A Replicated Molecular Genetic Basis for Subtyping Antisocial Behavior in Children With Attention-Deficit/Hyperactivity Disorder

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Context: Attention-deficit/hyperactivity disorder (ADHD) is a heterogeneous neurodevelopmental disorder that in some cases is accompanied by antisocial behavior.

Objective: To test if variations in the catechol-O-methyltransferase gene (COMT) would prove useful in identifying the subset of children with ADHD who exhibit antisocial behavior.

Design: Three independent samples composed of 1 clinical sample of ADHD cases and 2 birth cohort studies.


Main Outcome Measures: Diagnosis of ADHD and measures of antisocial behavior.

Results: We present replicated evidence that the COMT valine/methionine polymorphism at codon 158 (COMT Val158Met) was associated with phenotypic variation among children with ADHD. Across the 3 samples, valine/valine homozygotes had more symptoms of conduct disorder, were more aggressive, and were more likely to be convicted of criminal offenses compared with methionine carriers.

Conclusions: The findings confirm the presence of genetic heterogeneity in ADHD and illustrate how genetic information may provide biological evidence pointing to clinical subtypes.

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Attention-deficit/hyperactivity disorder (ADHD) is a common childhood-onset neurodevelopmental disorder characterized by severe inattention, overactivity, and impulsiveness. Attention-deficit hyperactivity disorder is a major burden to health services. Moreover, the burden associated with childhood ADHD extends into adulthood to include educational failure, drug and alcohol misuse, and crime. The disorder is also notable for its heterogeneity, with wide variation in clinical features and outcome. Clinical, epidemiological, and behavioral genetic studies point to the presence of childhood-onset antisocial behavior in ADHD as an important marker of the disorder’s heterogeneity. About 50% of young people with ADHD exhibit antisocial behavior, and longitudinal studies demonstrate that ADHD leads to antisocial behavior, rather than vice versa. Attention-deficit/hyperactivity disorder that is accompanied by antisocial behavior is not only more strongly familial than ADHD alone, it is more heritable, has a worse prognosis, and is associated with more pronounced neurocognitive deficits.

The covariation between antisocial behavior and ADHD has been shown to be accounted for, in part, by common genetic factors. Although the specific genes involved have yet to be identified, it has been suggested that the catechol O-methyltransferase gene (COMT) may play a role in influencing the manifestation of antisocial behavior in children with ADHD. Herein, we provide molecular genetic support for dividing ADHD according to the presence of antisocial behavior by showing that the antisocial features of ADHD are related to variations in the gene encoding the COMT enzyme that plays a major role in modulating prefrontal cortex (PFC) dopamine levels. Prefrontal cortex dopamine is relevant to executive cognitive dysfunctions that characterize early-onset and persistent antisocial behavior. The human COMT gene is located on chromosome 22q11 and contains a valine/methionine (Val/Met) poly-
morphism at codon 158 (Val158Met; codon numbering based on the largest known protein isoform). The Met allele is associated with a 40% reduction in enzymatic activity in the PFC and (it is inferred) a higher level of PFC dopamine as a consequence. The biochemical consequences of this polymorphism in COMT may be of broad functional relevance, as carriers of the Val allele have been shown to exhibit less efficient PFC processing as indicated by a worse performance on measures of executive functioning. These findings led us to test whether individuals with ADHD who are carriers of the Val allele would be at greater risk of developing early-onset antisocial behavior and its sequelae. In 3 independent studies of children with ADHD, we confirmed that children homozygous for the high-activity Val allele exhibited early-onset, pervasive, and persistent antisocial behavior and were convicted of a disproportionate share of crimes as adults.

METHODS

SAMPLES

Participants in the first sample were drawn from the Cardiff ADHD Genetic Study. This sample of 376 children of white British origin had been drawn from child psychiatry and pediatric clinics across northwest and southwest England and Wales between 1997 and 2003. Genotyping for COMT was completed for the first set of individuals collected, which consisted of 241 children (214 boys and 27 girls [the expected sex distribution for clinic cases with this diagnosis]) aged 5 to 14 years (mean age, 9 years 3 months [SD, 2 years 2 months]) who met DSM-IV criteria for ADHD or International Statistical Classification of Diseases, 10th Revision (ICD-10), criteria for hyperkinetic disorder. Data on COMT and antisocial behavior in the Cardiff ADHD Genetic Study have been previously reported, but were reanalyzed herein to facilitate cross-study comparisons and pooled analysis.

Participants in the second sample were members of the Environmental Risk (E-Risk) Study, which tracks the development of a birth cohort of 2232 British children. This E-Risk sample was drawn from a larger 1994-1995 birth registry of twins born in England and Wales. The E-Risk sample was constructed in 1999-2000, when 1116 families with same-sex 5-year-old twins (93% of those eligible) participated in home-visit assessments, forming the base cohort for the longitudinal E-Risk Study. Details about the sample are reported elsewhere and have been described in the Archives. At the assessment when the children were aged 5 years, with parents’ permission, questionnaires were posted to the children’s teachers, who returned questionnaires for 94% of children. Two years later, a follow-up home visit was conducted for 98% of the 1116 E-Risk families when the children were aged 7 years and teacher questionnaires were obtained for 91% of the 2232 E-Risk twins (93% of those followed up). Because each study family contains 2 children, all statistical analyses in this report were corrected conservatively for the nonindependence of the twin observations by using tests based on the sandwich or Huber-White variance estimator (Stata, version 8.2; Stata Corp, College Station, Texas).

Participants in the third study were members of a New Zealand birth cohort study (the Dunedin Longitudinal Study), which tracks the development of 1037 children. This sample was constituted at age 3 years when the investigators enrolled 91% of consecutive 1972-1973 births in Dunedin, New Zealand. Cohort families represent the full range of socioeconomic status in the general population of New Zealand’s South Island. Details about the sample are reported elsewhere. Follow-up has been carried out at ages 5, 7, 9, 11, 13, 15, 18, 21, 26, and most recently at 32 years when 96% of the living cohort members were assessed.

ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

In the Cardiff ADHD Genetic Study, at baseline all children included in the study met DSM-IV criteria for ADHD or ICD-10 criteria for hyperkinetic disorder (89% male). Symptoms of ADHD and comorbid disorders were assessed using the Child and Adolescent Psychiatric Assessment, a semi-structured research diagnostic interview. Symptom reports were obtained before starting treatment with medication. Both DSM-IV and ICD-10 require the presence of symptoms or impairment in more than one setting. This criterion was assessed using information from the Child Attention-Deficit Hyperactivity Disorder Teacher Telephone Interview.

In the E-Risk Study, ADHD was ascertained on the basis of mother and teacher reports at ages 5 and 7 years (1999-2002). In the mothers’ interviews, their children’s symptomatology was assessed with 18 items concerning hyperactivity, impulsivity, and inattention, representing symptom criteria for ADHD specified by DSM-IV (eg, “very restless, has difficulty staying seated for long,” “impulsive, acts without thinking,” “inattentive, easily distracted”). Symptoms were reported for the preceding 6 months. Teachers rated the same set of items. A research diagnosis of ADHD was made following DSM-IV criteria: children received the diagnosis if they had 6 or more of the hyperactivity/impulsivity symptoms or 6 or more of the inattention symptoms according to either the mother or teacher report. In addition, the other rater had to indicate 2 or more symptoms to ensure pervasiveness across home and school. Onset before age 7 years was required. The prevalence of this research diagnosis of ADHD was 8% (70% male).

In the Dunedin Longitudinal Study, ADHD was ascertained on the basis of child, mother, and teacher reports. At ages 11, 13, and 15 years (1983-1988), adolescents’ symptoms were measured with the Diagnostic Interview Schedule for Children–Child Version, with a reporting period of 12 months at each age. Interviews were conducted by a psychiatrist or clinical psychologist in private, standardized sessions. In addition, each adolescent’s parent and teacher completed ADHD symptom scales that were used to confirm the diagnosis, ensure pervasiveness of the symptoms, and confirm onset of the disorder before age 7 years. A research diagnosis of ADHD was made based on the (then) current DSM-III criteria. The prevalence of this research diagnosis of ADHD was 6% (80% male).

ANTISOCIAL OUTCOMES

In the Cardiff ADHD Genetic Study, assessments of conduct disorder symptoms were gathered using the parent version of the Child and Adolescent Psychiatric Assessment. The DSM-IV conduct disorder symptoms were coded as present or absent and summed to yield a total antisocial symptom score. Items included behaviors such as “physical cruelty to other people,” “sets fires,” “nontrivial stealing,” and “crime involving confrontation with the victim.” All DSM-IV conduct disorder symptoms in this sample were childhood onset (onset < 10 years).

In the E-Risk Study, aggressive behavior at age 7 years was assessed using the parent and teacher versions of the Child Behavior Checklist, the most widely used measure of children’s behavior problems. Mother and teacher reports of children’s...
aggressive behavior problems were totaled to create a measure that reflects pervasive aggressive behavior across settings. Sample items from the aggression subscale include “physically attacks people,” “destroys things that belong to others,” and “gets in many fights.”

In the Dunedin Longitudinal Study, we analyzed a composite index of antisocial behavior in adolescence and in adulthood (aged ≤26 years) that counts the number of antisocial outcomes observed for each study member, including whether a study member (1) met diagnostic criteria for adolescent conduct disorder, (2) was convicted of a violent crime, (3) had elevated scores on a self-reported disposition toward violence measure, or (4) had elevated scores on informant reports of antisocial symptoms. Details about this index are provided in an article by Caspi et al.29 In addition, the Dunedin cohort has now been followed up to age 32 years, enabling us to test whether genotype accounted for heterogeneity in criminal offending through that age. We obtained conviction data by searching the computerized New Zealand Police database, with the informed consent of the study participants. Computerized records covered all courts in Australia, New Zealand, and the surrounding islands. Twenty percent of study participants had been convicted of a criminal offense, including nonviolent (eg, drug trafficking, theft, burglary) and violent (eg, assault, rape, robbery, manslaughter) offenses; traffic offenses were excluded.

DNA EXTRACTION AND GENOTYPING

In the Cardiff ADHD Genetic Study, DNA was obtained from venous blood or mouthwash samples from all participants. In the E-Risk Study, DNA was obtained via buccal swabs from 90% of participants. In the Dunedin Longitudinal Study, DNA was obtained from 97% of participants (93% via blood and 7% via buccal swabs). To avoid potential problems of population stratification, DNA from Dunedin cohort members of Maori origin was not included. Genotyping protocols are summarized in “Supplementary Methods” (available at http://archpsyc.ama-assn.org). Allele frequencies in all samples were consistent with reported allele frequencies in white individuals.10 Participants in each sample were split into 3 groups on the basis of genotype: individuals homozygous for the low COMT–activity allele (Met/Met, 25% of the Cardiff, 26% of the E-Risk, and 25% of the Dunedin cohorts), individuals homozygous for the high COMT–activity allele (Val/Val, 21% of the Cardiff, 25% of the E-Risk, and 25% of the Dunedin cohorts), and heterozygotes (Val/Met, 44% of the Cardiff, 49% of the E-Risk, and 50% of the Dunedin cohorts). We have previously demonstrated that there is no evidence for significant association between the COMT Val158Met variant and ADHD in the Cardiff sample, using family-based association analysis (χ²=0.02, P=.88).31 Similarly, the COMT Val158Met variant was also not associated with ADHD in the E-Risk cohort (χ²=0.18, P=.91). The percentage of children meeting diagnostic criteria for ADHD in each genotype group was 8% in the Met/Met, 8% in the Val/Met, and 7% in the Val/Val genotypes (nor in the Dunedin cohort (χ²=0.50, P=.78) the percentage of children meeting diagnostic criteria for ADHD in each genotype group was 5% in the Met/Met, 6% in the Val/Met, and 5% in the Val/Val genotypes).

STATISTICAL ANALYSIS

Association with antisocial behavior was tested using multiple regression analysis. Possession of at least 1 Met allele (vs the Val/Val genotype) was the independent (predictor) variable. To test whether the association of the Val158Met variant with antisocial behavior was stronger among children with diagnosed ADHD than among children without ADHD, an interaction term (ADHD × COMT Val158Met) was also included. We used ordinary least squares regression to analyze continuous outcome measures and binomial regression to analyze categorical outcome measures. For all samples, genotyping was performed blind to phenotype and the hypothesis of this study. Study protocols were approved by the institutional review boards of the participating universities, and informed consent was obtained from study participants.

Among ADHD cases in the Cardiff sample, we observed a significant association between COMT Val158Met and the total number of conduct disorder symptoms (Figure 1). Individuals homozygous for the Val allele had significantly more conduct disorder symptoms than Met allele carriers (b=0.43, SE=0.21, t=2.02, P=.05). Haplotypes at COMT have been reported to be associated with alteration in COMT expression and thus may modify the functional effects at the Val/Met locus.32 We therefore examined the 2 other markers in COMT (rs737865 near exon 1 and rs165599 near the 3′-untranslated region), which, together with the Val158Met variant (rs4680), define those haplotypes. Neither marker alone was found to be associated with ADHD in a previous analysis of this sample,33 nor were haplotypes constructed from any combination of the 3 markers. Similarly, neither marker showed a trend for association with childhood conduct disorder symptoms (rs737865, b=−0.23, SE=0.17, t=1.36, P=.17; rs165599, b=−0.12, SE=0.18, t=0.68, P=.50). These findings indicated that the COMT Val158Met variant was associated with antisocial behavior in this sample.

We turned to the E-Risk Study to test replication of findings in the Cardiff clinical sample. The E-Risk cohort faithfully represents population heterogeneity within ADHD cases and within children without ADHD. The cases were not subject to factors that could bias recruit-
ment into clinic-identified samples, and the children without ADHD represent the distribution of aggression in the non-ADHD population. This design thus allowed us to test whether COMT is a susceptibility gene for aggression specifically within children with ADHD or more generally in the entire population. We compared aggressive behavior in children as a function of the COMT Val158Met variant (Figure 2). Among children with diagnosed ADHD, those homozygous for the Val allele were characterized as having significantly more aggression than Met allele carriers \( (b=6.20, SE=3.03, \text{t} = 2.05, P = .04) \). In contrast, among children without ADHD, there was no significant association between the Val158Met variant and aggression \( (b=-0.49, SE=0.56, \text{t} = 0.88, P = .38) \). The association between the Val158Met variant and aggression was significantly stronger among children with ADHD than those without \( (b=6.69, SE=3.05, \text{t} = 2.20, P = .03) \), suggesting that the COMT polymorphism is useful as one marker for indexing individuals with ADHD at risk for antisocial behavior but not a susceptibility gene for aggression in the general population.

Next, we examined individuals in the Dunedin cohort, who have been followed to adulthood, to test whether the Val158Met variant modified long-term antisocial outcomes among children with ADHD. We compared the composite measure of antisocial behavior in children as a function of the COMT Val158Met variant. Among children with diagnosed ADHD, those homozygous for the Val allele had higher mean scores on the composite index of antisocial behavior than Met allele carriers \( (b=0.98, SE=0.44, \text{t} = 2.25, P = .03) \) (Table 1). In contrast, among children without ADHD, there was no significant association between the Val158Met variant and antisocial behavior \( (b=0.068, SE=0.076, \text{t} = 0.89, P = .37) \). The association between the Val158Met variant and antisocial behavior was significantly stronger among children with ADHD than those without \( (b=0.91, SE=0.34, \text{t} = 2.71, P = .007) \). In addition, in subsets of children, we compared their adult criminal behavior through age 32 years as a function of the COMT Val158Met variant (Figure 3). Among individuals with diagnosed ADHD, those homozygous for the Val allele were 2.3 times (95% confidence interval, 1.3-4.2) more likely to have been convicted of a crime than Met allele carriers \( (b=0.83, SE=0.30, z=2.76, P = .006) \). In contrast, among children without ADHD, there was no significant association between the Val158Met variant and criminal behavior \( (b=-0.01, SE=0.18, z=0.06, P = .95) \). The association between the Val158Met variant and criminal behavior was significantly stronger among children with ADHD than among children without \( (b=0.85, SE=0.35, z=2.41, P = .02) \), confirming that the COMT Val158Met variant is a risk factor for antisocial behavior in ADHD cases but is not a susceptibility gene for antisocial behavior in the general population.

Five points are relevant for interpreting the findings across the 3 samples. First, the COMT Val/Val genotype was not associated with ADHD symptom severity (Table 1) and the association between this genotype and antisocial behavior remained after controlling for ADHD symptom severity (Cardiff cohort, \( b=0.40, SE=0.21, \text{t} = 1.91, P = .06 \); E-Risk cohort, \( b=6.26, SE=2.83, \text{t} = 2.21, P = .03 \); Dunedin cohort, \( b=0.83, SE=0.42, \text{t} = 2.00, P = .05 \)). Second, the COMT Val/Val genotype was not associated with lower IQ scores (Table 1), and the association between this genotype and antisocial behavior remained after controlling for IQ (Cardiff cohort, \( b=0.51, SE=0.20, \text{t} = 2.55, P = .01 \); E-Risk cohort, \( b=5.69, SE=3.03, \text{t} = 1.87, P = .06 \); Dunedin cohort, \( b=0.97, SE=0.44, z=2.19, P = .03 \)). (Post hoc analysis also revealed no association between the COMT genotype and maternal smoking during pregnancy, a putative risk factor for conduct disorder.) Third, the association between the COMT Val/Val genotype and antisocial behavior among children with ADHD was not an artifact of ethnic stratification: The Cardiff ADHD Genetic Study enrolled only white children; our analyses of the New Zealand birth cohort (Dunedin Longitudinal Study) excluded individuals of Maori origin; and in the E-Risk Study, the relationship between genotype risk and antisocial behavior was reestimated excluding non-white children with ADHD \( (n=15) \), yielding nearly identical results \( (b=6.16, SE=3.15, \text{t} = 1.96, P = .05) \). Fourth, the association between the COMT Val/Val genotype and antisocial behavior among children with diagnosed ADHD is unlikely to be because of selective receipt of or in response to medication. In the Cardiff clinical sample, children with the Val/Val genotype were no less likely to have ever received medication than Met carriers \( (\chi^2=0.06, P = .81) \), and among medicated children, there was no association between the Val158Met variant and positive medication response assessed by the Clinical Global Impressions scale \( (b=-0.07, SE=0.11, \text{t} = -0.67, P = .51) \). Fifth, the molecular genetic basis for dividing children with diagnosed ADHD into those with and without risk for antisocial behavior showed some specificity to the COMT Val158Met single nucleotide polymorphism, as the association was not found with polymorphisms in 2 dopamine-system candidate genes widely hypothesized to be relevant in the pathogenesis of ADHD and its clinical
features: the 10-repeat allele of a variable number of tandem repeats in the 3′-untranslated region of the dopamine transporter gene (DAT1) and the 7-repeat allele of a variable number of tandem repeats polymorphism in the dopamine D4 receptor gene (DRD4). In contrast to COMT Val158Met, these were not consistent, significant predictors of antisocial behavior in children with ADHD across our 3 samples (Table 2).

**COMMENT**

We found evidence in 3 independent studies that heterogeneity, in terms of antisocial behavior, among children with diagnosed ADHD is associated with variation in the COMT gene. Pooling results across these 3 samples, along with results from a Canadian clinical study (the Douglas Hospital Study39), the mean effect size for the difference in antisocial behavior between COMT Val/Val homozygotes and Met carriers was 0.32 (95% confidence interval, 0.05-0.59; \( z = 2.30; P = .02 \)) (Figure 4). The replicability is notable for 3 reasons: Val/Val homozygotes were observed to be more antisocial than Met carriers in both clinic-referred and community samples; were assessed using different, albeit age-appropriate, measures of the same putative antisocial phenotype; and were assessed in different stages of life. The replicability is not perfect, and a false-positive association cannot be ruled out with certainty. Future studies can build on the meta-analysis reported here to refine the estimate of the association between COMT and antisocial behavior among children with ADHD.
The neurobiological route by which the observed COMT effect is achieved remains speculative. One possibility is that COMT is related to impaired executive functions. The COMT gene is relevant for dopamine metabolism in the PFC, and is a candidate gene for modulating PFC executive functions. Emerging imaging data are consistent regarding the importance of the COMT variant in PFC function, which is impaired in those with antisocial behavior and ADHD. Executive dysfunctions interfere with children's ability to control their own behavior, impairing them to consider the future implications of their acts. Such children may have difficulty understanding the negative effect their behavior has on others, fail to hold abstract ideas of ethical values and future rewards in their minds, and fail to inhibit inappropriate behavior or adapt behavior to changing social circumstances. For this reason, Sapolsky has noted that the PFC “is the closest thing we possess to a superego.” This hypothesis merits further scrutiny, using developmentally appropriate measures of prefrontally guided behaviors, though initial reports, including data from the Cardiff ADHD Genetic Study, did not find an association between the COMT genotype and several tests of executive functioning. Another possibility is that the COMT variant reflects a genetic predisposition that contributes to emotional dysregulation. Imaging findings suggest that COMT Val alleles are related to reduced responsiveness to unpleasant stimuli, which may be a marker of aggressive, sometimes violent, behavior in a subset of individuals.

Importantly, COMT does not appear to be a susceptibility gene for aggression or antisocial behavior; there was no evidence of an association between the COMT ValMet variant and antisocial behavior (nor with ADHD) in the general population. Rather, the COMT ValMet variant influenced phenotypic variation within children with ADHD and predicted which of these chil-
children would engage in antisocial behavior. That the association between genotype and antisocial behavior was conditional on ADHD diagnosis suggests that the involvement of COMT in antisocial behavior relies on the interaction(s) with other genes or other etiological factors involved in ADHD (hence, the association between genotype and antisocial behavior was not observed among children without ADHD). Such a situation is anticipated by animal research showing that genetic variants are associated with highly variable phenotypes depending on genetic background (eg, knockouts lead to different phenotypic effects in different strains); COMT may thus operate as a modifier gene, acting against a background of other etiological factors to affect clinical features and ADHD course rather than as a direct susceptibility gene. For example, in the Carduff sample, the association between COMT and antisocial behavior is most pronounced among children with low birth weight, but given the relatively small number of ADHD cases, we could not explore this possibility in the 2 birth cohort studies.

The clinical implications of these findings are premature. However, the results illustrate how genetic information may provide biological evidence in favor of clinical subtypes. Disorders such as ADHD are diagnosed on the basis of symptom syndromes only. However, children with identical core symptoms often differ markedly on associated clinical features, treatment response, prognosis, and presumably etiology. Currently, ICD-10 distinguishes hyperkinetic conduct disorder among hyperkinetic disorders, whereas DSM-IV does not. Our findings confirm the presence of genetic heterogeneity in ADHD, suggesting that ADHD may consist of clinically and biologically validated subgroups, some of which are at high risk for antisocial behavior and may warrant more vigorous treatment, and that these subgroups arise through the action of different genes and etiological pathways. Ultimately, knowledge of the molecular etiology of the ADHD family may become a useful tool for assigning risk and designing preventions.

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