Characterization of a Family with Rare Deletions in *CNTNAP5* and *DOCK4* Suggests Novel Risk Loci for Autism and Dyslexia

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Background: Autism spectrum disorders (ASDs) are characterized by social, communication, and behavioral deficits and complex genetic etiology. A recent study of 517 ASD families implicated *DOCK4* by single nucleotide polymorphism (SNP) association and a microdeletion in an affected sibling pair.

Methods: The *DOCK4* microdeletion on 7q31.1 was further characterized in this family using QuantiSNP analysis of 1M SNP array data and reverse transcription polymerase chain reaction. Extended family members were tested by polymerase chain reaction amplification of junction fragments. *DOCK4* dosage was measured in additional samples using SNP arrays. Since QuantiSNP analysis identified a novel *CNTNAP5* microdeletion in the same affected sibling pair, this gene was sequenced in 143 additional ASD families. Further polymerase chain reaction-restriction fragment length polymorphism analysis included 380 ASD cases and suitable control subjects.

Results: The maternally inherited microdeletion encompassed chr7:110,663,978-111,257,682 and led to a *DOCK4-IMMP2L* fusion transcript. It was also detected in five extended family members with no ASD. However, six of nine individuals with this microdeletion had poor reading ability, which prompted us to screen 606 other dyslexia cases. This led to the identification of a second *DOCK4* microdeletion co-segregating with dyslexia. Assessment of genomic background in the original ASD family detected a paternal 2q14.3 microdeletion disrupting *CNTNAP5* that was also transmitted to both affected siblings. Analysis of other ASD cohorts revealed four additional rare missense changes in *CNTNAP5*. No exonic deletions of *DOCK4* or *CNTNAP5* were seen in 2091 control subjects.

Conclusions: This study highlights two new risk factors for ASD and dyslexia and demonstrates the importance of performing a high-resolution assessment of genomic background, even after detection of a rare and likely damaging microdeletion using a targeted approach.

Key Words: Autistic, *CNTNAP5*, CNV, *DOCK4*, dyslexia, neurexin

Autism spectrum disorders (ASDs) are a subset of complex neurodevelopmental disorders characterized by deficits in three core domains: 1) reduced reciprocal social interaction, 2) impaired ability to communicate, and 3) a narrow range of interests and repetitive behaviors. Autism spectrum disorders are clinically heterogeneous and often show comorbidity with other conditions such as epilepsy and learning disability (1,2).

Although autism has consistently been shown to demonstrate high levels of heritability, it has only recently become clear that many of the genes recently implicated in autism are involved with the initiation and maintenance of synaptic connections. For example, a pathway-based analysis using data from the first published ASD genome-wide single nucleotide polymorphism (SNP) association study implicated the cadherin gene family. Stronger enrichment was seen when these 25 cadherins were combined with three neurexins and five neurexin-related *CNTNAP* genes (3). Mutations in neuroligin proteins, which interact with neurexins across the synaptic cleft, were associated with autism before neurexins were implicated (4). Other studies have shown that genomic copy number variations (CNVs) also play a significant role in autism susceptibility (5,6). Hemizygous disruption of the neurexin 1 gene (*NRXN1*) was first seen in sisters with ASD (7). In addition, rare structural variants involving *CNTNAP2* have also recently been associated with autism susceptibility (8,9),

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further implicating this gene family. Drosophila orthologues of *CNTNAP2* and *NRXN1* can both reorganize synaptic morphology and regulate another synaptic protein bruchpilot (10). It is thus proposed that a shared synaptic mechanism underlies the similar clinical outcomes for patients with *NRXN1* or *CNTNAP2* mutations.

An emerging trend from recent literature is that many microdeletion syndromes exhibit considerable phenotypic variability. For instance, variable dosage of *CNTNAP2* has also been documented in epilepsy and schizophrenia (11), while deletion of 15q13.3 has been implicated in autism (12), generalized learning disability (13), epilepsy (14), and schizophrenia (15). One possible hypothesis is that additional CNVs elsewhere in the genome may act as genetic modifiers. In support of this hypothesis, recent studies on schizophrenia have detected more than one rare CNV $>$ 100 kb within the same affected individual (16).

The autism susceptibility locus 1 (*AUTS1*), situated on chromosome 7q, has been identified in at least four genome-wide linkage scans (17–20) and two meta-analyses (21,22). A recent *AUTS1* fine-mapping study, using both family-based and case-control association analyses, detected SNPs within *DOCK4* (dedicator of cytokinesis 4) and *IMMP2L* (IMP2 inner mitochondrial membrane protease-like) that may be indexing autism susceptibility factors. A rare genomic deletion disrupting both *DOCK4* and *IMMP2L* was also detected and shown to be transmitted to both members of an affected sibling pair (ASP), further implicating this gene region (23). *DOCK4* is a plausible ASD candidate gene, as recent RNA interference studies using rat hippocampal neurons have shown that this gene may influence dendritic branching and growth (24). Genes at this locus have previously been linked to Tourette syndrome, through association (25) or cytogenetic aberration (26). Disruption of a related gene, *DOCK3*, has also been linked to attention-deficit/hyperactivity disorder (27). To understand its potential effect, we first characterized the *IMMP2L-DOCK4* deletion and resulting fusion transcript at base-pair resolution, using a variety of methods. Due to reports of additional neurological and psychiatric disorders in the extended family, relatives for whom DNA was available were also screened for this deletion. The finding that three relatives with poor reading ability had also inherited the same deletion led us to further assess an additional dyslexia cohort for genomic variants in this gene region.

We also assessed the genomic background in the autism family with the *IMMP2L-DOCK4* deletion and detected a second rare microdeletion disrupting *CNTNAP5* in the ASP. As this gene is related to *CNTNAP2*, it was sequenced in additional ASD families from the International Molecular Genetic Study of Autism Consortium (IMGSAC) cohort to search for other rare variants that might be of etiological relevance to autism.

Methods and Materials

Clinical Details

The Dutch multiplex autism family 15-0084 volunteered to participate in the IMGSAC research study, as approved by the medical ethical commission of the University Medical Center Utrecht. Extended family members were also asked to participate and donate saliva samples. Informed consent was obtained from all participants. Case reports for the proband (15-0084-003), his affected brother (15-0084-004), and other family members are given in Supplement 1.

Clinical Assessment

Social communication ability was assessed in all participating family members using the Social Responsiveness Scale (SRS) (28). Depending on the age of the participant, either the children's version or the adult informant version was used. The *t* scores are reported for the SRS children's version. For adults, *t* scores are unavailable, so the total scores are compared with the mean total scores in the Dutch population (Ilse L. Noens, Ph.D., written communication to MVdJ, November 2009). The participants with a formal diagnosis of autism scored within the severe range of the SRS. The total SRS scores of the unaffected family members are all between the mean and $+1$ SD. Reading and spelling ability were assessed by means of a short battery of Dutch word reading, nonword reading, text reading, and spelling tests. An overall evaluation of communication ability and reading capacity is presented in Table S1 in Supplement 1.

DNA Extraction

DNA was extracted from the saliva samples of the extended family using the Oragene DNA Extraction Kit (DNA Genotek, Ontario, Canada), following the manufacturer's protocol. DNA from family 15-0084 had been extracted, as described previously (18).

1M SNP Array and CNV Analysis

A total of 750 ng of genomic DNA was run on the single-sample Infinium 1M SNP BeadArray (Illumina, San Diego, California) according to manufacturer's instructions, with default SNP clustering. Log R ratios and B allele frequencies were used to call CNVs using the QuantiSNP algorithm (29), with $L = 2M$, expectation-maximization iterations $= 50$, MaxCopy $= 4$, and correcting for local GC content. High confidence calls with log Bayes Factor > 10 were used, whereby we expected approximately one false CNV call per sample.

Long-Range Polymerase Chain Reaction, Quantitative Multiplex PCR of Short Fluorescent Fragments, and CNV Validation

Long-range polymerase chain reaction (PCR) was carried out with Bio-X-ACT long DNA polymerase (Bioline, London, United Kingdom) using the manufacturer's suggested protocol. Primers TTTCACCTTTTGGGGTGCTA and TGGAGCCTGGGAATTAAAAA were used to amplify across the *IMMP2L-DOCK4* deletion. Nested *CNTNAP5* primer sequences are available on request.

Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF) was performed as previously described (30), using DNA from 197 multiplex IMGSAC families (320 affected individuals) and 461 Caucasian United Kingdom control subjects available from the European Collection of Cell Cultures. The QMPSF primers (sequences available on request) were designed in *DOCK4* exons 1, 8, 15, 25, 31, and 52 to complement our previous QMPSF analysis, which included *IMMP2L* exons and the last exon of *DOCK4* (23). Exon 7 of the *RNF20* gene was co-amplified as a control exon.

To ensure that all reported CNV results were verified by at least two methods, DNA samples from extended family members were also tested using quantitative PCR and/or 44 k array-based comparative genomic hybridization (Agilent, Santa Clara, California).

Real-Time-PCR

Total RNA was extracted from blood samples stored in RNA*later* solution using RiboPure Blood Kit (Ambion, Austin, Texas), according to the manufacturer's instructions. Comple-

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