way to success in future GWAS investigations. This could be tackled by examining symptom level phenotypes or separating childhood anxiety and depression into two distinct phenotypes. However, the most promising approach would be to eliminate heterogenous effects entirely through factor analysis. Factor analysis can be used to derive a stable core phenotype by focusing on what multiple measurements have in common. Evidence from both twin and molecular research shows that focusing on the common part of multi-informant assessments results in a more reliable phenotype, free from rater bias, which shows higher heritability than that captured by individual measurements separately (44, 61-63).

To conclude, in this large GWAS of childhood and adolescent internalising symptoms, no individual loci with strong associations with the outcome were detected. However, the identification of three genes, and strong genetic correlations with adult internalising traits, as well as childhood psychiatric traits indicate that there is signal buried in the noise. Future GWAS success is likely to lie in reducing heterogeneity in childhood samples by focusing on a more stable phenotype of internalising symptoms.
Acknowledgements

We extend a warm thank you to all participants, their parents, and teachers for taking part in this study. The study was supported by the “Childhood and Adolescence Psychopathology: unravelling the complex etiology by a large Interdisciplinary Collaboration in Europe” project (CAPICE). CAPICE received funding from the European Union’s Horizon 2020 research and innovation programme, Marie Skłodowska Curie Actions – MSCA-ITN-2016 – Innovative Training Networks, under grant agreement number 721567. Author and cohort-specific acknowledgements and funding information are described in the Supplementary Text.

Conflict of Interest

H Larsson has served as a speaker for Evolan Pharma and Shire/Takeda and has received research grants from Shire/Takeda, all outside the submitted work. All other authors declare no conflicts of interest.
References

1. Erskine H, Moffitt TE, Copeland W, Costello E, Ferrari A, Patton G, et al. A heavy burden on young minds: the global burden of mental and substance use disorders in children and youth. Psychological medicine. 2015;45(7):1551-63.
2. Organization WH. Depression and other common mental disorders: global health estimates. World Health Organization; 2017.
3. Steel Z, Marnane C, Iranpour C, Chey T, Jackson JW, Patel V, et al. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980–2013. International journal of epidemiology. 2014;43(2):476-93.
4. Polanczyk GV, Salum GA, Sugaya LS, Caye A, Rohde LA. Annual Research Review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. Journal of Child Psychology and Psychiatry. 2015;56(3):345-65.
5. Husky MM, Boyd A, Bitfoi A, Carta MG, Chan-Chee C, Goelitz D, et al. Self-reported mental health in children ages 6–12 years across eight European countries. European child & adolescent psychiatry. 2017:1-11.
6. Weissman MM, Wolk S, Wickramaratne P, Goldstein RB, Adams P, Greenwald S, et al. Children with prepubertal-onset major depressive disorder and anxiety grown up. Archives of general psychiatry. 1999;56(9):794-801.
7. Roza SJ, Hofstra MB, van der Ende J, Verhulst FC. Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: A 14-year follow-up during childhood, adolescence, and young adulthood. American Journal of Psychiatry. 2003;160(12):2116-21.
8. Gregory AM, Eley TC. Genetic Influences on Anxiety in Children: What we’ve Learned and Where we’re Heading. Clinical Child and Family Psychology Review. 2007;10(3):199-212.
9. Franić S, Dolan CV, Borsboom D, van Beijsterveldt CE, Boomsma DI. Three-and-a-half-factor model? The genetic and environmental structure of the CBCL/6–18 internalizing grouping. Behavior genetics. 2014;44(3):254-68.
10. Nivard M, Dolan C, Kendler K, Kan K-J, Willemsen G, van Beijsterveldt C, et al. Stability in symptoms of anxiety and depression as a function of genotype and environment: a longitudinal twin study from ages 3 to 63 years. Psychological medicine. 2015;45(5):1039-49.
11. Polderman TJ, Benyamin B, De Leeuw CA, Sullivan PF, Van Bochoven A, Visscher PM, et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nature genetics. 2015;47(7):702.
12. Hannigan L, Walaker N, Waszczuk M, McAdams T, Eley T. Aetiological influences on stability and change in emotional and behavioural problems across development: a systematic review. Psychopathology review. 2017;4(1):52.
13. Franić S, Middeldorp CM, Dolan CV, Ligthart L, Boomsma DI. Childhood and Adolescent Anxiety and Depression: Beyond Heritability. Journal of the American Academy of Child & Adolescent Psychiatry. 2010;49(8):820-9.
14. Kendler KS, Gardner CO, Lichtenstein P. A developmental twin study of symptoms of anxiety and depression: evidence for genetic innovation and attenuation. Psychological Medicine. 2008;38(11):1567-75.
15. Trzaskowski M, Eley TC, Davis OS, Doherty SJ, Hanscombe KB, Meaburn EL, et al. First genome-wide association study on anxiety-related behaviours in childhood. PloS one. 2013;8(4):e58676.
16. Benke KS, Nivard MG, Velders FP, Walters RK, Pappa I, Scheet PA, et al. A genome-wide association meta-analysis of preschool internalizing problems. Journal of the American Academy of Child & Adolescent Psychiatry. 2014;53(6):667-76. e7.
17. Trzaskowski M, Dale PS, Plomin R. No genetic influence for childhood behavior problems from DNA analysis. Journal of the American Academy of Child & Adolescent Psychiatry. 2013;52(10):1048-56. e3.

18. Pappa I, Fedko IO, Mileva-Seitz VR, Hottenga J-J, Bakermans-Kranenburg MJ, Bartels M, et al. Single nucleotide polymorphism heritability of behavior problems in childhood: genome-wide complex trait analysis. Journal of the American Academy of Child & Adolescent Psychiatry. 2015;54(9):737-44.

19. Sallis H, Evans J, Wootten R, Krapohl E, Oldehinkel AJ, Smith GD, et al. Genetics of depressive symptoms in adolescence. BMC psychiatry. 2017;17(1):321.

20. Cheesman R, Selzam S, Ronald A, Dale PS, McAdams TA, Eley TC, et al. Childhood behaviour problems show the greatest gap between DNA-based and twin heritability. Translational psychiatry. 2017;7(12):1284.

21. Jami ES, Eilertsen EM, Hammerschlag AR, Qiao Z, Evans DM, Ystrom E, et al. Maternal and paternal effects on offspring internalizing problems: Results from genetic and family-based analyses. American Journal of Medical Genetics Part B-Neuropsychiatric Genetics. 2020;183(5):258-67.

22. Cheesman R, Eilertsen EM, Ahmadzadeh YI, Gjerde LC, Hannigan LJ, Havdahl A, et al. How important are parents in the development of child anxiety and depression? A genomic analysis of parent-offspring trios in the Norwegian Mother Father and Child Cohort Study (MoBa). medRxiv. 2020:2020.04.14.20064782.

23. Howard DM, Adams MJ, Clarke T-K, Hafferty JD, Gibson J, Shirali M, et al. Genomewide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. Nature neuroscience. 2019;22(3):343.

24. Purves KL, Coleman JR, Meier SM, Rayner C, Davis KA, Cheesman R, et al. A major role for common genetic variation in anxiety disorders. Molecular psychiatry. 2019:1-12.

25. Wray NR, Ripke S, Mattheisen M, Trzaskowski M, Byrne EM, Abdellaoui A, et al. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nature genetics. 2018;50(5):668.

26. Middeldorp CM, Felix JF, Mahajan A, McCarthy MI, consortium EGG. The Early Growth Genetics (EGG) and EArly Genetics and Lifecourse Epidemiology (EAGLE) consortia: design, results and future prospects. European journal of epidemiology. 2019;34(3):279-300.

27. Goodman R. The Strengths and Difficulties Questionnaire: a research note. Journal of child psychology and psychiatry. 1997;38(5):581-6.

28. Achenbach TM. The Achenbach system of empirically based assessment (ASEBA): Development, findings, theory, and applications: University of Vermont, Research Center for Children, Youth, & Families; 2009.

29. Rutter M. A children's behaviour questionnaire for completion by teachers: preliminary findings. Journal of child Psychology and Psychiatry. 1967;8(1):1-11.

30. Rutter M, Tizard J, Whitmore K. Education, Health and Behaviour. London: Longman; 1970.

31. Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Mägi R, et al. Quality control and conduct of genome-wide association meta-analyses. Nature protocols. 2014;9(5):1192.

32. Baselmans BM, Jansen R, Ip HF, van Dongen J, Abdellaoui A, van de Weijer MP, et al. Multivariate genome-wide analyses of the well-being spectrum. Nature genetics. 2019;51(3):445-51.

33. Ip HF, van der Laan CM, Brikell I, Sánchez-Mora C, Nolte IM, St Pourcain B, et al. Genetic Association Study of Childhood Aggression across raters, instruments and age. bioRxiv. 2019:854927.
34. Bulik-Sullivan BK, Loh P-R, Finucane HK, Ripke S, Yang J, Patterson N, et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nature genetics. 2015;47(3):291-5.
35. Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh P-R, et al. An atlas of genetic correlations across human diseases and traits. Nature genetics. 2015;47(11):1236.
36. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. PLoS computational biology. 2015;11(4).
37. Watanabe K, Taskesen E, Van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. Nature communications. 2017;8(1):1-11.
38. Vilhjálmsson Bjarni J, Yang J, Finucane Hilary K, Gusev A, Lindström S, Ripke S, et al. Modeling Linkage Disequilibrium Increases Accuracy of Polygenic Risk Scores. The American Journal of Human Genetics. 2015;97(4):576-92.
39. Nagel M, Jansen PR, Stringer S, Watanabe K, de Leeuw CA, Bryois J, et al. Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways. Nature genetics. 2018;50(7):920-7.
40. Nagel M, Watanabe K, Stringer S, Posthuma D, Van Der Sluis S. Item-level analyses reveal genetic heterogeneity in neuroticism. Nature communications. 2018;9(1):1-10.
41. Speed D, Hemani G, Speed MS. Investigating the causal relationship between neuroticism and depression via Mendelian Randomization. Acta Psychiatrica Scandinavica. 2019;139(4):395.
42. Middeldorp CM, Cath DC, Van Dyck R, Boomsma DI. The co-morbidity of anxiety and depression in the perspective of genetic epidemiology. A review of twin and family studies. Psychol Med. 2005;35(5):611-24.
43. Griffith JW, Zinbarg RE, Craske MG, Mineka S, Rose RD, Waters AM, et al. Neuroticism as a common dimension in the internalizing disorders. Psychological medicine. 2010;40(7):1125-36.
44. Cheesman R, Purves KL, Pingault J-B, Breen G, Plomin R, Eley TC. Extracting stability increases the SNP heritability of emotional problems in young people. Translational psychiatry. 2018;8(1):1-9.
45. Akingbuwa WA, Hammerschlag AR, Jami ES, Allegrini AG, Karhunen V, Sallis H, et al. Genetic Associations Between Childhood Psychopathology and Adult Depression and Associated Traits in 42 998 Individuals: A Meta-Analysis. JAMA Psychiatry. 2020.
46. Musci RJ, Masyn KE, Benke K, Maher B, Uhl G, Ialongo NS. The effects of the interplay of genetics and early environmental risk on the course of internalizing symptoms from late childhood through adolescence. Development and psychopathology. 2016;28(1):225-37.
47. Nivard MG, Gage SH, Hottenga JJ, van Beijsterveldt CE, Abdellaoui A, Bartels M, et al. Genetic Overlap Between Schizophrenia and Developmental Psychopathology: Longitudinal and Multivariate Polygenic Risk Prediction of Common Psychiatric Traits During Development. Schizophrenia Bulletin. 2017;sbx031.
48. Riglin L, Collishaw S, Richards A, Thapar AK, Rice F, Maughan B, et al. The impact of schizophrenia and mood disorder risk alleles on emotional problems: investigating change from childhood to middle age. Psychological medicine. 2017:1-6.
49. Jansen PR, Polderman TJ, Bolhuis K, Ende J, Jaddoe VW, Verhulst FC, et al. Polygenic scores for schizophrenia and educational attainment are associated with behavioural problems in early childhood in the general population. Journal of Child Psychology and Psychiatry. 2018;59(1):39-47.
50. Lee PH, Anttila V, Won H, Feng Y-CA, Rosenthal J, Zhu Z, et al. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. Cell. 2019;179(7):1469-82. e11.
51. Neumann A, Nolte IM, Pappa I, Ahluwalia TS, Pettersson E, Rodriguez A, et al. A genome-wide association study of total child psychiatric problems scores. medRxiv. 2020;2020.06.04.20121061.

52. Rhee SH, Lahey BB, Waldman ID. Comorbidity Among Dimensions of Childhood Psychopathology: Converging Evidence From Behavior Genetics. Child Development Perspectives. 2015;9(1):26-31.

53. Allegrini AG, Cheesman R, Rimfeld K, Selzam S, Pingault J-B, Eley TC, et al. The p factor: genetic analyses support a general dimension of psychopathology in childhood and adolescence. Journal of Child Psychology and Psychiatry. 2020;61(1):30-9.

54. Levinson DF, Mostafavi S, Milaneschi Y, Rivera M, Ripke S, Wray NR, et al. Genetic studies of major depressive disorder: Why are there no GWAS findings, and what can we do about it? Biological psychiatry. 2014;76(7):510.

55. Wray NR, Maier R. Genetic Basis of Complex Genetic Disease: The Contribution of Disease Heterogeneity to Missing Heritability. Current Epidemiology Reports. 2014;1(4):220-7.

56. Cai N, Choi KW, Fried EI. Reviewing the genetics of heterogeneity in depression: operationalizations, manifestations and etiologies. Human Molecular Genetics. 2020.

57. Otowa T, Hek K, Lee M, Byrne EM, Mirza SS, Nivard MG, et al. Meta-analysis of genome-wide association studies of anxiety disorders. Molecular psychiatry. 2016;21(10):1391.

58. de Vlaming R, Okbay A, Rietveld CA, Johannesson M, Magnusson PKE, Uitterlinden AG, et al. Meta-GWAS Accuracy and Power (MetaGAP) Calculator Shows that Hiding Heritability Is Partially Due to Imperfect Genetic Correlations across Studies. PLoS Genet. 2017;13(1):e1006495-e.

59. Ask H, Torgersen S, Seglem KB, Waaktaar T. Genetic and environmental causes of variation in adolescent anxiety symptoms: A multiple-rater twin study. Journal of anxiety disorders. 2014;28(4):363-71.

60. Fedko IO, Hottenga J-J, Medina-Gomez C, Pappa I, van Beijsterveldt CE, Ehli EA, et al. Estimation of genetic relationships between individuals across cohorts and platforms: application to childhood height. Behavior genetics. 2015;45(5):514-28.

61. Wesseldijk LW, Fedko IO, Bartels M, Nivard MG, van Beijsterveldt CE, Boomsma DI, et al. Psychopathology in 7-year-old children: Differences in maternal and paternal ratings and the genetic epidemiology. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2017;174(3):251-60.

62. Lubke GH, Miller PJ, Verhulst B, Bartels M, van Beijsterveldt T, Willemsen G, et al. A powerful phenotype for gene-finding studies derived from trajectory analyses of symptoms of anxiety and depression between age seven and 18. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2016;171(7):948-57.

63. Fedko IO, Wesseldijk LW, Nivard MG, Hottenga J-J, van Beijsterveldt CE, Middeldorp CM, et al. Heritability of Behavioral Problems in 7-Year Olds Based on Shared and Unique Aspects of Parental Views. Behavior genetics. 2017;47(2):152-63.
Figure 1 Manhattan plot of overall meta-analysis for childhood and adolescent internalising symptoms (INToverall). The solid line represents the significance threshold ($p < 5 \times 10^{-08}$), and the dotted line represents the suggestive threshold ($p < 1 \times 10^{-05}$). Red dots represent SNPs that were included in the significant genes from the gene-based analysis. The corresponding significant genes are annotated on top of these SNP towers.
**Figure 2** SNP heritability estimates based on N-weighted meta-analyses of internalising symptoms. Error bars represent 95% confidence intervals.
Figure 3 Genetic correlations with external phenotypes. The left panel shows genetic correlations with the meta-analysis for overall internalising symptoms in childhood and adolescence (INT_{overall}), and the right panel shows genetic correlations with self-reported internalising symptoms (INT_{self}). Error bars represent 95% confidence intervals. Correlation points in red are statistically significant after correction for multiple testing.