High loading of polygenic risk for ADHD in those with comorbid aggression

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**Conflicts of Interest**
The following report no conflicts of interest


**Reports of conflict of interest:**

In the past year, Stephen Faraone received consulting income and/or research support from Shire, Otsuka and Alcobra and research support from the National Institutes of Health (NIH). He is also on the Clinical Advisory Board for Akili Interactive Labs. In previous years, he received consulting fees or was on Advisory Boards or participated in continuing medical education programs sponsored by: Shire, McNeil, Janssen, Novartis, Pfizer and Eli Lilly. Dr. Faraone receives royalties from books published by Guilford Press: Straight Talk about Your Child’s Mental Health and Oxford University Press: Schizophrenia: The Facts.
H.C. Steinhausen has worked as an advisor and speaker for the following pharmaceutical companies: JanssenCilag, Eli Lilly, Novartis, Medice, Shire, and UCB. In the past, he has received unrestricted grants for postgraduate training courses or conferences and research by JanssenCilag, Eli Lilly, Novartis, Medice, and Swedish Orphan International.

H. Roeyers is a member of an Advisory Board to Shire and has received research funding and conference attendance support from Shire and Eli Lilly.

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Edmund SonugaBarke has been on recent speaker boards for Shire, UCB Pharma. Current & recent consultancy: UCB Pharma, Shire. Current & recent research support: Janssen Cilag, Shire, Qbtech, Flynn Pharma and been on the Advisory Board for Shire, Flynn Pharma, UCB Pharma, Astra Zeneca. Conference support: Shire.
Aribert Rothenberger served on the Advisory Board and Speakers Bureau for: Lilly, Shire, Medice and Novartis and has received Research Support from Shire, German Research Society, Schwaabe, Travel Support from Shire, an Educational Grant from Shire and acts as a Consultant for UCB/Shire and Lilly.

Joseph Biederman is currently receiving research support from the following sources: Elminda, Janssen, McNeil, and Shire. In 2012, Joseph Biederman received an honorarium from the MGH Psychiatry Academy and The Children’s Hospital of Southwest Florida/Lee Memorial Health System for tuition-funded CME courses. In 2011, Dr. Joseph Biederman gave a single unpaid talk for Juste Pharmaceutical Spain, received honoraria from the MGH Psychiatry Academy for a tuition-funded CME course, and received honoraria for presenting at international scientific conference on ADHD. He also received an honorarium from Cambridge University Press for a chapter publication. Dr. Biederman received departmental royalties from a copyrighted rating scale used for ADHD diagnoses, paid by Eli Lilly, Shire and AstraZeneca; these royalties are paid to the Department of Psychiatry at MGH. In 2010, he received a speaker’s fee from a single talk given at Fundación Dr.Manuel Camelo A.C. in Monterrey Mexico. Dr. Biederman provided single consultations for Shionogi Pharma Inc. and Cipher Pharmaceuticals Inc.; the honoraria for these consultations were paid to the Department of Psychiatry at the MGH. Dr. Biederman received honoraria from the MGH Psychiatry Academy for a tuition-funded CME course. In previous years, Dr. Joseph Biederman received research support, consultation fees, or speaker’s fees for/from the following additional sources: Abbott, Alza, AstraZeneca, Boston University, Bristol Myers Squibb, Celltech, Cephalon, Eli Lilly and Co., Esai, Fundacion Areces (Spain), Forest, Glaxo, Gliatech, Hastings Center, Janssen, McNeil, Medice Pharmaceuticals (Germany), Merck, MMC Pediatric, NARSAD, NIDA, New River, NICHD, NIMH, Novartis, Noven, Neurosearch, Organon, Otsuka, Pfizer, Pharmacia, Phase V Communications, Physicians Academy, The Prechter Foundation, Quantia Communications, Reed Exhibitions, Shire, the Spanish Child Psychiatry Association, The Stanley Foundation, UCB Pharma Inc., Veritas, and Wyeth.
Jan K Buitelaar has in the past 3 years been a consultant to / member of advisory board of / and/or speaker for Janssen Cilag BV, Eli Lilly, BristolMyer Squibb, Shering Plough, UCB, Shire, Novartis and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties.

Christine Freitag has been on the Speaker’s Bureau of Eli Lilly and Novartis during the last 3 years.
Abstract

Objective: Although ADHD is highly heritable, genomewide association studies (GWAS) have not yet identified any common genetic variants that contribute to risk. There is evidence that conduct disorder/aggression in those with ADHD indexes higher genetic loading and clinical severity. Here, we investigate whether common genetic variants considered en masse as polygenic scores for ADHD are especially enriched in those with comorbid conduct disorder.

Method: Polygenic scores derived from an ADHD GWAS metaanalysis were calculated in an independent ADHD sample (452 cases 5,081 controls). Multivariate logistic regression analyses were employed to compare polygenic scores in the ADHD group and controls and test for higher scores in ADHD cases with comorbid conduct disorder vs. controls and vs. those without comorbid conduct disorder. Association with symptom scores was tested using linear regression.

Results: Polygenic risk for ADHD, derived from the metaanalysis, was higher in the independent ADHD sample than in controls (p=0.010). Polygenic score was significantly higher in ADHD cases with conduct disorder compared to ADHD cases without conduct disorder (p=0.013). ADHD polygenic score showed significant association with comorbid conduct disorder symptoms. This relationship was explained by the aggression items (β=0.151, t=3.152, p=0.002).

Conclusions: Common genetic variation is relevant to ADHD, especially in those with comorbid aggression. The findings suggest that the previously published ADHD GWAS metaanalysis contains weak but true associations to common variants, support for which falls below genomewide significance levels. The findings also highlight that aggression in ADHD indexes genetic as well as clinical severity.
Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is an earlyonset highly heritable neurodevelopmental disorder, characterised by marked clinical heterogeneity and a preponderance of affected males (24). It has a complex genetic architecture, as is the case for most common disorders. Although associated rare variants have been identified (58), it has generally been considered that the most satisfactory explanatory model of inheritance is a multifactorial, polygenic liability threshold one, where the combined effects of multiple common genetic variants with environmental factors contribute to ADHD risk.

With respect to common risk variants, there are no genomewide significant findings for ADHD (1, 9). Although it has been proposed that this simply reflects inadequate sample sizes (6, 10), others suggest the lack of findings is a consequence of psychiatric disorders, including ADHD, being explained mainly or solely by rare high penetrance variants (11). So far, for ADHD, rare variants in the form of copy number variants (CNVs) have been found to be associated (58), and studies show that other classes of rare mutations make some contribution to autism spectrum disorder (ASD) (1214), another childhoodonset disorder that strongly overlaps with ADHD (15). While the issue about the relative contributions of common and rare variants is very far from being empirically resolved, it has previously been shown through pathway analytic approaches that not only do both contribute to ADHD, they tend to act on similar functional classes of genes (6, 16).

Given a contribution from both common and rare alleles (and presumably also alleles with intermediate frequencies), the multifactorial, polygenic liability threshold model predicts that forms of the disorder in groups of people less often affected (e.g. ADHD in females) or with more severe forms of the disorder should carry a greater genetic load, including greater enrichment of ADHD common risk alleles.
The presence of conduct disorder in those with ADHD is known to index greater clinical severity (17). Previous twin and family studies have also shown that in those with ADHD, the presence of conduct disorder/symptoms indexes higher ADHD familial and genetic loading (18-22). For example, the relative risk (RR) for ADHD in biological relatives of probands who have ADHD that is accompanied by Conduct Disorder (RR 9.5), is almost double that of relatives of those with ADHD alone (RR 5.4) (18). These studies suggest that in ADHD, the presence of conduct disorder likely indexes greater genetic load. Previously, it has only been possible to infer this indirectly by measuring recurrence rates in various classes of relatives, but recent developments now allow the component attributable to relatively common alleles to be estimated using genomewide molecular genetic data in the form of polygenic load (23-25). In the present study, using ADHD polygenic risk scores derived from the largest published genomewide association metaanalysis (1), we set out to test in an independent sample whether ADHD accompanied by conduct disorder is characterised by greater enrichment of ADHD “risk alleles” and also to investigate the relationship between polygenic score and conduct disorder symptoms.

Methods

Subjects

Participants for what we term in this study the “Cardiff Sample” were recruited from Child and Adolescent Mental Health Services or Community Paediatric outpatient clinics in the UK. The sample of 452 children met criteria for a lifetime diagnosis of DSMIIIIR or DSMIV ADHD, confirmed by a research diagnostic assessment (26). Children with bipolar disorder, schizophrenia, ASD, Tourette’s syndrome, IQ<70 (assessed using the WISCIII/IV) (27, 28), epilepsy or any other neurological or genetic disorder were excluded. Written informed consent from parents and assent/consent from children was obtained for all individuals. The study protocol was approved by NorthWest England and Wales Multicentre Research Ethics Committees. The control sample comprised 5,081 individuals from the Wellcome Trust Case Control Consortium – Phase 2 (WTCCC2).
Clinical measures

For the Cardiff sample, ADHD diagnoses were confirmed using the Child and Adolescent Psychiatry Assessment (26), a research diagnostic interview undertaken with the child's parents. Interviews were completed by trained psychologists, supervised weekly by a child psychiatrist and a psychologist. Interrater reliability for diagnosis of ADHD subtype assessed using 60 cases, was excellent ($\kappa=1.0$). Information on symptom pervasiveness and school impairment in school was obtained using the Child ADHD Teacher Telephone Interview (29), the DuPaul (30) or Conner’s teacher rating scales (31).

The Child and Adolescent Psychiatric Assessment or CAPA was also used to assess comorbid psychiatric disorders. Interrater reliability for parentrated conduct disorder symptoms was very good (intra class correlation=0.98). Summed scores for total number of DSMIV conduct disorder symptoms were obtained in addition to diagnoses. In line with previous studies and factor analyses (32, 33), aggressive (DSMIV conduct disorder criteria labelled as “aggression to people and animals”) and covert (DSMIV conduct disorder criteria labelled as “Destruction of property” or “Deceitfulness or theft”) symptom totals were generated (see Appendix 1 for a full list of DSMIV aggressive and covert conduct disorder symptoms).

Genotyping

DNA was obtained through saliva and blood samples. Genotyping for cases was performed on the Illumina (San Diego, CA, USA) Human660WQuad BeadChip. Genotyping for controls was performed using the Illumina Human 1.2M BeadChip. The samples, quality control assessment, and GWAS results are described in detail elsewhere (6).

Published data used to derive ADHD polygenic risk scores

We made use of the international metaanalysis of ADHD vs. control data described in detail elsewhere (1). This data set contains 2,064 trios, 896 cases and 2,455 control individuals and 1,206,461 single nucleotide polymorphisms (SNPs). Cases had been assessed with the same inclusion criteria and similar methods to the cases in the Cardiff study. A total of 54 individuals affected with ADHD were removed from the Cardiff sample as they
had been included in the metaanalysis (1). Control groups were not overlapping.

**Polygenic Analysis**

We used the analytic approach described by the International Schizophrenia Consortium (25) with the published metaanalysis of ADHD (1) as the discovery data set and Cardiff sample ADHD data as the target set. Each individual in the target set was assigned a polygenic score, based on information in the discovery set. We made comparisons between ADHD cases (with and without conduct disorder) and controls to determine whether the ADHD-derived polygenic scores were significantly different. As comorbid conduct disorder indexes higher ADHD familial loading, our key aim was to test whether polygenic scores for ADHD were higher in ADHD cases with vs. without conduct disorder.

We first selected a set of SNPs in relative linkage equilibrium in our ADHD and control samples using a sliding window of 200 SNPs, moving it along the genome in steps of 5 SNPs and dropping a SNP when the pairwise estimate of linkage disequilibrium ($r^2$) was greater than 0.2 (PLINK command: indeppairwise 200 5 0.2) (34). In the discovery metaanalysis data set, we identified corresponding p-values and associated alleles for the selected SNPs. Based on the findings from the International Schizophrenia Consortium (25) and Psychiatric Genetics Consortium (23, 24), which identified a relaxed $p<0.5$ as the optimal ‘association’ threshold in discovery samples of the size equivalent to those used here, we a priori defined this as the threshold from which to derive score alleles from our discovery sample. Alleles that are more common in the discovery cases at $p<0.5$ for two-tailed p-values are termed the “score” alleles (34). For each individual in the target sample, we used PLINK (34) to obtain a polygenic score which corresponds to the mean number of score alleles across the set of SNPs. We employed logistic regression analysis to compare the polygenic scores for ADHD in the target set to the controls. To allow for population stratification in our data, we conditioned on two covariates (the first two principal components estimated from our GWAS data using EIGENSTRAT software designed for this purpose (35, 36)). We are a priori hypothesised that ADHD “risk alleles” derived from the published GWAS would be enriched in our independent ADHD sample (compared to controls), and in particular in those with conduct disorder. Therefore for each analysis we report a onetailed pvalue and pseudoR$^2$, the latter being a measure of the estimated variability in casecontrol status explained.
Using logistic regression analysis, we then compared polygenic score in those with and without a conduct disorder diagnosis from the set of ADHD cases (ADHD with conduct disorder vs. ADHD without conduct disorder).

Linear regression analyses were used to investigate whether polygenic score was significantly associated with total DSM-IV conduct disorder symptom score, as well as aggression and covert conduct disorder symptom scores (see Appendix 1 for a list of the items). Our rationale for further dividing conduct disorder symptoms into two subgroups was based on factor analyses (33), showing that they can be split into aggressive symptoms (such as cruelty to people or animals, fighting and stealing with confrontation of the victim) and covert symptoms (such as firesetting, breaking into a building or car, or vandalism).

Results

A total of 452 children from the Cardiff sample met inclusion criteria and had genetic and phenotypic data available. They were aged 6-17 years (mean=10.7 years, SD=2.8 years), of whom 389 were male (86.1%) and 63 were female (13.9%). The mean full-scale IQ was 87.13 (SD=11.24). The gender ratio and IQ scores are typical of UK ADHD clinic cases. The mean number of ADHD symptoms was 14.68 (SD=2.87, 25th percentile ADHD score =13.00, 50th percentile=15.00 75th percentile=17.00). Within this sample, 77 (17.0%) individuals had a diagnosis of ADHD with conduct disorder and 375 (83.0%) had no comorbid conduct disorder. The mean numbers of DSM-IV conduct disorder symptoms were 3.60 (SD=1.86) and 0.53 (SD=0.76) for the ADHD groups with conduct disorder and without conduct disorder, respectively. There was no association between conduct disorder scores, age and gender. In addition, 229 (50.7%) subjects met criteria for a DSMIV diagnosis of Oppositional Defiant Disorder; 65 of those with a conduct disorder diagnosis (84.4%) also had a diagnosis of Oppositional Defiant Disorder. A comorbid diagnosis of anxiety disorder was present for 22 individuals (4.9%) whilst comorbid depressive disorder was present in 3 individuals (0.66%).

Polygenic scores predicting ADHD in the target sample

ADHD risk as defined from weakly associated alleles (n=46,156) in the discovery GWAS was significantly higher in ADHD cases than controls (p=0.010) (Table 1). Thus, as we postulated, risk for ADHD is in part attributable to common alleles tagged by the genomewide genotyping arrays.
Polygenic score enrichment in those with ADHD accompanied by conduct disorder

The polygenic score representing ADHD risk was significantly higher in ADHD cases with a conduct disorder diagnosis compared to ADHD cases without conduct disorder (p=0.013). The magnitude of the effect (as defined by $R^2$) was 1.1%, larger than that observed when comparing ADHD cases and controls (Table 1).

To test if our findings could be attributable to higher ADHD total symptom count in those with conduct disorder, we tested association between ADHD symptom count and polygenic risk score. Within ADHD cases, the total number of ADHD symptoms was not significantly associated with polygenic score ($\beta=0.018$, t=0.374, p=0.709). As expected, total ADHD scores were significantly associated with total conduct disorder scores ($\beta=0.159$, t=-2.900, p=0.004). ADHD polygenic risk scores were significantly higher in female cases than male cases ($\beta=0.104$, t=2.159, p=0.031) and thus all the data were reanalysed allowing for sex as a covariate. The results were unchanged (data not shown).

Polygenic score predicting conduct disorder symptom scores

Within cases, ADHD polygenic risk scores increased with total conduct disorder scores ($\beta=0.118$, t=2.530, p=0.006). At the level of individual composite phenotypes, they also increased with aggressive conduct disorder symptoms ($\beta=0.151$, t=3.152, p=0.002), but not covert conduct disorder symptoms ($\beta=0.045$, t=0.922, p=0.357). These associations remained significant when controlling for sex. The distribution of risk scores showed increasing polygenic score with respect to increasing total conduct scores (Figure 1).

Discussion

We initiated this study to investigate the contribution of common genetic variants to ADHD and to test whether comorbid conduct disorder, defined categorically and dimensionally, indexed greater genetic risk at a molecular level. Our data support a polygenic component to ADHD, in that the risk score was higher in our independent sample of ADHD cases than the controls. This is the first report to suggest that the previously published ADHD GWAS metaanalysis (1) harbours common risk alleles that do show contribution to ADHD, when they are considered en masse. More importantly, as studies which suggest that comorbid conduct disorder indexes higher familial and genetic loading in ADHD, we found that ADHD risk score is particularly elevated in those with ADHD and conduct disorder compared with those who have just ADHD.
A within case analysis of conduct disorder symptoms as a dimension rather than a category revealed similar findings, there being a positive linear relationship between ADHD polygenic scores and comorbid conduct disorder symptoms. Interestingly, this association related to aggressive, rather than covert, conduct disorder symptoms (see Appendix 1). Twin studies also suggest that these different symptom dimensions may be distinct in their genetic aetiology, with stronger genetic loading for overt aggressive symptoms (32, 37).

Overall, our study confirms the hypothesis that common genetic variants are relevant to ADHD risk. The findings of the present study also highlight that comorbid conduct disorder indexes heterogeneity in terms of genetic loading at a molecular level. Our findings that individual symptom groups of total conduct disorder scores and aggressive conduct disorder scores are significantly associated with polygenic score further underscore the point that specific clinical phenotypes can index differential genetic loading. Most of the evidence to date suggests that conduct problems index ADHD cases that are quantitatively rather than qualitatively different from the remaining ADHD cases (38), in terms of the patterns of association with clinical, cognitive, genetic and environmental correlates. This is in keeping with the approach currently taken by ICD10, where Hyperkinetic conduct disorder is considered a subtype of ADHD/Hyperkinetic Disorder (39). However, some associated factors also appear to be unique to conduct disorder in ADHD. Notably, the functional COMT Val158Met variant has been found to be associated with conduct problems in ADHD (40), a finding that has been replicated in six independent samples (32, 4143), while metaanalysis shows that this variant is not associated with ADHD risk (44). A separate future question of interest would be to investigate the independent contribution of genetic liability associated with conduct disorder (regardless of ADHD). There is evidence to suggest shared liabilities but as yet, large scale Conduct Disorder GWAS data sets are unavailable.
We also incidentally found that female cases had significantly higher ADHD risk scores than male cases. This finding requires replication, but is intriguing as it supports the hypothesis that ADHD is less common in females because a more extreme genetic load is required for the liability threshold to be surpassed. This would predict that relatives of female ADHD probands have a greater risk for ADHD than relatives of male ADHD probands, although evidence here is lacking (4). However, there is the possible impact of ascertainment bias. Females with ADHD may be less likely to be diagnosed (or referred for diagnosis) than males with comparable severity of disorder (45) and therefore our findings might simply reflect that females must have more severe forms of the disorder to be diagnosed and ascertained. However, females in our sample did not have significantly more ADHD (p=0.919) or conduct disorder symptoms (p=0.511) than males.

As expected, ADHD severity (i.e. total ADHD scores) showed association with conduct disorder scores, but the genetic findings were driven by conduct disorder as there was no association between ADHD severity and polygenic risk. As all clinical cases have high ADHD scores by definition, and thus variance is limited, this may not be surprising. It is of note that case control comparisons were significant for the total ADHD group and the ADHD with conduct disorder group but did not achieve significance in the ADHD without conduct disorder group. This might simply reflect sample size, whereby groups with lower genetic load (those with ADHD without conduct disorder) would need larger samples than those with higher genetic load (those with ADHD with conduct disorder) to demonstrate statistically significant differences.

This paper utilised a well characterised sample of children who underwent careful phenotyping. The diagnosis of ADHD was confirmed using a semistructured research diagnostic interview, which also enabled detailed information to be obtained on conduct disorder symptoms and measures showed high reliability. There are no means of obtaining clinical data on the controls, although failure to exclude controls with ADHD or conduct disorder would reduce our power, not generate false positives. One limitation of case control and within-case studies is the possibility of population stratification. However, we limited the impact of stratification by the well-accepted approach of including derived principal components that allow for population variation (35, 36) in our ADHD GWAS, and by using hypothesis driven analyses. Furthermore, for stratification to be an issue, it would have to be refractory to inclusion of the principle components and the same uncorrected ethnic variation be overrepresented in the cases in both the Cardiff sample and the samples used in the metaanalysis (46), and would
also have to be specifically overrepresented in those with comorbid conduct disorder compared with the full ADHD sample.

The magnitudes of the plygenic effect (as defined by $R^2$) are small, as typically found using this method. The magnitude is also smaller than some figures published for schizophrenia ($R^2$ = 3 to 6% (24, 25)) but not all (47). This method is also very sensitive to sample sizes and available GWAS data sets for ADHD are very much smaller than is the case for schizophrenia. In the most recent schizophrenia analysis (24) of over 9,000 cases and 12,000 control individuals, the total amount of variance explained was estimated to be 6%, whereas in an earlier analysis of 3,000 cases and 3,000 control individuals by the International Schizophrenia Consortium (25), the estimate was 3%. Our estimate of percentage variance explained of 0.1% is based on a much smaller sample size of 452 ADHD cases and 5,081 control individuals. Another factor that would reduce explained variance is heterogeneity across samples. This could plausibly be higher for some disorders than others; for example, through international differences in clinical service provision and thus, ascertainment as well as other variability such as ethnic composition. It is also important to note that GWAS SNP arrays do not completely capture relevant genetic variation; they “tag” potentially causal variants and the arrays do not capture the full spectrum of allelic frequencies or allele types (e.g. repeat sequence polymorphisms such as variable number tandem repeats). Overall, we expect that as more ADHD and control samples with more comprehensive genetic capture become available, more variance will be explained. Finally, we also acknowledge the contribution of environmental risk factors and gene-environment interplay, although this was not the focus of the present analyses.

These findings suggest that ADHD, like other psychiatric disorders, can be considered a polygenic disorder, the architecture of which includes common as well as rare alleles (10). They are also compatible with our earlier work showing overlap between the biological processes enriched for weak ADHD SNP association signals and those enriched for rare copy number variants (6). Given the evidence for contribution of common variants, the future acquisition and genetic analysis of much larger ADHD samples in an attempt to capture relevant genetic variation is crucial. The aim is not to simply identify single “significant” SNPs of small effect size, but rather to utilise the spectrum of associated common and rare genetic risk variants to uncover novel clues for risk mechanisms and underlying biology and to inform our conceptualisation of ADHD.
In conclusion, common genetic variation appears relevant to ADHD and a higher loading for common ADHD genetic risk variants is indexed by comorbid conduct disorder, especially aggressive symptoms. We also provide support that the previously published ADHD GWAS metaanalysis contains true associations to common variants, support for which falls below currently accepted genomewide significance levels. The findings highlight that aggression in ADHD, as an index of clinical severity, is underpinned by higher genetic loading at a molecular level. They also illustrate, that for hypothesis-driven research, careful phenotyping is still useful in psychiatric genetic studies.


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Table 1: Summary of results using the published ADHD metaanalysis (1) as the discovery data and the Cardiff data set as the target sample. In all analyses, the ADHD cases had more risk alleles than the controls. +CD: diagnosis of conduct disorder, CD: no diagnosis of conduct disorder. All zstatistics are distributed with one degree of freedom and all pvalues are onetailed.

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Figure 1: Box plot showing the distribution of polygenic scores for ADHD by total conduct disorder score. The whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box.