Complexities of Cognition: The Case of ADHD

As for autism and schizophrenia, the closer we look at attention deficit hyperactivity disorder (ADHD), the more complicated it seems. Found in 4% of children, this syndrome of attention, hyperactivity, and impulsiveness is highly heritable, yet we know only a few of the responsible genetic variants. Here, Lionel et al. assessed a particularly well-defined population of 248 children with ADHD, plus many of their parents, for extra copies or deletions of genes. The 306 rare copy number variations (CNVs) found in these individuals were of various kinds—only 1.7% were de novo mutations in brain-specific genes, 7.7% were clearly inherited and occurred in genes known to be associated with ADHD or defined new culprit genes, and several were in genes already implicated in other disorders such as autism. To take a closer look at possible genes that confer risk for more than one developmental disorder, the authors examined the CNVs in a separate group of patients with autism. In four autism patients and two of the patients with ADHD, a cluster of rare disorder-associated CNVs occurred on chromosome 9 in and around two genes: ASTN2, necessary for mammalian brain development, and TRIM32, a neuronal stem cell–associated gene. This region has also been associated with CNVs in bipolar disorder, intellectual disability, and schizophrenia. In all, the authors found rare inherited CNVs at sites that had been previously implicated in ADHD or in other neurodevelopmental disorders in 8% of the individuals with ADHD. Their results implicate common genes and pathways for several neuropsychiatric disorders, which is consistent with the common clinical co-occurrence of ADHD with other such conditions.
Rare Copy Number Variation Discovery and Cross-Disorder Comparisons Identify Risk Genes for ADHD

Attentive deficit hyperactivity disorder (ADHD) is a common and persistent condition characterized by developmentally atypical and impairing inattention, hyperactivity, and impulsiveness. We identified de novo and rare copy number variations (CNVs) in 248 unrelated ADHD patients using million-feature genotyping arrays. We found de novo CNVs in 3 of 173 (1.7%) ADHD patients for whom we had DNA from both parents. These CNVs affected brain-expressed genes: DCLK2, SORCS1, SORCS3, and MACROD2. We also detected rare inherited CNVs in 19 of 248 (7.7%) ADHD probands, which were absent in 2357 controls and which either overlapped previously implicated ADHD loci (for example, DRD5 and 15q13 microduplication) or identified new candidate susceptibility genes (ASTN2, CPLX2, ZBBX, and PTPRN2). Among these de novo and rare inherited CNVs, there were also examples of genes (ASTN2, GABRG1, and CNNTN5) previously implicated by rare CNVs in other neurodevelopmental conditions including autism spectrum disorder (ASD). To further explore the overlap of risks in ADHD and ASD, we used the same microarrays to test for rare CNVs in an independent, newly collected cohort of 349 unrelated individuals with a primary diagnosis of ASD. Deletions of the neuronal ASTN2 and the ASTN2-intronic TRIM32 genes yielded the strongest association with ADHD and ASD, but numerous other shared candidate genes (such as CHCHD3, MACROD2, and the 16p11.2 region) were also revealed. Our results provide support for a role for rare CNVs in ADHD risk and reinforce evidence for the existence of common underlying susceptibility genes for ADHD, ASD, and other neuropsychiatric disorders.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a common psychiatric disorder characterized by developmentally atypical and impairing inattention, hyperactivity, and impulsiveness, which affects 4% of school-age children worldwide (1, 2). It appears initially in the preschool years and persists in ~50% of cases through adolescence into adulthood (3). ADHD increases the risk for conduct, anxiety, and learning disorders in childhood, as well as for substance use, school failure, and motor vehicle accidents in adolescence. Increased health care costs and loss of workplace productivity (4) are also observed in adulthood. More than 2 million children in North America, about half of all diagnosed cases, take stimulant medication to treat ADHD (5). Some receive substantial benefit from pharmacotherapy, but in most cases, considerable impairment remains (6).

Family, twin, and adoption studies indicate that genetic factors contribute to ADHD. First-degree family members of ADHD probands are 8 to 10 times more likely to have ADHD than are relatives of non-ADHD individuals (7). Twin studies of ADHD show high average heritability of ~80%, which is comparable to the ~90% heritability observed in autism spectrum disorders (ASDs) (8). ADHD is heritable irrespective of how it is measured, for example, according to The Diagnostic and Statistical Manual—Fourth Edition (DSM-IV) criteria, as the extreme of a trait, as a continuous trait throughout its distribution, or as a trait derived from latent class analysis (9). Both dimensions of inattentive and hyperactivity-impulsivity, as well as total ADHD trait or symptom scores, are heritable, and the inattention and hyperactivity-impulsivity dimensions share most, but not all, of their genetic risk (10).

ADHD appears to be clinically and genetically heterogeneous. More boys than girls are affected by ADHD (with gender ratios of 3:1 to 8:1) (11). Several ADHD subtypes (primarily inattentive, primarily hyperactive-impulsive, and combined) are identified in DSM-IV contribute to heterogeneity, although the etiological implication of these distinctions is debated (12, 13). Most individuals with ADHD present as comorbid with one or more other disorder including those of conduct, oppositional defiance, reading, language, anxiety, and/or mood disorders. Attention deficits and hyperactivity are clinically significant problems in up to 75% of individuals with ASD (14–16), and surveys of ADHD populations reveal a higher than expected rate of
autistic symptoms (14, 17, 18). In summary, ADHD rarely occurs in isolation, suggesting that genetic risk factors may overlap with other neuropsychiatric conditions.

Although ADHD is highly heritable, the underlying genetic determinants are still largely unknown. Genetic association studies examining functional candidate genes (19) such as DAT1, DRD4, DRD5, and SNAP25 have yielded some positive findings accounting for perhaps 3% of ADHD variance (20). These locus-specific findings, however, have not yet been replicated through genome-wide association (GWA) studies, which themselves have yet to reveal obvious candidate regions meeting genome-wide significance (21–23). The lack of significant GWA findings implies that common genetic variants likely confer low relative risk for ADHD and further suggests that rare genetic variants might have important etiologic roles.

ADHD traits are also observed in ~30% and ~70% of individuals with the DiGeorge (24) and Williams-Beuren microdeletion syndromes (25), respectively. These observations, along with the growing recognition of the role of gene dosage imbalances in other neuropsychiatric disorders (26), set the stage for testing the hypothesis that copy number variations (CNVs) may also be involved in ADHD risk. Three ADHD case-control studies involving subjects from the United States (27), Germany (28), and the UK (29) have found evidence for the enrichment of rare CNVs in ADHD, including CNVs that affect genes also associated with other neurodevelopmental disorders.

In this study of a well-characterized Canadian cohort, we sought to further explore the etiologic role of rare inherited CNVs in ADHD, as well as to determine whether de novo CNV mutations might also contribute. Our primary study design emphasized the use of identical high-resolution genotyping microarrays and detection algorithms for all cases and controls, yielding a robust discovery data set (30). We also compared our ADHD-CNV data with CNVs from a newly characterized ASD cohort genotyped with the same microarray platform and analysis design to test whether neuropsychiatric risk genes might have pleiotropic effects across—or be expressed as traits within—disorders (31–33).

RESULTS

Detection of CNVs in ADHD cases and population controls

We genotyped 248 unrelated ADHD probands (73 female and 175 male) collected in Canada using the Affymetrix single-nucleotide polymorphism (SNP) 6.0 microarray, which contains 1.8 million markers for CNV interrogation. In 173 of these families, DNA was genotyped in both parents. We also analyzed 2357 population-based controls (1234 from Ontario and 1123 from Germany; see Materials and Methods) for CNVs using the same array platform and calling strategy as the cases (Fig. 1). Similar CNV profiles were observed in cases and controls with no significant difference in overall CNV call rate or length (table S1). We defined a data set of rare, stringently defined CNVs in ADHD probands that were at least 20 kb in length, spanning a minimum of five array probes, had support from two or more calling algorithms, and were not found in controls (less than 50% overlap by length). On the basis of these criteria, a total of 306 rare CNVs were detected in the ADHD probands (table S2). Using quantitative polymerase chain reaction (qPCR), we validated 33 of 34 (97.1%) rare stringent calls that were tested, including all 23 CNVs in Table 1. Moreover, in our previous experience with this approach, >90% of stringent CNV calls could be validated (34, 35). To define ADHD candidate loci, we prioritized de novo CNVs and rare inherited CNVs that overlapped genetic loci previously implicated in ADHD or in other neuropsychiatric disorders (Table 1, Fig. 2, and table S3).

Loci implicated by de novo CNVs in ADHD cases

Using data from probands and both parents (trio data), we determined the de novo CNV rate in ADHD probands to be ~2% (3 of 173) (Table 1A and Fig. 2A). De novo CNVs were validated by either qPCR or fluorescence in situ hybridization (FISH) after parentage in all trios with array genotypes was confirmed (36). In one family, a 33-kb de novo deletion at 4q31.3 was detected in a male proband (27696.3) with ADHD and anxiety traits who also exhibited seizure-like symptoms. The deletion eliminates two exons of the DCLK2 (doublecortin-like kinase 2) gene, which is expressed in both proliferating neural cells and postmitotic neurons (37). Mutations in the paralogous gene DCX have been associated with epilepsy and periodic limb movements (38), and the mouse double-knockout model for DCX and DCLK2 exhibits spontaneous seizures and a disorganized hippocampal network with aberrant positioning of excitatory and inhibitory neurons (39). The DCLK1 region was one of the stronger signals in a GWA study of 958 ADHD probands, but did not meet genome-wide significance (21).

![Fig. 1. CNV analysis workflow](https://www.sciencemag.org/lookup/doi/10.1126/science.1213197)
In one male ADHD proband (30600.3) of a second family, a pair of adjacent de novo CNVs at 10q25.1 duplicated regions of sizes 242 and 318 kb, overlapping the genes SORCS3 and SORCS1, respectively. SORCS3 and SORCS1 are paralogs encoding sortilin-related VPS10 domain–containing receptor proteins, which are involved in intracellular sorting of surface membrane proteins and are highly expressed in the developing and mature central nervous system (40). Upon phenotypic follow-up, it was discovered that the proband, who had met criteria for ADHD at age 8, was later diagnosed with bipolar disorder at age 15. Indeed, variants at SORCS2 (a paralog of SORCS3 and SORCS1 mapping to 4p16.1) have been shown by GWA studies to be potential risk factors for both ADHD (41) and bipolar disorder (42, 43). Neither the proband’s father nor brother exhibited these CNVs, although both had a diagnosis of ADHD, but not bipolar disorder. Thus, the de novo duplications may contribute to the bipolar disorder phenotype of the proband.

Last, a 109-kb de novo exonic deletion was detected in a male ADHD proband (113400.3) overlapping MACROD2 at 20p12.1. Further support for the potential pathogenicity of this locus comes from the presence of a rare, maternally inherited deletion exonic to MACROD2 and FLRT3, a neuronal cell adhesion gene intrinsic to MACROD2, in another ADHD proband (108300.3) in this study, and from reports of rare CNVs at this locus in two previous ADHD CNV scans (27, 29) (table S4). Although little is known about its function, the MACROD2 gene is expressed in the brain and represented the most significant association in a recent GWA analysis of ASD (44).

### Table 1. De novo and rare inherited CNVs at candidate loci in ADHD probands

<table>
<thead>
<tr>
<th>Sample Sex CNV</th>
<th>Locus</th>
<th>Size (kb)</th>
<th>Inheritance</th>
<th>Ancestry</th>
<th>Genes</th>
<th>Previous reports</th>
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<tr>
<td>A. Loci implicated by de novo CNVs in ADHD probands</td>
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<td></td>
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<td></td>
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<tr>
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<td>Loss</td>
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<td>D</td>
<td>EA</td>
<td><strong>DCLK2</strong>†</td>
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<td>EA</td>
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<tr>
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<td>Loss</td>
<td>20p12.1</td>
<td>109</td>
<td>D</td>
<td>EA</td>
<td><strong>MACROD2</strong>‡</td>
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<td>B. Rare CNVs at loci previously implicated in ADHD</td>
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<tr>
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<td>Gain</td>
<td>4q31.2</td>
<td>482</td>
<td>P</td>
<td>AS</td>
<td><strong>DRD5, WDR1, SLC2A9</strong></td>
</tr>
<tr>
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<td>Gain</td>
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<td>M</td>
<td>EA</td>
<td><strong>CPLX2</strong></td>
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<td>7q32.3</td>
<td>159</td>
<td>M</td>
<td>EA</td>
<td><strong>CHCD3</strong></td>
</tr>
<tr>
<td>19896.3 M</td>
<td>Gain</td>
<td>7q36.3</td>
<td>1023</td>
<td>M</td>
<td>EA</td>
<td><strong>PTPRN2, WDR60, NCPAG2, ESY2</strong></td>
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<tr>
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<td>11q13.4</td>
<td>147</td>
<td>U§</td>
<td>MIX</td>
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<td>P</td>
<td>EA</td>
<td><strong>16p11.2 locus (40 genes)</strong></td>
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<td>C. Overlapping rare CNVs in unrelated ADHD probands</td>
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<td>EA</td>
<td><strong>ZB9X</strong></td>
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<tr>
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<td>Loss</td>
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<td><strong>ZB9X</strong></td>
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<td><strong>GCNT2</strong></td>
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<tr>
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<td>Loss</td>
<td>9q33.1</td>
<td>177</td>
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<td>EA</td>
<td><strong>ASTN2, TRIM32</strong></td>
</tr>
<tr>
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<td>Loss</td>
<td>9q33.1</td>
<td>148</td>
<td>U§</td>
<td>EA</td>
<td><strong>ASTN2, TRIM32</strong></td>
</tr>
<tr>
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<td>Loss</td>
<td>20p12.1</td>
<td>542</td>
<td>M</td>
<td>EA</td>
<td><strong>MACROD2, FLRT3</strong></td>
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<tr>
<td>D. Rare CNVs at loci implicated in other neurodevelopmental disorders</td>
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<td></td>
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<td></td>
</tr>
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<td>97</td>
<td>M</td>
<td>EA</td>
<td><strong>STK32B</strong></td>
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<td>Loss</td>
<td>4p12</td>
<td>64</td>
<td>M</td>
<td>EA</td>
<td><strong>GABRG1</strong></td>
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<tr>
<td>125700.3 M</td>
<td>Loss</td>
<td>11q22.1</td>
<td>49</td>
<td>P</td>
<td>EA</td>
<td><strong>CNTN5</strong> (intronic)</td>
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<tr>
<td>89700.3 M</td>
<td>Gain</td>
<td>12q24.33</td>
<td>211</td>
<td>P</td>
<td>EA</td>
<td><strong>ANKLE2, POLE, PGAMS, PXMP2</strong></td>
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<td>27075.3 M</td>
<td>Loss</td>
<td>Xp22.11</td>
<td>388</td>
<td>M</td>
<td>EA</td>
<td><strong>DDX53, upstream of PTCHD1</strong></td>
</tr>
</tbody>
</table>

*This column lists the neuropsychiatric disorder(s) in connection to which each locus has been reported by previous studies: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; BPD, bipolar disorder; ID, intellectual disability; SZ, schizophrenia. †Paralogs of these genes have been implicated by previous studies in the neuropsychiatric disorders listed in the last column. ‡Loci highlighted in bold had overlapping rare CNVs in both the ADHD and the ASD data sets. §DNA was not available from the father for testing. ||DNA was not available from the mother for testing.
Rare inherited CNVs at loci previously implicated in ADHD
Among rare inherited CNVs that overlapped loci previously reported in genetic studies of ADHD (Table 1B and Fig. 2B) were large inherited duplications at 16p11.2 and 15q13 in ADHD probands 19839.3 and 19752.3, respectively, consistent with reports of ADHD being a frequent phenotypic component in patients with microdeletions and duplications at these two loci (45, 46). Other genes of interest implicated by overlap with rare duplications included DRD5 (at 4p16.1) and PTPRN2 (at 7q36.3). Genetic variants within the dopamine receptor subtype D5 gene (DRD5) have been associated with ADHD (19, 47), and in our study, a duplication encompassing DRD5 was transmitted to a male proband (27027.3) from a father having a presumptive ADHD diagnosis. The duplication at 7q36.3 was transmitted to two brothers with ADHD (19896.3) from their mother and overlaps PTPRN2, which encodes a protein tyrosine phosphatase. This genetic locus has been associated with ADHD traits (48) and with behavioral and learning disturbances in mice with deletion of the PTPRN2 homolog (49).

We also detected CNVs at ZBBX (two exonic deletions) (Table 1C and Fig. 2C), CPLX2, CHCHD3, and 11q13.4 overlapping loci previously reported in ADHD CNV scans (27, 28) (fig. S1 and table S4). ZBBX was implicated previously by a de novo deletion in a male ADHD proband (28), and separately by a CNV gain disrupting ZBBX (27). The observation of CNVs at complexin 2 (CPLX2) across multiple studies is intriguing given its colocalization with the SNAP-25 protein in the SNARE complex (50). The SNAP25 gene has been implicated in ADHD via candidate gene association testing (51, 52), and Cplx2 knockout mice exhibited behavioral deficits and cognitive abnormalities (53).

Rare CNVs at loci previously implicated in other neurodevelopmental disorders
We also observed overlap of rare CNVs in our ADHD cohort with those previously implicated in other neuropsychiatric disorders, notably ASD, including deletions at genes CNTN5, GABRG1, GCNT2, and STK32B (Table 1D and Fig. 2D). There is evidence from multiple CNV studies for the involvement of contactins in neuropsychiatric disorders (54). Moreover, disruption of the GABRG1 (55) and GCNT2 (56) genes has previously been reported in ASD.

Overlap between rare CNV findings in ADHD and ASD
Given the observation that several rare CNVs in our ADHD data set have been previously found in ASD CNV studies, and the reported

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### Table 1

<table>
<thead>
<tr>
<th>Family</th>
<th>Chromosome</th>
<th>CNV Description</th>
<th>Genes Implicated</th>
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<tbody>
<tr>
<td>27027</td>
<td>15q13</td>
<td>482 kb gain (DRD5, WDFT1, SLC2A9)</td>
<td>DRD5, WDFT1, SLC2A9</td>
</tr>
<tr>
<td>27060</td>
<td>7q36.3</td>
<td>131 kb gain (CPLX2)</td>
<td>CPLX2</td>
</tr>
<tr>
<td>63900</td>
<td>11q13.4</td>
<td>159 kb gain (CHCHD3)</td>
<td>CHCHD3</td>
</tr>
<tr>
<td>19896</td>
<td>11q13.4</td>
<td>154 kb gain (ZBBX)</td>
<td>ZBBX</td>
</tr>
<tr>
<td>19752</td>
<td>16p11.2</td>
<td>3.6 Mb gain</td>
<td>GABRG1, GCNT2</td>
</tr>
<tr>
<td>19939</td>
<td>16p11.2</td>
<td>789 kb gain (16p11.2)</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 2.** Pedigrees of ADHD families with rare CNVs of interest. (A to D) Pedigrees of ADHD families with variants of interest, which were absent in controls and (A) were de novo or (B) occurred at loci previously implicated in ADHD or (C) were present in two unrelated ADHD probands or (D) overlapped loci previously implicated in other neurodevelopmental disorders. Circles and squares denote females and males, respectively, whereas arrows highlight the index proband in each family. Black filled objects indicate ADHD diagnosis, unfilled symbols signify unaffected family members, gray symbolizes individuals with non-ADHD neuropsychiatric conditions, and stripes represent individuals with some ADHD traits, but not having a definitive ADHD diagnosis. N/A denotes individuals from whom no DNA was available for testing. Detailed overview of phenotypes is presented in table S3. Proband in family 30600 also has a diagnosis of bipolar disorder.
phenotypic overlap between ASD and ADHD, we decided to explore further the hypothesis that rare CNVs absent in controls and present in both ASD and ADHD patients could represent putative candidates for genetic risk across these conditions. We therefore expanded our case data set to include 349 newly characterized ASD probands. ASD was selected as a first comparator because of the high rate of co-occurrence of ADHD in ASD. To standardize our initial data and allow the most robust comparison (30, 57), we used the same microarray platform and CNV calling strategy for the ASD, ADHD, and control cohorts. Genetic loci highlighted by the occurrence of rare CNVs in both the ASD and the ADHD data sets included those previously mentioned (16p11.2 locus, MACROD2, and CHCHD3) as well as the 12q24.33 region and the X-linked DDX53/PITCHD1 locus (table S5).

Our most intriguing finding was a significant enrichment of rare CNVs at 9q33.1 overlapping ASTN2 and TRIM32 in four ASD (~1%) and two ADHD probands (~1%) (Fig. 3A) (frequency of 6 of 597 in cases versus 0 of 2357 in controls; Fisher’s exact test two-tailed P = 6.68 × 10^-5). ASTN2 encodes astrotactin 2, a neuronal membrane protein exhibiting abundant expression in the cerebellum in both the fetal and the adult brain (58). Three of the ASD probands met criteria for ADHD as measured by the Conners’ questionnaire, whereas the fourth showed signs of ADHD but was below the threshold for diagnosis (Fig. 3B). These findings highlight the potential contribution of this locus to the presence of ADHD symptoms in ASD. Although exceptionally rare CNVs at this locus have been reported in earlier CNV studies of ASD (59), bipolar disorder (60), intellectual disability (61), and schizophrenia (62) (Fig. 3A), this is the first report of CNVs at this locus in ADHD.

**DNA sequencing of ASTN2 and TRIM32**

To further assess whether smaller nucleotide-resolution mutations might be found in ASTN2 and/or TRIM32, we performed DNA sequencing of all coding exons and splice sites in each of these genes in all of the ADHD and ASD cases, as well as in ancestry-matched controls. The five ASTN2/TRIM32 cases from this study carrying deletions at this locus were also sequenced. We detected no nonsense mutations that would have led to ASTN2 or TRIM32 haploinsufficiency in a manner similar to CNV. We did detect previously undescribed missense variants in the ASTN2 gene in 8 of 276 ADHD cases, 9 of 346 ASD cases, and 2 of 188 controls (tables S6 to S9). TRIM32 sequencing revealed missense variants in 4 of 257 ADHD cases, 6 of 357 ASD cases, and 2 of 157 controls (tables S10 to S12). From these data, we did not observe a significant enrichment of missense variants in the case cohorts compared to the population controls for either gene (table S6). One ASD proband had previously undescribed missense variants in both the TRIM32 and the ASTN2 genes. Several of the missense variants in the cases (six ADHD and seven ASD) occurred at positions highly conserved across all vertebrates. The screening of larger case and control data sets is needed to shed light on the clinical significance of these variants.

**DISCUSSION**

Our data delineate several rare CNVs identified in ADHD cases, which were not found in controls, suggesting that they may have a contributory role in the clinical phenotype observed in these individuals. In this systematic test for de novo CNVs in ADHD, we found a rate of ~1.7% (3 of 173), just slightly higher than the typical ~1% that has been reported in non-disease control trios (63),

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**Fig. 3.** Rare CNVs at the ASTN2/TRIM32 locus in ADHD and ASD probands. (A) Overview of rare CNVs detected at the 9q33.1 locus, overlapping ASTN2 and/or TRIM32 in six unrelated male probands in this study (two ADHD and four ASD). Variants reported at this locus from previous CNV studies of different neuropsychiatric disorders are also shown: six ASD probands (59), two schizophrenia probands (62), one intellectual disability proband (61), and one bipolar disorder proband (60). The blue and red bars denote deletions and duplications, respectively. No CNVs were seen at this locus in 2357 control individuals. Genomic coordinates and information about transcript isoforms are from Genome Build 36 (hg18). (B) Pedigrees of four ASD probands with maternally inherited CNVs overlapping ASTN2 and/or TRIM32. Black filled symbols represent individuals with an ASD diagnosis, whereas black filled symbols with white stripes symbolize ASD probands who also met criteria for ADHD.
but less than the 5 to 10% reported in ASD (34, 35, 64) and schizophrenia (65). The similar rate of de novo CNV observed in ADHD cases and controls implies that not all the de novo events observed in ADHD subjects may be pathogenic. However, we did identify one ADHD proband with seizure-like symptoms, who was carrying a de novo CNV disrupting DCLK2. There was also another ADHD proband with de novo CNVs disrupting SORCS1 and SORCS3, who developed bipolar disorder in adolescence. In both cases, the clinical presentation was consistent with what might be predicted on the basis of the annotated function of these genes. Our data, therefore, suggest that the contribution of de novo CNV to ADHD is likely small, but that careful analysis of phenotype and genes affected (and comparison to other cases and controls) may potentially be clinically revealing. Below, we provide potential scenarios based on case descriptions for the probands carrying these two de novo CNVs (DCLK2 and SORCS1/SORCS3), and a third case study of a male with a rare, maternally inherited X-linked CNV (at the PTCHD1 locus). These examples and others from this study support the need for both extensive and longitudinal phenotypic data including family histories in analyses such as these, particularly when considering subjects exhibiting additional medical complications. Moreover, in all instances, potential genotype-phenotype correlations will benefit from much larger sample sizes.

Case 1 (27696.3) was first diagnosed at age 7 with ADHD, with combined subtype and subthreshold anxiety symptoms that did not warrant separate diagnoses. At age 14, he continued to show ADHD and social difficulties, and the patient and his mother reported episodes during which the patient stared blankly and was unresponsive to events going on around him, including someone calling his name. He also reported having unusual sensory experiences such as hearing noises (for example, high-pitched screeches and whispers) and smelling odors. The family was concerned that he might have psychosis because of schizoaffective disorder in a maternal uncle and schizophrenia plus depression in the maternal grandfather. There was no family history of epilepsy or evidence of delusional or disordered thoughts, or mood symptoms. Neurological examination and electroencephalogram found no abnormality. The patient was found to have a de novo deletion at 4q31.3 affecting DCLK2, a gene whose predicted function may account for his seizure-like episodes.

Case 2 (30600.3), a male, was diagnosed with ADHD, combined subtype, at age 8. There were no concerns regarding his mood. Family history was positive for ADHD in father and brother, bipolar disorder and possible schizophrenia in a second-degree relative (maternal aunt), possible childhood schizophrenia in a maternal cousin, and alcoholism in multiple paternal and maternal second-degree relatives. At age 15, the patient was diagnosed with bipolar II disorder after several depressive episodes and a single manic episode characterized by inflated self-esteem, grandiosity, hypersensitivity to environmental stimuli, rapid thought patterns, rapid speech, and decreased need for sleep. He continued to meet diagnostic criteria for ADHD, combined subtype. At age 18, both ADHD and bipolar disorder persisted with some escalation of impairment and symptoms. Subsequent to the third assessment at age 18, de novo duplications at 10q25 were identified affecting SORCS1 and SORCS3. Variants at SORCS2 have been associated with bipolar disorder. The existence of these two CNVs in this patient may therefore have influenced the presentation of bipolar disorder.

Case 3 (27075.3), a male, was diagnosed with ADHD at age 7. He was noted to have poor social skills and anxious traits. He did not have a history of language delay, social isolation, or obsessive preoccupations. Subsequent to the initial assessment, a community psychiatrist diagnosed depression, generalized anxiety disorder, and learning disability. Assessment at ages 15 and 17 revealed continued ADHD and anxious traits, but no depression. His parents reported that he preferred to be alone or with adults. Upon entering university, he began to flourish academically but his anxiety increased with the heightened academic demands. At each assessment point, his preference for social isolation was noted, but no formal diagnosis of ASD was made, because he did not meet full criteria. Microarray analysis revealed a maternally inherited deletion at the PTCHD1 locus at Xp22.11. This CNV has recently been implicated in ASD (35, 66) and could potentially contribute to and explain the social interaction deficits in this individual.

Overall, we detected rare inherited CNVs at loci previously reported in ADHD or in other neurodevelopmental disorders in 8% of the ADHD sample, suggesting that they may be risk factors for ADHD and/or associated neuropsychiatric phenotypes in the individuals carrying them. CNVs involving previously implicated ADHD loci such as DRD5 and 15q13 microduplications are most compelling for further genomic analyses, but new candidate susceptibility genes such as CPLX2, ZBBX, and PTNPR2 were also discovered.

We also identified rare inherited CNVs in genes such as ASTN2, GABRG1, and CNTN5, which are affected by rare CNVs in other neurodevelopmental disorders. The apparent overlap of rare inherited CNVs in our ADHD cohort with those in cases with other neurodevelopmental disorders, in particular ASD, supports the idea that variants at a common set of genes could be involved in the etiology of several neuropsychiatric disorders (31–33). To further explore this possibility, we assessed rare CNVs in a new cohort of 349 unrelated individuals having a primary diagnosis of ASD. Deletions of ASTN2 and the ASTN2-intronic TRIM32 genes yielded the strongest association with ADHD and ASD. GWA (41) and linkage studies (67–69) provide additional evidence for involvement of the 9q33 region in ADHD. GWA studies have also suggested a role for this region in schizophrenia (70, 71) and bipolar disorder (71).

ASTN2 has recently been shown to play a vital role in the developing mammalian brain by forming a complex with its paralog astrotactin 1 (ASTN1) and regulating its expression on the surface of young cerebellar neuroblasts (58). Although ASTN2 and ASTN1 are highly homologous, their protein products have been found to function together in a complementary yet nonredundant manner (58). In vitro studies, using antibodies against ASTN1, have confirmed its role as a receptor for neuronal migration along astroglial fibers by facilitating glial-neuron binding (72, 73). Mice deficient in ASTN1 exhibit slowed neuronal migration, altered Purkinje cell morphology, and impairments in movement and coordination (74). TRIM32 encodes a tripartite motif containing E3 ubiquitin ligase protein, which has been reported to have a role in deciding the fate of neuronal stem cell lineages (75). Homozygous mutations in this gene have also been reported in a consanguineous family with Bardet-Biedl syndrome and cognitive impairment (76).

DNA sequencing of the ASTN2 and TRIM32 genes did not reveal obvious deleterious variants, such as nonsense or frameshift mutations, although several missense variants were identified. With the current sample size, no significant enrichment was observed in cases over controls. These results, together with the observation of deletions spanning ASTN2 and TRIM32 in ASD and ADHD patients, could indicate that disruption of both genes is associated with a higher risk for
neuropsychiatric disorder in general rather than risk for any specific disorder.

There has been recent debate about the possibility that intellectual disability, rather than ADHD, accounts for the large rare variants previously observed in ADHD (77). We believe that low IQ did not account for the rare CNVs reported in the current study, because we excluded ADHD probands with below-average IQ, defined as both a verbal and a performance IQ score below 80 (see Materials and Methods) and because the IQ of those subjects with rare CNVs did not differ significantly from that of the total sample (P = 0.07, unpaired two-tailed t test).

We also performed additional genetic CNV burden and gene set enrichment analyses [following our approach in (35)], but did not detect any genome-wide differences between ADHD cases and controls. This may be attributed to insufficient power because of the comparatively small sample size in our study.

One intriguing finding that emerges from CNV studies of neuropsychiatric conditions such as ADHD and ASD is that there seem to be common genes and pathways implicated in several disorders (31, 32). It is rare to find ADHD in the absence of any other neuropsychiatric disorder (14, 17, 18), and our genomic studies here and elsewhere (27–29) point to numerous rare variants that serve as potential risk factors. More recent genomic hypotheses to explain clinical overlap and pleiotropy put forth by Oti et al. (78), Rzhetsky et al. (79), and others suggest that different human disease-phenotype groups might arise from overlapping molecular causation. This could involve different functional domains of a single protein, the interaction between different proteins (such as a ligand and receptor), the interaction of proteins in a multiprotein complex, or different steps in a cellular pathway. Crespi and colleagues have also suggested that rare duplications and deletions at dosage-sensitive genes could be responsible for diametric models of neuropsychiatric disorders, for example, considering schizophrenia and autism as opposite conditions along a spectrum of social-brain phenotypes from hypodevelopment in autism to hyperdevelopment in schizophrenia (80). Given the extent and types of genes that we identified, it is likely that some or all of these mechanisms contribute to ADHD etiology. Moreover, all of these issues are exacerbated in complex and apparently heterogeneous disorders like ADHD, where the genetic liability of other risk alleles, protective genetic modifiers (both rare and common), and other factors like gender can influence expression and penetrance of neuropsychiatric phenotypes.

We also recognized the importance of obtaining detailed longitudinal phenotype data on the ADHD index cases and on their family members. In the three case studies (27696.3, 30600.3, and 27075.3) discussed above and others described in Table 1, the original ascertainment diagnosis of ADHD initially predominated our interpretation of the CNV association results, but these interpretations were broadened upon receiving new clinical assessments as the participants reached adolescence. Ascertainment issues will always present challenges in genetic studies of neuropsychiatric disorders. One example relevant to our work is that the DSM-IV will not allow a diagnosis of ADHD to a child with ASD, even though data suggest upward of 50% of children with ASD would otherwise meet criteria for ADHD. Ultimately then, we believe our new results should serve as a conservative starting point for much larger studies exploring the role of de novo and rare CNVs in ADHD, ADHD-related disorders, and other associated clinical comorbidities.

**MATERIALS AND METHODS**

**ADHD probands: Assessment protocol for diagnosis**

Participants were 175 boys (71%) and 73 girls (29%) ages 5 to 17 (mean = 9.5, minimum = 5.6, maximum = 16.9) who were referred for assessment of attention, learning, and behavior problems to the Hospital for Sick Children, Toronto. Participants were included if they met criteria for ADHD based on the results of a semistructured diagnostic interview of the participant’s parent(s) and of the proband’s teacher, and did not meet any of the exclusion criteria specified by DSM-IV (mental retardation, pervasive developmental disorder, autism, or comorbid psychiatric disorder that could better account for the disorder).

The Parent Interview for Child Symptoms (PICS) (81) is similar to the Schedule for Affective Disorders and Schizophrenia (KSADS) (82) with an enhanced module for ADHD and other disruptive behavior disorders. The PICS was conducted by a social worker with a master’s degree in social work (MSW), a clinical nurse specialist, or a clinical psychologist. Reliability of ADHD diagnoses was assessed through videotaped interviews in 48 cases and was found to be high (interclass correlation for total symptom score = 0.93). The Teacher Telephone Interview (TTI) (83) is a semistructured interview conducted by an interviewer with at least a master’s degree in psychology. Reliability of the TTI was assessed with audiotapes and found to be high (interclass correlation for total symptom score = 0.93). Intelligence and academic attainment were assessed by a clinical psychologist.

To receive a best-estimate diagnosis of ADHD arrived at through consensus between the assessing psychiatrist and psychologist, the participant had to present with impairing and developmentally atypical symptoms before age 7, meet DSM-IV criteria based on PICS and/or TTI, exhibit evidence of symptoms and impairment both at home and at school, and not present with any of the exclusion criteria for ADHD as stated in DSM-IV. Individuals were excluded if they had an IQ of less than 80 on both the verbal and the performance subscales of the WISC. The mean full-scale IQ for the entire sample was 102.12 (SD = 12.74) and 107.57 (SD = 16.25) for the subsample with a rare CNV of interest (Table 1).

Presumptive psychiatric status of each proband’s parents and siblings was established by family history. Parents and children over age 12 years provided consent, and younger participants gave assent. The protocol was approved by the Research Ethics Board of the Hospital for Sick Children, Toronto.

**ASD probands: Assessment protocol for diagnosis**

The participants in this study were 349 unrelated probands (257 boys and 92 girls) who had a clinical diagnosis for ASD by expert clinicians, according to the Autism Diagnostic Interview—Revised (ADI-R) and/or the Autism Diagnostic Observation Schedule (ADOS) (84). Individuals were recruited from four different Canadian sites: Hospital for Sick Children, Toronto, Ontario; McMaster University, Hamilton, Ontario; Memorial University of Newfoundland, St. John’s, Newfoundland; and University of Alberta, Edmonton, Alberta.

**Genotyping**

DNA collected from ADHD probands and their parents, and from ASD probands, was sent for genotyping at The Centre for Applied Genomics, Toronto. Samples were genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0 with standard protocols as
PCR validation of CNV calls was performed in triplicate with SYBR Green–based real-time quantitative PCR (qPCR) with controls at the FOXP2 loci at chromosome 7 (fig. S2). At least two independent qPCR assays were required for confirmation of a CNV. FISH validation was performed for the de novo gains at the SORCS1/SORCS3 locus (fig. S2).

Sequencing and mutation screening methods
All coding exons and intron-exon splice sites of TRIM32 and ASTN2 were sequenced in ADHD and ASD cases and in population-based controls of European ancestry from the Ontario Population Genomics Project with standard PCR-based Sanger sequencing. Primer3 software v. 0.4.0 (http://frodo.wi.mit.edu/primer3) was used to design PCR primers. For ASTN2, primers were chosen to cover the longest transcript isoform NM_198187 (Genome Build 36/hg18), which had 23 exons, as well as the first exon of the shorter isoform NM_198188 (Genome Build 36/hg18). The amplified products were sequenced with the ABI 3730XL capillary sequencer (Applied Biosystems). Variant detection was performed with SeqScape software from Applied Biosystems. Novel variants detected in the cases, not previously reported in the SNP database (dbSNP) build 130, were validated by resequencing in the case and in samples from both parents, when available. The Fisher’s exact test was used to test frequency of novel missense variants in ADHD and ASD cases versus controls (table S6).

SUPPLEMENTARY MATERIAL
www.sciencetranslationalmedicine.org/cgi/content/full/3/95/95ra75/DC1

Fig. S1. Examples of loci with overlapping rare CNVs in this study and in previous ADHD studies. Fig. S2. Examples of CNV validation.

Table S1. Summary statistics of stringent CNVs found in ADHD and control data sets. Table S2. List of rare stringent CNVs specific to ADHD data set. Table S3. Phenotypes of families with rare CNVs at ADHD candidate loci.

Table S4. Loci with overlapping rare CNVs in this study and in previous ADHD studies. Table S5. Rare CNVs in ASD probands at loci also implicated in ADHD probands. Table S6. Summary of newly described ASTN2 and TRIM32 sequence variants. Table S7. Newly described ASTN2 sequence variants in ASD probands. Table S8. Newly described ASTN2 sequence variants in controls. Table S9. Newly described TRIM32 sequence variants in ASD probands. Table S10. Newly described TRIM32 sequence variants in ADHD probands. Table S11. Newly described TRIM32 sequence variants in controls. Table S12. Newly described TRIM32 sequence variants in controls.

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Competing interests: R.S. is a consultant for Eli Lilly and Purdue Pharma. S.W.S. is on the Board of Trustees of Genome Canada, which is a funder of this project, on the Scientific Advisory Committee of Autism Speaks, which has funded some of the data collection, and on the Scientific Advisory Board of Population Diagnostics, a U.S. company that could use data from this study. S.W.S. and R.S. have filed a patent on the use of AstN2 and other CNVs in the diagnosis of ADHD based on the data from this paper. The other authors declare that they have no competing interests.

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