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Sequencing of sporadic Attention-Deficit Hyperactivity Disorder (ADHD) identifies novel and potentially pathogenic *de novo* variants and excludes overlap with genes associated with autism spectrum disorder

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Abstract

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Disclosures: The authors declare that they have no conflicts of interest.

Description of Appendices: Supplemental material includes four tables. Supplemental Table 4 describes clinical and demographic information for the 11 sporadic ADHD probands chosen for trio or quad exome sequencing for discovery of *de novo* variants potentially causative for ADHD. Supplemental Table 2 describes the quality control metrics for the 26 genes associated with ASD or ID that were MIP sequenced in our study. Supplemental Table 3 describes the primer design for validation of SNVs and CNVs identified in the study. Supplemental Table 4 describes SNVs that were identified as *de novo* but were present in other non-family members (e.g., SNV identified in ADHD proband in family 2, but same SNV is also present in the father in family 3).

Web Resources: The URLs for data presented herein are as follows:
1000 Genomes Browser, http://www.1000genomes.org/1000-genomes-browsers
Database of Genomic Variants, http://dgv.tcag.ca/dgv/app/home
ExAC Browser, http://exac.broadinstitute.org/
Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
GEMINI, https://gemini.readthedocs.org/en/latest/
Human Protein Atlas, http://www.proteinatlas.org/
SeattleSeq Annotation 138, http://snp.gs.washington.edu/SeattleSeqAnnotation138/
UCSC Genome Browser, http://genome.ucsc.edu

Attention-Deficit Hyperactivity Disorder (ADHD) has high heritability; however, studies of common variation account for <5% of ADHD variance. Using data from affected participants without a family history of ADHD, we sought to identify *de novo* variants that could account for sporadic ADHD. Considering a total of 128 families, two analyses were conducted in parallel: first, in 11 unaffected parent/affected proband trios (or quads with the addition of an unaffected sibling) we completed exome sequencing. Six *de novo* missense variants at highly conserved bases were identified and validated from 4 of the 11 families: the brain-expressed genes *TBC1D9, DAGLA, QARS, CSMD2, TRPM2,* and *WDR83*. Separately, in 117 unrelated probands with sporadic ADHD, we sequenced a panel of 26 genes implicated in intellectual disability (ID) and autism spectrum disorder (ASD) to evaluate whether variation in ASD/ID-associated genes were also present in participants with ADHD. Only one putative deleterious variant (*Gln600STOP*) in *CHD1L* was identified; this was found in a single proband. Notably, no other nonsense, splice, frameshift, or highly conserved missense variants in the 26 gene panel were identified and validated. These data suggest that *de novo* variant analysis in families with independently adjudicated sporadic ADHD diagnosis can identify novel genes implicated in ADHD pathogenesis. Moreover, that only 1 of the 128 cases (0.8%, 11 exome and 117 MIP sequenced participants) had putative deleterious variants within our data in 26 genes related to ID and ASD suggests significant independence in the genetic pathogenesis of ADHD as compared to ASD and ID phenotypes.

Keywords

attention deficit hyperactivity disorder (ADHD); exome sequencing; molecular inversion probe (MIP) sequencing; sporadic ADHD; autism spectrum disorder (ASD); intellectual disability (ID)

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a childhood onset disorder characterized by symptoms of hyperactivity, inattention, and/or impulsivity, with an estimated pooled prevalence of 3–4% (Erskine et al., 2013). The heritability of ADHD has been estimated to be between 70–90% (Stevenson, 1992; Levy et al., 1997; Faraone et al., 2012). Despite this strong evidence of inheritance of ADHD, genome-wide association studies (GWAS) of ADHD have not found any single nucleotide variant (SNV) to be associated with ADHD at the level of genome-wide significance ($P < 5\times10^{-8}$) (Zayats et al., 2016; Middeldorp et al., 2016; Stergiakouli et al., 2012; Neale et al., 2010). Familial and candidate gene studies have identified numerous significant associations with ADHD; however, SNVs in these genes were not significant in larger GWAS of unrelated participants, and do not account for large amounts of ADHD variance when considered in aggregate (<5%) (Franke et al., 2009; Hawi et al., 2015).

Alternative to the use of GWAS and candidate gene studies of common SNVs, investigators have focused on rare SNVs identified through exome sequencing (Krumm et al., 2014), an approach that has been particularly fruitful for neurodevelopmental phenotypes. This approach was first used to identify pathogenic variants in genes likely causative for Mendelian-inherited disorders, such as Kabuki syndrome (Ng et al., 2010). Later, this

emphasis on rare variants was extended to sporadic (occurring only in the proband, without evidence of inheritance from either parent) (Fischbach and Lord, 2010) and non-syndromic childhood psychiatric disorders, such as autism spectrum disorder (ASD) and intellectual disability (ID). Notably, these disorders - similar to ADHD – had not yielded genome-wide significant SNV associations (Zayats et al., 2016; Middeldorp et al., 2016). Through use of familial data from trios (affected proband and parents) or quads (affected proband, unaffected sibling, parents), investigators identified numerous *de novo* mutational events in the proband that were potentially causative for ASD (O'Roak et al., 2011; O'Roak et al., 2012; Neale et al., 2012; Iossifov et al., 2012; Sanders et al., 2012) or ID (Rauch et al., 2012; de Ligt et al., 2012). Proliferation of this approach to sporadic and non-syndromic ASD and ID has resulted in the identification of numerous candidate genes with potentially causative *de novo* mutations (Krumm et al., 2014). Twenty-six (26) of these candidate genes for ASD and ID currently compose a genetic panel used for rapid and efficient targeted exonic sequencing using molecular inversion probes (MIPs) (Coe et al., 2014).

There is growing evidence of shared genetic etiology for numerous neuropsychiatric disorders. In particular, the Psychiatric Genomics Consortium (PGC) has identified genetic variants within L-type calcium channels, *CACNA1C* and *CACNB2*, which are significantly associated across the five major PGC phenotypes of study: ASD, ADHD, bipolar disorder, schizophrenia, and major depressive disorder (Smoller et al., 2013). In addition, there has been increasing scrutiny of the symptom overlap between ASD and ADHD (Mayes et al., 2011), with investigators suggesting candidate genes that may underlie risk for both disorders (Rommelse et al., 2010). Despite this speculation, few studies have yet evaluated the potential shared genetic etiology between ASD and ADHD specifically.

In the present study, we applied the exome sequencing approach to familial data to identify rare, *de novo* SNVs that may be causative for sporadic and non-syndromic ADHD. In addition, we used MIP sequencing of 26 ASD and ID candidate genes in a large number of unrelated sporadic ADHD probands to determine whether these ASD and ID risk-genes are also associated with ADHD.

MATERIALS AND METHODS

Ethics Statement

The Institutional Review Boards of the University of Washington, University of California Irvine, Oregon Health and Science University (OHSU), and Michigan State University approved of this study. Written, informed consent was received from the parent or guardian of each minor.

Selection of Participants

Families were recruited from the local community via public advertisements and mass mailings to parents of children in the target age range asking for volunteers for a study of attention, development, and ADHD, and specifying a need for healthy children as well as children with suspected or diagnosed ADHD. Identical procedures were followed at two different sites, one in Michigan and one in Oregon, thus covering two different geographic

regions. An additional trio was identified at Washington and evaluated in the same manner, as outlined below. Through this process 846 children were identified who met our research criteria for ADHD (any subtype, using DSM-IV criteria). The definition of sporadic cases (below) was applied to this sample; 17.0% met our definition of sporadic, yielding an N=155 for this study for. Eleven of those families were set aside for the sequencing described below, based on (a) availability of sufficient quality and quantity of DNA from both parents and (b) older parental age (see Supplemental Table 1 for a summary of the demographic and clinical characteristics of the 11 sporadic ADHD probands who underwent exome sequencing). The numbers and demographic and clinical features of the entire cohort, and the sporadic cases identified and studied here are described in Table 1.

Procedure for Child Evaluation and Cohort Selection

Step 1. Selection of Cohort—Interested families volunteered by contacting the study and then completed a multi-stage screening to identify participants for the study. Parents first completed a short screen to evaluate numerous exclusion criteria, including history of autism spectrum disorder, history of >1 non-febrile seizure; head injury with loss of consciousness > 1 minute, history of diagnosis with intellectual disability, and any long acting psychoactive medication that could not wash out for companion studies of cognitive functioning.

Parents and teachers then completed nationally standardized rating scales (The Conners-III (Conners, 2014), the DuPaul ADHD Rating Scale (DuPaul, 2016), and the Strengths and Difficulties Questionnaire (SDQ, Goodman, 1997)). A parent completed a semi-structured clinical interview with a trained, masters-degree level clinician (KSADS-E (Orvaschel, 1994)). Inter-interviewer reliability with a gold standard trainer was satisfactory (k>.75 for all disorders discussed here). The clinician also briefly interviewed and observed the child. A full scale intellectual quotient (IQ) screening test (WISC-IV Vocabulary, Block Design, and Information (Wechsler, 1991)) and an academic screening test (WIAT-II Word Reading and Math Problem Solving (Wechsler, 2005)) were administered by different clinicians, who made detailed written notes on their behavioral observations.

A team of experienced clinicians (a board certified child psychiatrist and a licensed clinical child psychologist) then reviewed all available information independently to arrive at a best estimate diagnostic profile for ADHD and all other child disorders, blind to all study hypotheses or genetic data. Inter-clinician agreement was acceptable (k>.80 for all disorders discussed here). Disagreements were resolved by consensus. To be eligible, clinicians had to see cross-informant agreement at a minimal level i.e., (1) at least 3 symptoms of inattention or hyperactivity endorsed by both parent and teacher AND (2) at least T>60 on at least one nationally normed rating of ADHD symptoms by both reporters. They also had to show (3) impairment by at least one source (KSAD clinician rating or SDQ impairment at moderate or severe by at least one reporter). Once passing that screen-in, clinicians employed the “or” rule to count symptoms and conducted a further evaluation of rule outs, impairment, and other DSM criteria.

Additional exclusion criteria were applied at that point, including appearance of autism spectrum disorder on the interview, estimated FSIQ < 80, history of ever had psychosis, or

current major depressive episode (which made it difficult to evaluate symptoms of inattention), or failure of minimal convergence between parent and teacher ratings.

Step 2. Parent ADHD and Sporadic Status—Determination of sporadic ADHD in the proband was evaluated by assessing each parent for ADHD on at least one of the following (a) a Structured Clinical Interview that included both a SCID-I (First et al., 2002) and modified KSADS for adults on current and past symptoms of ADHD, (b) the Conners Adult ADHD Rating Scale (CAARS (Conners, 2014)), and/or (c) the ADHD Rating Scale on current and childhood symptoms (DuPaul, 2016). For each case, a parent provided a rating regarding history of ADHD in relatives of the child including the child's siblings. If a parent was unavailable to complete an interview or ratings, the available parent rated the missing parent on the ADHD Rating Scale.

Determination of Sporadic ADHD: Diagnosis of sporadic ADHD was then based on the following:

- **a.** Both parents denied history of confirmed or suspected ADHD in self, sibling, or parent (no child 1st or 2nd degree relative) on a Family Background Questionnaire; AND
- **b.** None of the child's siblings had ever been diagnosed or treated for ADHD per parent report; AND
- **c.** Both parents scored < 6 symptoms of ADHD hyperactivity/impulsivity and < 6 inattention on current and past ratings using the Barkley & Murphy Adult ADHD Rating Scale and the KSADS retrospective interview, and < T=65 on the Conners Adult ADHD Rating Scale. (In one case the ADHD RS and KSAD conflicted on our decision rule; the interview was used as the gold standard; in two cases the ADHD RS indicated no symptoms but the Conners > 65; we relied on the ADHD RS as the standard. In all other cases measures agreed on the case selection). The < 6 symptom cutoff was used in congruence with DSM-IV criteria (American Psychiatric Association), which were in force at the time this study was planned and data were collected.

As noted, 155 families were identified as sporadic ADHD cases, 17.0% of the total cohort of 846 ADHD cases. From these, 144 sporadic ADHD probands were sequenced for a panel of 26 genes of prior interest in ASD and ID, of which 117 passed quality control and were used in analyses. The remaining 11 families were tested for pilot exome sequencing to identify potential *de novo* SNVs responsible for sporadic ADHD. The 11 families selected for exome sequencing had an average paternal age of 34.8 years (range 27.1 to 46.0 years); maternal average age was 33.1 years (range 23.5 to 44.0 years).

Exome Sequencing/De Novo Variant Identification in 11 Families with Sporadic ADHD

The NimbleGen Big Exome 2011 Library (EZ Exome v3 with approximately 64 Mb of target sequence) was used for exome sequencing of all 11 families' DNA samples. FastQ files were aligned to the human genome reference sequence 19 (hg19) with the Burrows-Wheeler Aligner for the generation of BAM files (Li and Durbin, 2009). Realignment of

regions with indels, recalibration of base qualities, and variant detection and calling was performed with the Genome Analysis Toolkit (GATK) UnifiedGenotyper to produce VCF files (McKenna et al., 2010). Variants sites of low quality and likely to be false positives were removed via the GATK Variant Quality Score Recalibrator. CNVs were separately called from exome sequence data by CoNIFER (Krumm et al., 2012). Annotation of variants was performed with SeattleSeq 138 (http://snp.gs.washington.edu/). Relatedness between families was determined by comparing probands for relatedness up to the third degree (first cousins) using the software KING (Manichaikul et al., 2010).

GEMINI was used to identify SNVs, CNVs, and indels present in the affected ADHD proband that were not present in the unaffected parents or sibling (if available) (Paila et al., 2013). Brain expression of the genes of the identified SNVs and CNVs was performed using the Human Protein Atlas. SNVs, CNVs, and indels were examined for evolutionary conservation through GERP, with a minimum cut-off of 3, indicative of high importance across species (Cooper et al., 2005). Finally, these high-impact variants were filtered for presence in any database (NHLBI ESP Exome Variant Server, 1000 Genomes, or ExAC and, separately, the Database of Genomic Variants for indels). Variants not found in any known database were carried forward as *de novo* variants. As a secondary analysis, we considered the same classes of *de novo* variants, but relaxed the allele frequency to < 1% in the Exome Aggregation Consortium (ExAC) database if the same variant was found in a second affected proband, even when the occurrence in the second proband was not *de novo*.

MIP Resequencing of ID/ASD Genes in 144 Unrelated, Sporadic ADHD Probands

Targeted molecular inversion probe (MIP) resequencing of 144 sporadic ADHD probands (excluding the 11 probands who already had data from exome sequencing) from OHSU and Michigan State University was performed for 26 candidate genes for ASD and ID (see Table 3 in prior work by Coe *et al* and note that *ARHGAP11A* has subsequently been identified as a duplicated gene with no association with ASD/ID) (Coe et al., 2014), as previously described (O'Roak et al., 2012; 2014). In total, the coding sequence and splice-donor/splice-acceptor sites of the 26 candidate genes were targeted using 1,388 MIPs. Participant DNA was barcoded and sequenced using an Illumina HiSeq 2000. Quality control metrics for the MIPs used to sequence the genes implicated in ASD and ID are presented in Supplemental Table 2.

Participants were filtered for quality control on the basis of the total percentage of MIPs with at least 20 reads (the minimum for variant calling). The sporadic ADHD probands were required to have a minimum of 75% MIP coverage at the threshold of 20x. This resulted in the inclusion of 117 of 144 samples (81.25%) for analyses of GEMINI-determined loss-of-function stop-gain, stop-loss, splice-acceptor, splice-donor, frameshift, and missense variants in the 26 candidate genes for ASD and ID.

Validation of De Novo and MIP Sequencing Variants

All identified *de novo* SNVs identified in the sporadic ADHD families were separately validated using custom designed TaqMan assays (see Supplemental Table 3 for primer design). Similarly, SNVs and CNVs that were likely loss-of-function or high GERP

missense variants in ASD or ID candidate genes were validated using Taqman assays (for SNVs) and quantitative PCR (qPCR, for CNVs).

RESULTS

Demographic and parental ADHD scale information for the 11 sporadic ADHD probands who underwent exome sequencing are presented in Table 2. Eight of the 11 exome sequenced families were European Ancestry (EA, 73%), while nine of the 11 probands were male (82%). The mean paternal age at birth of the ADHD proband was 34.8 years; notably, the father's age was higher in the families for whom a *de novo* SNV was identified: 39.2 years as compared to 32.3 years in families for whom no *de novo* SNV was found (one-sided t-test P=0.050). Additional clinical information about each of the 11 sporadic ADHD probands is presented in Supplemental Table 1.

A total of 8 *de novo* missense SNVs were called in 4 of the 11 families with sporadic and non-syndromic ADHD who underwent whole exome sequencing. Of these 8 *de novo* missense variants, 2 SNVs (in *SARM1* and *SPTBN2*) in 2 unique families (4 and 6, respectively) did not subsequently validate in an orthogonal test (i.e., showed the reference, non-*de novo* SNV). The 6 validated *de novo* missense SNVs were all expressed in the brain and found in 4 unique ADHD probands (Table 3). Single Taqman validated *de novo* missense SNVs were observed in $TBC1D9_{His1179Tyr}$, and $WDR83_{Gly127Arg}$ in ADHD probands, but not parents or unaffected siblings (when available), in families 3 and 11, respectively. Families 4 and 10 each had 2 *de novo* and validated missense SNVs identified. In family 4, the sporadic ADHD proband carried $DAGLA_{Asp1017His}$ (rs199764983) and $QARS_{Cys471Tyr}$ variants unobserved in family members. In family 10, the sporadic ADHD proband was observed to have both a $CSMD2_{Ile891Val}$ and $TRPM2_{Arg687Cys}$ (rs139554968) variant. Notably, 4 of the 6 validated *de novo* missense SNVs were not observed in any database. These results are summarized in Table 3. Additional SNVs that were identified as *de novo* in a proband but which were subsequently noted to be present in another proband, even if transmitted, and had MAF <1% are presented in Supplemental Table 4.

No CNVs or indels were identified as *de novo* and passed filtering criteria in the 11 families examined for sporadic ADHD.

A MIP-based panel of 26 genes implicated in ASD and ID (Coe et al., 2014) was separately performed for the 117 probands with sporadic ADHD. Within the 26 genes, 3 total nonsense, splice, frameshift, or highly conserved missense variants were called among the 117 sporadic ADHD probands and the 11 sequenced trios and quads described above (see Table 4). Of these 3 called variants, only 1 variant ($CHD1L_{Gln600STOP}$) in a single proband validated in TaqMan assay orthogonal testing. The other MIP called variants ($ARHGAP11A_{TACA>TA}$ frameshift and $ADNP_{TT>TTGT}$ frameshift) each subsequently showed homozygosity for the reference allele for all identified ADHD probands. The exomes of the 11 probands from the trios/quad tests were evaluated for variants in these same 26 genes and none were found. Thus, only 1 of 128 ADHD probands (0.68%) had a putative deleterious variant identified in these 26 ASD/ID genes.

DISCUSSION

Previous studies of the genetic determinants of ADHD have not yielded SNVs or CNVs that are strongly associated with the ADHD phenotype (Neale et al., 2010; Stergiakouli et al., 2012; Franke et al., 2009). Given the high heritability of ADHD (Stevenson, 1992; Levy et al., 1997; Faraone et al., 2012), efforts have shifted from GWAS to alternative methods, such as statistical modeling of complex gene-by-gene/gene-by-environment interactions (Zuk et al., 2012) or the use of endophenotypes (Hawi et al., 2015) and/or continuous measures of ADHD severity (Groen-Blokhuis et al., 2014). In this work, we present an alternative view by performing the first known study to combine whole exome sequencing to identify rare, *de novo* variants of likely high impact (Eichler et al., 2010), with stringent phenotype classification to identify cases of sporadic and non-syndromic ADHD.

Specifically in this work, we identified *de novo* missense variants present only in the sporadically affected proband that are possibly responsible for the sporadic ADHD observed in the proband. The 6 validated missense SNVs are each exceedingly rare (maximum allele frequency of 4.24×10^{-5}), highly conserved, offering several additional possible candidates of study in the pathogenesis of sporadic ADHD. Notably, *CSMD2* and *TRPM2*, both of which were identified in the same sporadic ADHD proband in Family 10, each have been reported in association with other psychiatric disorders; adult ADHD (Lesch et al., 2008) and schizophrenia (Håvik et al., 2011) for *CSMD2*, and bipolar disorder for *TRPM2* (McQuillin et al., 2005; Jang et al., 2015). Two additional *de novo* mutations identified in ADHD probands (see Supplemental Table 3) were found to have been transmitted to the probands in other families. The possibility that these are related to ADHD, but with incomplete penetrance and or additive effects, cannot be excluded.

We MIP sequenced 26 genes associated with ASD and ID in 117 children with sporadic ADHD and evaluated their sequence from exomes in the 11 family probands to assess overlap in these genes with ADHD risk. These 26 genes are listed in Table 3 of work by Coe *et al* (Coe et al., 2014); MIP coverage of these genes in our data is presented in Supplemental Table 1. In these 128 children affected with ADHD (117 MIP sequenced and 11 exome sequenced), we identified and validated only 1 highly-conserved, called loss-of-function nonsense, splice, frameshift, or missense variant in the 26 ASD/ID genes, which was found in a single ADHD proband. This relative paucity of highly-damaging variants in these genes associated with ASD/ID suggest independent genetic pathways of pathogenesis for sporadic ADHD, as compared to the more frequently studied ASD/ID phenotypes (Krumm et al., 2014).

Both common and rare variants are expected to contribute to the heritability of disease (Eichler et al., 2010). A large GWAS examining common SNVs have found significant associations with two L-type calcium channel genes, *CACNA1C* and *CACNB2*, when looking for phenotypic overlap between 5 psychiatric phenotypes (ASD, ADHD, bipolar disorder, major depressive disorder, and schizophrenia) as the outcome (Smoller et al., 2013). These and other prior results suggested a common genetic etiology in low penetrance risk variants between the disparate psychiatric disorders. Notably, prior study of psychiatric disease overlap did not specifically study ID (Smoller et al., 2013), nor have GWAS been

performed on ID. Our work, which examined rare variants, suggests that high penetrance gene associations with sporadic ADHD do not overlap with ASD and ID. However, as noted above, genes that may also be associated with schizophrenia and bipolar disorder (*CSMD2* and *TRPM2*) did contain *de novo* mutations in sporadic ADHD probands.

Several limitations of this study should be considered. First, our study did not identify any *de novo* CNVs in affected probands that could account for the occurrence of sporadic ADHD. Prior studies of ADHD have noted an enrichment of large CNVs in ADHD cases (Stergiakouli et al., 2012; Elia et al., 2010), particularly in genes related to neurodevelopment (Williams et al., 2010). Likely explanations for this paucity of CNVs are likely related to the difficulty in identifying large CNVs, which often span intergenic regions not captured by exome and MIP probes (Krumm et al., 2012). Second, our study was composed of two aims, which occurred independently and temporally parallel to each other: to identify *de novo* variants in sporadic ADHD probands and to exclude the possibility of overlap with genes implicated in ASD and ID. As we used an existing gene panel for ASD and ID (Coe et al., 2014); we did not have the capability to examine the unique genes implicated in sporadic ADHD in the 117 independent samples used to exclude genetic overlap with ASD and ID. Third, it is notable that while nine of the 11 families studied were EA, two of the four families in which *de novo* missense SNVs were identified were non-EA (see Table 2). Finally, our analyses of *de novo* variation included only 11 families. Our study was also underpowered to detect recurrent *de novo* variants in the same gene; this phenomena has been noted to have only occurred 3 times thus far for ASD/ID (in genes *CHD8*, *NTNG1*, and *SCN2A*) after the sequencing of several thousand probands (Krumm et al., 2014).

In summary, ADHD is a prevalent disease of childhood (Thomas et al., 2015) that has been reported to cause an approximate 2-fold increased mortality rate ratio in a recent large cohort study (Dalsgaard et al., 2015) in addition to the other challenges the affected patients face. Despite the importance of ADHD, little is yet known of the specific genetic factors that influence its pathogenesis (Neale et al., 2010; Hawi et al., 2015). Within this context, we have performed the first known exome sequencing analysis of sporadic (non-inherited) ADHD and identified six candidate genes with *de novo* missense SNVs. We also report the novel finding that the genetic etiology of sporadic ADHD appears to be independent of ASD and ID, contradicting past findings from GWAS. These results emphasize the importance of identifying and evaluating specific genetic predictors of ADHD, to better understand the pathogenesis of this prevalent and morbid disorder of childhood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Summary of the selected sporadic ADHD cases for MIP and exome sequencing versus total cohort.

	Excluded Familial ADHD Cases (N=691)	Sporadic ADHD Cases MIP Sequenced (N=144)	Sporadic ADHD Cases Trio or Quad Exome Sequenced (N=11)
Male, %	70%	67%	82%
Age, years	9.9	10.1	9.8
Full scale IQ[a]	105	107	106
Word reading score[a]	104	101	111
Family income, thousands, $	64.1	64.3	101
Lifetime ODD diagnosis[b], %	24%	21%	18%
Lifetime anxiety diagnosis[b], %	30%	35%	27%
Lifetime mood disorder[b], %	12%	14%	0%
ADHD Hyperactivity T-Score[c]	70	65	72
ADHD Inattention T-Score[c]	74	70	79

Abbreviations: ADHD = attention-deficit hyperactivity disorder; IQ = intellectual quotient; ODD = oppositional defiant disorder.

[a]IQ and reading assessment are described in the text; these are standard scores with a mean of 100 and standard deviation of 15.

[b]Comorbid disorders are identified by the KSADS interview and diagnostic team review as described in the text.

[c]ADHD Rating Scale Parent rating T-scores have a mean of 50 and standard deviation of 10.

Table 2

Demographic and parental ADHD characteristic comparison among 11 exome sequenced families.

	European Ancestry (n), %	Male Proband (n), %	Mean Paternal Age
11 families	9 (82%)	9 (82%)	34.8 years
4 families with *de novo* SNV	2 (50%)	4 (100%)	39.2 years
7 families without *de novo* SNV	7 (100%)	5 (71%)	32.3 years

Abbreviations: ADHD = attention-deficit hyperactivity disorder; SNV = single nucleotide variant.

Table 3

Summary of *de novo* SNVs identified by trio or quad sequencing of sporadic ADHD in 11 families.

Gene Affected	Variant	Chr:Position[a]	rsID	GERP	MAF[b]	Brain Expression [c]	Study Family
Validated de novo missense SNVs							
TBC1D9	His1179Tyr	4:141543615	None	5.01	0	Yes	3
DAGLA[d]	Asp1017His	11:61511880	rs199764983	4.14	$4.24x10^{-5}$	Yes	4
QARS[e]	Cys471Tyr	3:49137057	None	5.86	0	Yes	4
CSMD2[f]	Ile891Val	1:34190974	None	3.28	0	Yes	10
TRPM2[g]	Arg687Cys	21:45819235	rs139554968	4.19	$3.34x10^{-5}$	Yes	10
WDR83	Gly127Arg	19:12781425	None	4.51	0	Yes	11

Abbreviations: Chr = chromosome; GERP = genomic evolutionary rate profiling; MAF = minor allele frequency; N/A = not available

[a] Position based on hg19 build.

[b] Minor allele frequency from ExAC pooled data.

[c] Brain tissue gene expression data from the Human Tissue Atlas.

[d] A *DAGLA* CNV has been implicated in autosomal dominant spinocerebellar ataxia 20 (OMIM 608687) based on a 260 kb duplication on chromosome 11q12.2-11q12.3 (Knight et al., 2004; Coutinho et al., 2006).

[e] *QARS* rare variants have been implicated in two cases of autosomal recessive progressive microcephaly with seizures and cerebral/cerebellar atrophy (OMIM 603727 and (Zhang et al., 2014).

[f] *CSMD2* common variants have previously been associated with adult ADHD (Lesch et al., 2008) and schizophrenia (Håvik et al., 2011).

[g] *TRPM2* common variants have previously been associated with bipolar disorder (McQuillin et al., 2005; Jang et al., 2015).

Table 4

Called loss of function or highly conserved missense variants in 26 gene autism spectrum disorder/intellectual disability MIP panel considering 117 ADHD probands (excluding the 11 probands who underwent whole exome sequencing).

Gene	Variant	Chr:Position[a]	rsID	GERP	MAF[b]	#Affected ADHD Probands	#Validated/N
ARHGAP11A	Frameshift (TACA>TA)	15:32917411-32917415	None	4.95	0	1	0/1
CHD1L	**Gln600STOP**	**1:146765310**	**rs149664186**	**5.27**	**0.00034**	**1**	**1/1**
ADNP	Frameshift (TT>TTGT)	20:49510414-49510416	None	4.81	0	1	0/1

[a]Position based on hg19 build.

[b]Minor allele frequency from ExAC pooled data