Single Nucleotide Polymorphism Heritability of Behavior Problems in Childhood: Genome-Wide Complex Trait Analysis

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Objective: Genetic factors contribute to individual differences in behavior problems. In children, genome-wide association studies (GWAS) have yielded the first suggestive results when aiming to identify genetic variants that explain heritability, but the proportion of genetic variance that can be attributed to common single nucleotide polymorphisms (SNPs) remains to be determined, as only a few studies have estimated SNP heritability, with diverging results.

Method: Genomic-relationship-matrix restricted maximum likelihood (GREML) as implemented in the software Genome-Wide Complex Trait Analysis (GCTA) was used to estimate SNP heritability (SNP h^2) for multiple phenotypes within 4 broad domains of children's behavioral problems (attention-deficit/hyperactivity symptoms, internalizing, externalizing, and pervasive developmental problems) and cognitive function. We combined phenotype and genotype data from 2 independent, population-based Dutch cohorts, yielding a total number of 1,495 to 3,175 of 3-, 7-, and 9-year-old children.

Results: Significant SNP heritability estimates were found for attention-deficit/hyperactivity symptoms (SNP $h^2 = 0.37–0.71$), externalizing problems (SNP $h^2 = 0.44$), and total problems (SNP $h^2 = 0.18$), rated by mother or teacher. Sensitivity analyses with exclusion of extreme cases and quantile normalization of the phenotype data decreased SNP h^2 as expected under genetic inheritance, but they remained statistically significant for most phenotypes.

Conclusion: We provide evidence of the influence of common SNPs on child behavior problems in an ethnically homogenous sample. These results support the continuation of large GWAS collaborative efforts to unravel the genetic basis of complex child behaviors.

Key Words: genome-wide complex trait analysis (GCTA), heritability, children, behavior problems

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Complex behaviors are shaped by both genetic and environmental influences,[1,2] and numerous twin, family, and adoption studies have estimated significant contributions of genetic factors to individual differences in behavioral and psychiatric traits.[3-5] In addition, longitudinal population-based studies provide evidence of the genetic stability of common behavioral problems (e.g., anxiety and depression symptoms,[6] attention problems[7]) across the lifespan, with higher heritability estimates in childhood (e.g., for attention problems, heritability estimates decreased from 0.70 in childhood to 0.40 in adulthood[7]).

In adult samples, genome-wide association studies (GWAS) identified genes and pathways related to complex traits.[8,9] This approach has also yielded positive findings in studies of important traits in children (e.g., birth weight[10] and length[11]). For childhood psychiatric traits and problem behaviors, successes have been limited,[12-15] which can probably be ascribed to the very modest sample sizes in these studies.[16] The relatively small or absent genetic associations with complex traits of interest in GWAS[12-15] may seem in contrast to the large heritability estimates from twin and family studies but are indeed in line with recent evidence that the small effect sizes of individual SNPs may be responsible for the nonreplicability of these associations.[17] To assess whether GWAS of child behavior problems can be expected to yield important findings regarding biological pathways, we address the question of what part of the heritability of childhood behavior problems is captured by common (minor allele frequency >1%) single nucleotide polymorphisms (SNPs) included in standard genotyping arrays.

The genetic variance explained by genome-wide SNPs[18] can be estimated by using the genetic similarity among unrelated individuals as a predictor of their phenotypic resemblance. When individual-level genotype data are available, these can be used to obtain a measure of genetic similarity between all possible pairs of (unrelated)

This article is discussed in an editorial by Drs. Philip Asherson and Paul F. O'Reilly on page 702.

Supplemental material cited in this article is available online.

individuals in the study. In a second step, this genetic relatedness matrix (GRM) is used to predict the phenotype similarity between individuals, just as the different similarity of monozygotic (MZ) and dizygotic (DZ) twin pairs predicts their different phenotype resemblances. This approach has been implemented in the software package Genome-Wide Complex Trait Analysis (GCTA).[18] The heritability estimates from GCTA (SNP h^2) are commonly considered an indicator of the upper limit of the variance that can be explained by current GWAS efforts. Power estimations have indicated that for quantitative traits, a sample size of 3,000 individuals is required to detect an SNP h^2 of 0.30 with 80% power.[19] Thus, large sample sizes are required to reliably estimate the SNP heritability of complex behavioral traits, which can imply the need to pool data from multiple studies.

To date, few SNP heritability estimates are available for behavioral problems in childhood. Some studies indicate substantial additive genetic heritability of normative differences in children's social communication difficulties[20] and in clinical cases of attention-deficit/hyperactivity disorder (ADHD)[21,22] and childhood-onset obsessive-compulsive disorder (OCD).[23] However, other studies indicate modest, statistically nonsignificant SNP heritability estimates for children's internalizing problems,[12] anxiety,[24] and callous-unemotional (CU) traits[13] in population-based samples. A study from the Twins Early Development Study (TEDS) indicated no significant SNP heritability for parent-, teacher-, and self-reported behavioral problems (i.e., attention problems, internalizing, and externalizing problems) in contrast to cognitive and anthropomorphic traits in a population-based sample (n = 2,500) of 12-year-old children.[25]

Here we focus on 4 domains of children's behavioral problems: attention deficit problems, externalizing, internalizing, and pervasive developmental problems. Genetic influences on nonverbal cognitive abilities were also estimated. To obtain sufficient power, we combined genotype and phenotype data from 2 independent, population-based Dutch cohorts: the Generation R Study (GEN-R) and the Netherlands Twin Register (NTR). Genotyped SNP data from both studies were used to construct a GRM.[26] For both studies, behavior problems of a total number of 1,495 to 3,175 of 3-, 7-, and 9-year-old children were rated by mothers and/or teachers. We estimated the SNP heritability in each of these traits, and we compared our findings to the SNP heritability estimates previously reported.

METHOD

Participants

This study included data from children from 2 population-based Dutch cohorts, GEN-R and NTR. GEN-R is a prospective cohort based in Rotterdam. The characteristics of the study have been previously described in detail.[27] NTR is a nationwide longitudinal sample of twins and their family members followed from birth onward after voluntary registration.[28] In both studies, parents gave informed consent for participation and also to approach teachers of the children. Study protocols were approved by the local ethics committees.

Measures

All phenotypes analyzed in this study have been described in detail in previous publications of GEN-R and NTR, and twin-based heritabilities in the Dutch population were reported for these traits (see Table S1, available online).

Conners' Parent Rating Scale. ADHD symptoms and related comorbid symptoms were assessed using the Conners' Parent Rating Scale (CPRS-R)[26] completed by the mothers. Four scales of the CPRS-R were used: ADHD Combined; ADHD Inattentive; ADHD Hyperactive-Impulsive; and Oppositional Defiant Disorder (ODD) scale.

Child Behavior Checklist. We assessed child behavior problems using the well-validated Child Behavior Checklist (CBCL),[27] completed by the mother. Internalizing, externalizing, and total problems were assessed using the appropriate CBCL syndrome scales. For the CBCL Internalizing, Externalizing, and Total Problems scores, the GEN-R study used the CBCL for ages 1.5 to 5 years,[28] and NTR used the CBCL for ages 6 to 18 years.[29] In the CBCL for ages 1.5 to 5 years, the Internalizing scale consists of 4 scales (Emotionally Reactive, Anxious/Depressed, Somatic Complaints, and Withdrawn), and the Externalizing scale consists of 2 scales (Attention Problems and Aggressive Behavior). In the CBCL for ages 6 to 18 years, the Internalizing scale consists of 3 scales (Anxious/Depressed, Withdrawn/Depressed, and Somatic Complaints), and the Externalizing scale consists of 2 scales (Rule-Breaking Behavior and Aggressive Behavior). The Total Problems score is computed by summing the ratings of all problem items included in the CBCL. To avoid phenotypic heterogeneity in the combined dataset due to differences in the items between the 2 CBCL versions, we selected only overlapping items to compute the scores (see Table S2, available online).

We assessed pervasive developmental problems using the Pervasive Developmental Disorder (PDD) subscale of the CBCL for children 1.5 to 5 years.[28] The PDD subscale has been shown to be a valid screening tool for autism spectrum disorders (ASD).[30]

Teacher's Rating Form. The Teacher's Rating Form (TRF) for ages 6 to 18 years[29] was used to assess attention problems (Attention Problems scale) and behavioral problems (Externalizing scale), rated by the teacher. We used the teachers' ratings of externalizing and not internalizing problems, since it has been previously shown that they can better identify children with externalizing rather than internalizing problems.[31] The teacher reports were also selected to assess behavior in a different environment, and to avoid informant effects, which could bias estimates of genetics contribution to common child behavior problems.[32,33]

Nonverbal Cognitive Abilities. Nonverbal cognitive abilities were assessed with the Snijder–Oomen Nonverbal Intelligence Test[34] (SON-R 2.5–7 years) in the GEN-R study, and the nonverbal subtest of the Revised Amsterdam Children Intelligence Test[35] (RAKIT) in the NTR. Both measurements are well validated and correlate substantially with the Wechsler Preschool and Primary Scale of Intelligence–Revised (WPPSI-R)[36] and the Wechsler Intelligence Scale for Children (WISC).[37] The nonverbal cognition scores in both samples were transformed to mean = 100 and SD = 15.

Genotyping and Imputation

A total of 3,102 children from the GEN-R study and 2,826 children from NTR, all of white ethnicity, were genotyped on Illumina (660W, 610K) and Affymetrix 6.0 platforms, respectively. Because the number of overlapping SNPs between platforms was small ($n \sim 120K$), both cohorts were cross-platform imputed using MaCH-Admix imputation software[38] as described in Fedko *et al.*[26] Cross-platform imputation supplies all participants from both cohorts with genetic information from all SNPs genotyped on both