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Association between Microdeletion and Microduplication at 16p11.2 and Autism

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ABSTRACT

BACKGROUND

Autism spectrum disorder is a heritable developmental disorder in which chromosomal abnormalities are thought to play a role.

METHODS

As a first component of a genomewide association study of families from the Autism Genetic Resource Exchange (AGRE), we used two novel algorithms to search for recurrent copy-number variations in genotype data from 751 multiplex families with autism. Specific recurrent de novo events were further evaluated in clinical-testing data from Children's Hospital Boston and in a large population study in Iceland.

RESULTS

Among the AGRE families, we observed five instances of a de novo deletion of 593 kb on chromosome 16p11.2. Using comparative genomic hybridization, we observed the identical deletion in 5 of 512 children referred to Children's Hospital Boston for developmental delay, mental retardation, or suspected autism spectrum disorder, as well as in 3 of 299 persons with autism in an Icelandic population; the deletion was also carried by 2 of 18,834 unscreened Icelandic control subjects. The reciprocal duplication of this region occurred in 7 affected persons in AGRE families and 4 of the 512 children from Children's Hospital Boston. The duplication also appeared to be a high-penetrance risk factor.

CONCLUSIONS

We have identified a novel, recurrent microdeletion and a reciprocal microduplication that carry substantial susceptibility to autism and appear to account for approximately 1% of cases. We did not identify other regions with similar aggregations of large de novo mutations.

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AUTISM IS A PERVASIVE DEVELOPMENTAL disorder defined by a neurobehavioral phenotype that includes social disability, communication impairment, repetitive behaviors, and restricted interests. The onset is generally before the age of 3 years, and the disorder has a prevalence of 0.6% in the population, affecting many more boys than girls.[1] Results of twin and family studies have shown that the heritability of autism is approximately 90%, making it one of the most heritable complex disorders.[2] In approximately 10% of patients, autism can be explained by genetic syndromes and known chromosomal anomalies (most of which have recognizable features in addition to autism), including the fragile X syndrome, tuberous sclerosis, the Smith–Lemli–Opitz syndrome, the Potocki–Lupski syndrome, and maternally inherited duplications of the region (15q11–13) that is affected in the Prader–Willi and Angelman syndromes.[2] However, despite the high heritability of autism, genetic studies have not provided substantial insight into the 90% of cases of autism whose cause is idiopathic.

The relative genetic contribution to a susceptibility to autism from de novo mutations, rare mutations, and common polymorphisms has been debated extensively.[3] Recent whole-genome studies assessing copy-number variation[4] reported an excess of large de novo copy-number variants, with such events reported in 7 to 10% of simplex families, 2 to 3% of multiplex families, and only 1% of control families.[5,6] Although these data imply a role for de novo copy-number variation, no recurrent events were identified and implicated as having an unequivocal association with autism.

We therefore carried out a high-resolution genomewide analysis of a sample of multiplex families in the Autism Genetic Resource Exchange (AGRE)[7] with the use of the Affymetrix 5.0 genotyping platform. This platform offers not only single-nucleotide polymorphism (SNP) probes but also a dense collection of SNP invariant probes, which combine to enable detection of copy-number variation. In this study, we describe a screening technique for recurrent de novo autosomal copy-number variants that could influence susceptibility to autism with follow-up analysis of clinical genetic-testing data from Children's Hospital Boston and a large population sample from Iceland (studied at deCODE Genetics).

METHODS

STUDY DESIGN

The samples and methods that we used are summarized in Table 1. Detailed materials and methods describing sample collections and methods of copy-number analysis (the copy-number polymorphism evaluation routine [COPPER] and Birdseye algorithms) are provided in the Supplementary Appendix, available with the full text of this article at www.nejm.org. These novel methods have good power to detect deletions spanning at least 10 probes (approximately 30 kb) on the Affymetrix 5.0 platform. We screened AGRE samples only from persons without known chromosomal anomalies, the fragile X syndrome, and other established syndromes.

The study was approved by the institutional review boards at the Massachusetts Institute of Technology (for the AGRE samples) and Children's Hospital Boston (for the Children's Hospital samples) and by the Data Protection Authority and National Bioethics Committee of Iceland (for the deCODE samples). Written informed consent was obtained from all subjects in the AGRE and deCODE research studies. Children's Hospital Boston carried out comparative genomic hybridization for clinical diagnostic purposes; since results were anonymous and obtained by chart review, research-based informed consent was not required by the institutional review board that approved the study.

RESULTS

COPY-NUMBER ABNORMALITIES

To discover recurrent deletions or duplications conferring a risk of autism in multiple families, we used the COPPER algorithm to identify regions in which three or more patients with autism had overlapping copy-number (or genomic "dosage") abnormalities — that is, we looked for regions that had either fewer than or more than two genomic copies. To ensure that these regions were not sites of common copy-number polymorphism, we focused on regions that were variant in less than 1% of parents of subjects with autism. For each event predicted by COPPER and meeting these criteria, we used visual inspection of intensity data, whether the same event was predicted with Birdseye, and analysis of mendelian inheritance to assess our confidence in the observation.

We identified 32 high-confidence regions and 15 lower-confidence regions, and all but 1 of these regions appeared to be normally segregating variants. Of these regions, 16 (including 8 with high confidence) had at least one de novo event in which both parents were negative for the copy-number variant, suggesting the possibility of recurrent mutation. Although the majority of these variants did not cosegregate with autism (and probably constitute rare, neutral copy-number variations), one region stood out as having multiple de novo events and no inherited events.

MICRODELETION ON CHROMOSOME 16P11.2

A region on chromosome 16p11.2 (from genomic coordinates 29.5 Mb to 30.1 Mb) was unique in our data. Five children (four boys and one girl) with autism in four independent families carried de novo deletions; we observed no deletions in the parents. One pair of siblings who were not monozygous twins shared the de novo event, presumably inherited from a parent with germline mosaicism. In the children with autism, we observed that the 16p11.2 deletion occurred on chromosomes derived from both the mother and the father.

The region coincides perfectly with a segment of 593 kb flanked by a 147-kb segmental duplication with 99.5% sequence identity. The identification of this cluster of de novo events by COPPER was confirmed by analysis of the same data with the use of Birdseye, with perfect agreement in identification of five samples with this deletion (Fig. 1). In addition, three of these samples overlapped with subjects who were genotyped at Johns Hopkins with Affymetrix 500K chips in an autism sample (provided by the National Institute of Mental Health), where the same deletions were identified by CNAT 4.0 (Affymetrix). The size of these deletions (593 kb, containing 86 distinct sites with SNP or copy-number probes) generates confidence that this observation is genuine, with all five subjects having a logarithm of the odds (LOD) of more than 50 in favor of a dosage of 1 (i.e., a hemizygous deletion) (Table 1 of the Supplementary Appendix). We did not observe the deletion in the parents of these five children (LOD >50 in favor of a normal dosage of 2 in all parents), nor did we observe it in any of the 1420 parents in this study.

Table 1. Detection of 16p11.2 Copy-Number Variants, According to Sample.*

Sample	Case Subjects	Control Subjects	Experiment	Analysis	Case		Control		P Value†
					Deletion	Duplication	Deletion	Duplication	
					number				
AGRE	751 Families, with 1441 case subjects‡	1420 AGRE parents and 2814 samples with bipolar disorder or NIMH controls	Affymetrix 5.0 for AGRE families, Affymetrix 500K for controls	COPPER and Birdseye for AGRE families, COPPER for controls	5	7	3	2	1.1×10^{-4}
Children's Hospital Boston§	512 Children	434 Children	Agilent comparative genomic hybridization	ADM-2	5¶	4	0	0	7.1×10^{-3}
deCODE	299 Subjects with autism spectrum disorder‖	18,834 Subjects not screened for a psychiatric or language disorder	Illumina HumanHap300 BeadChip	HMM	3	0	2	5	4.2×10^{-4}

* ADM denotes aberration detection method, AGRE Autism Genetic Resource Exchange, COPPER copy-number polymorphism evaluation routine, HMM hidden Markov model, and NIMH National Institute of Mental Health.

† P values for deletions plus duplications are for the comparison between case subjects and control subjects, as calculated by Fisher's exact test.

‡ Subjects were assessed according to the Autism Diagnostic Interview — Revised (for details, see the Supplementary Appendix).

§ Case subjects had received the diagnosis of developmental delay, mental retardation, or autism spectrum disorder after clinical evaluation. Control subjects had been referred for congenital anomalies not including developmental delay, mental retardation, or autism spectrum disorder.

¶ The number includes a monozygotic twin pair.

‖ The diagnosis of autism spectrum disorder was made according to the definition in the *International Classification of Diseases,* 10th revision.

Deletion and normal dosage were positively confirmed by multiplex ligation-dependent probe amplification (MLPA) for all subjects in all four of these AGRE families (Fig. 1C). However, in 2814 samples from other studies (unpublished data), three female control subjects (who participated in a study of bipolar disorder but were not screened for autism) carried the deletion (see the Supplementary Appendix). The deletion rate in this population is much lower than the rate in the sample of children with autism ($P=0.03$ for the deletion), although it suggests that the deletion does not cause severe autism in every case.

DUPLICATION IN FAMILIES WITH AUTISM

We observed reciprocal duplication of the 593-kb deleted region in three AGRE families (with at least one family member with a LOD >30) (Fig. 1). This duplication was inherited in two families: it was transmitted from a parent to two of two affected offspring (male and female) as well as to one unaffected daughter and from another parent to four of four affected sons. In the third family, the duplication appeared to be a de novo event in one of two affected male offspring. The full duplication was not observed in any of the 2814 samples from other studies analyzed and thus appears to be a high-penetrance risk factor conferring risk to seven additional subjects with autism in the AGRE sample ($P=1.1\times10^{-4}$ for both deletions and duplications) (Table 1, and Table 1 of the Supplementary Appendix).

ADDITIONAL DUPLICATIONS IN AGRE FAMILIES

We identified five large duplications of three different sizes in the 15q11–13 region associated with the Prader–Willi and Angelman syndromes (Table 2). Of these duplications, one was maternally inherited, one occurred in a subject whose father had a normal dosage and whose mother was unobserved, and three were de novo duplications; the smallest extended from genomic position 23 Mb to 25 Mb on chromosome 15. This relatively small duplication could help to focus candidate-gene studies since it included only two genes — *ATP10A* and *GABRB3*.

We did not observe de novo deletion or duplication of a recently implicated gene, *NRXN1* on chromosome 2,[5,8] although we observed six families with deletions within the *NRXN1* locus. The deletions did not cosegregate with autism in four of the six families (i.e., not all affected persons inherited the deletion) and were not associated with autism on the basis of a transmission disequilibrium test. We observed deletions at this locus in 5 of the 2814 control samples. Other events coincident with regions that were highlighted in two recent studies[5,6] are listed in Table 2 of the Supplementary Appendix.

To obtain a more complete tally of potentially causal recurrent events, we used the Birdseye algorithm to search for de novo deletions and duplications of 20 kb or larger in genome-wide data obtained from the AGRE samples. We found no additional de novo events in multiple subjects that were not observed in the International HapMap Project or in 2814 samples from control subjects. Although we detected approximately 50 de novo events of more than 100 kb that were not seen in HapMap, a number of these events did not cosegregate with autism in other families or they have been observed in samples from subjects who did not have autism; all such events require further study in additional subjects and much larger control samples before those that may confer susceptibility can be identified.

Figure 1 (facing page). Regions of Microdeletion and Microduplication on Chromosome 16p11.2.

In Panel A, normalized intensity data are averaged every 11 to 12 probes across a 2-Mb region on chromosome 16. Means (closed circles) and standard deviations (vertical bars) for subjects with normal copy numbers are depicted in blue; subjects with duplication are denoted with red open circles, and those with deletions are denoted with green triangles. Annotated genes in the region of interest are shown (not to scale), with gray denoting brain expression and black denoting unknown or little brain expression. Arrows represent the segmental duplications mediating the rearrangements, with three genes located within the segmental duplication. In Panel B, both the deletion and duplication graphs show shifted overlaying of two traces of multiplex ligation-dependent probe amplification (MLPA). Red tracings in both graphs represent a normal control sample. In the deletion graph, blue tracings show a sample with a 16p11.2 deletion; in the duplication graph, blue tracings show a sample with a 16p11.2 duplication. The MLPA profiles were generated by ABI 3730 Genetic Analyzer and normalized by GeneMarker software (SoftGenetics). The four amplicons that are underlined with a black bar and shown with arrows are from probes located within the imbalanced 16p11.2 region. Amplicons labeled with C are control probes located either on chromosome 16 but outside the imbalanced region or on other chromosomes.

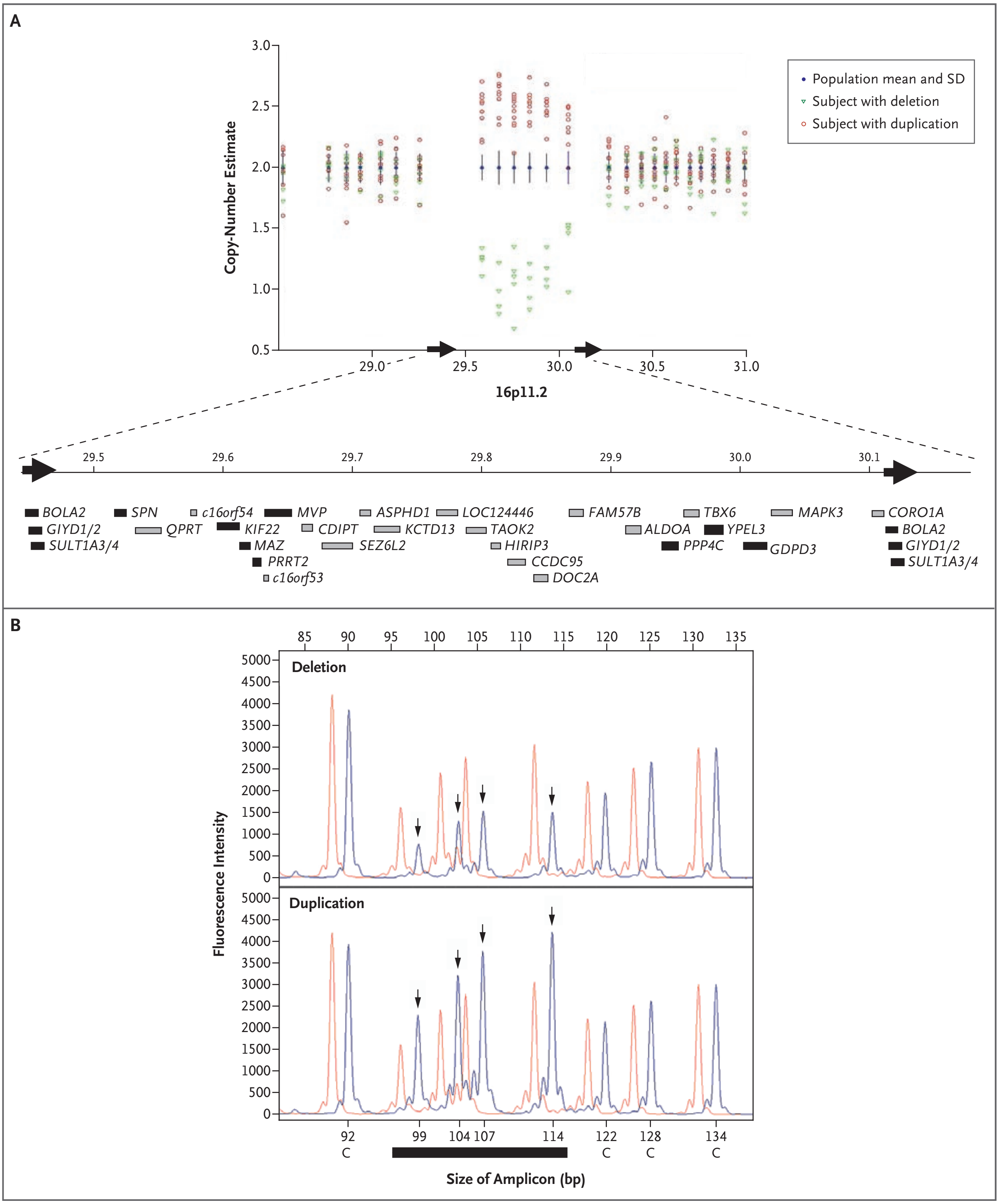
A
Copy-Number Estimate
3.0
2.5
2.0
1.5
1.0
0.5
29.0
29.5
30.0
30.5
31.0
16p11.2
Population mean and SD
Subject with deletion
Subject with duplication
29.5
29.6
29.7
29.8
29.9
30.0
30.1
BOLA2
GIYD1/2
SULT1A3/4
SPN
QPRT
c16orf54
KIF22
MAZ
PRRT2
c16orf53
MVP
CDIPT
SEZ6L2
ASPHD1
KCTD13
LOC124446
TAOK2
HIRIP3
CCDC95
DOC2A
FAM57B
ALDOA
PPP4C
TBX6
YPEL3
GDPD3
MAPK3
CORO1A
BOLA2
GIYD1/2
SULT1A3/4
B
85
90
95
100
105
110
115
120
125
130
135
Deletion
Duplication
Fluorescence Intensity
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
92
C
99
104 107
114
122
C
128
C
134
C
Size of Amplicon (bp)

Table 2. Duplication of Chromosome 15q11–13 in the AGRE Sample.*

Chromosome and Region	Inheritance and Transmission
Chromosome 15	
21.2–26.3 (BP2–BP3)	2 De novo copy-number variants
18.8–26.4 (BP1–BP3)	Inherited copy-number variant, 2 transmitted and 0 not transmitted; 1 unknown
23.3–24.7	De novo copy-number variant

* Duplications of the region associated with the Prader–Willi and Angelman syndromes were detected by applying novel algorithms to Affymetrix 5.0 genotype data. For inherited events, listed are the number of transmitted copy-number variants and the number of copy-number variants that were not transmitted to affected offspring for whom data were available. AGRE denotes Autism Genetic Resource Exchange.

CONFIRMATION IN CLINICAL SAMPLES

We tested for replication of the association between the 16p11.2 microdeletion and autism in a sample of 512 children with developmental delay, mental retardation, or autism spectrum disorder who were identified independently at Children's Hospital Boston with the use of comparative genomic hybridization. We identified five additional 16p11.2 deletions (all in boys, including one pair of monozygotic twins); the boundaries of the deletion in each case were identical to that described above (Fig. 1 and Table 3 of the Supplementary Appendix). One deletion was inherited from a mother with mild mental retardation, and the rest were de novo.

By contrast, we observed no deletions of this region in samples from 434 patients at Children's Hospital Boston that were tested by comparative genomic hybridization in the same laboratory. Samples from these children were submitted for diagnostic testing because they had dysmorphic features, multiple congenital anomalies, congenital heart disease, seizures, or other phenotypes in which developmental delay, mental retardation, or autism spectrum disorder was not indicated by the ordering physician.

The duplication at 16p11.2 was also observed in four independent samples (two from boys and two from girls) from the 512 children who were tested with the use of array comparative genomic hybridization for developmental delay, mental retardation, or autism spectrum disorder; the duplication was not observed in any of the 434 children who were tested for other diagnoses. This finding significantly reinforces the association of dosage abnormalities at 16p11.2 and phenotypes associated with autism spectrum disorder and developmental delay ($P=0.007$ for both deletions and duplications) (Table 1). Clinical features are described in Table 4 of the Supplementary Appendix. All deletions and duplications in this sample were positively confirmed with the use of MLPA and fluorescence in situ hybridization (FISH) (Fig. 1C and 2).

REPLICATION IN AN ICELANDIC SAMPLE

We observed that 3 of 299 subjects with autism spectrum disorder from Iceland carried the 16p11.2 deletion, a finding that was consistent with the 1% frequency observed in children at Children's Hospital Boston who had sporadic developmental delay or autism spectrum disorder (Table 5 of the Supplementary Appendix). One of these deletions was de novo, the origin of the second deletion was not known, and one was inherited from a father who had attention deficit–hyperactivity disorder (ADHD). By contrast, in a control sample of 18,834 subjects who did not undergo screening for a psychiatric or language disorder, only two deletions were observed — in other words, the deletion was observed more often in patients with autism by a factor of 100 ($P=3.7\times10^{-5}$). However, in a study of the same population by investigators at deCODE Genetics, this deletion was observed at a markedly increased rate in subjects with a psychiatric or language disorder. This study showed that the deletion was present in 1 of 648 patients with schizophrenia, 1 of 420 patients with bipolar disorder, 1 of 203 patients with ADHD (the father of a child with autism, as noted above), and 1 of 3000 patients with panic disorder, anxiety, depression, or addiction. In addition, 1 of 748 patients with dyslexia carried the deletion. Overall, in the Icelandic samples, the carrier frequency among patients with autism was 1%; the frequency was approximately 0.1% among patients with a psychiatric or language disorder and 0.01% in the general population.

We did not observe the duplication of this region in any of the Icelandic subjects with autism spectrum disorder but did observe it in two subjects with bipolar disorder and five unscreened control subjects, with a carrier frequency of 0.04% in subjects with a psychiatric or language disorder and in 0.03% of the general population.

In total, we have observed the identical deletion of nearly 600 kb in 13 subjects with autism or developmental or language delay (10 confirmed de novo mutations, 2 confirmed inherited mutations from parents with ADHD or mental retardation, and 1 mutation of unknown inheritance), with the reciprocal duplication of the same region documented in 11 additional subjects. The very low frequency (less than 0.1%) of dosage abnormalities in this region in the general population and the fact that we have yet to identify an instance in which the deletion was transmitted from an asymptomatic parent indicate strong natural selection, particularly against the recurrent microdeletion. A dosage abnormality in this region was present in 1% of autism samples from multiplex families, 1% of subjects with autism in a general population sample, and more than 1.5% of subjects with a developmental or language delay in a clinical setting.

DISCUSSION

We identified regions of rare copy-number variation in families with autism and observed an association between a microdeletion on chromosome 16 (and the inherited reciprocal duplication) and autism. Both the deletion and the duplication are likely to be mediated by the 147-kb segmental duplication flanking the deleted or duplicated sequence.[9] Microdeletion or microduplication through intrachromosomal recombination between segmentally duplicated sequences is an established mechanism associated with congenital developmental disorders such as the Smith–Magenis syndrome, the Williams syndrome,[10] the Potocki–Lupski syndrome (17p11.2 duplication),[11] and the DiGeorge syndrome (22q11 deletion).[12] The deleted or duplicated region on 16p11.2 contains 25 annotated genes or transcripts, and the flanking segmental duplications include an additional 3 genes (Fig. 1B). Several of the genes contained in this region could be considered good candidates for driving the phenotype on the basis of their expression in the brain or function in neurodevelopment.

We performed a simple calculation of differences in carrier frequency in subjects with autism and population controls for these events and assigned P values for the comparisons, achieving statistical significance in all three components of the study (the AGRE screening, the Children's Hospital Boston replication, and the deCODE Icelandic replication). However, such calculations underrepresent the full evidence in favor of causation because they do not reflect the fact that most of the deletions are de novo. If this deletion were neutral, the single-generation mutation rate implied by the observation of nine de novo events in fewer than 2500 subjects with autism would demand that the frequency of the deletion itself be extremely high, if not fixed, in the absence of very strong selective pressure against it. The fact that it is seen extremely rarely in the general population not only establishes a significant difference between rates in autism and control populations but also unambiguously establishes that strong natural selection is acting against transmission of this dele-

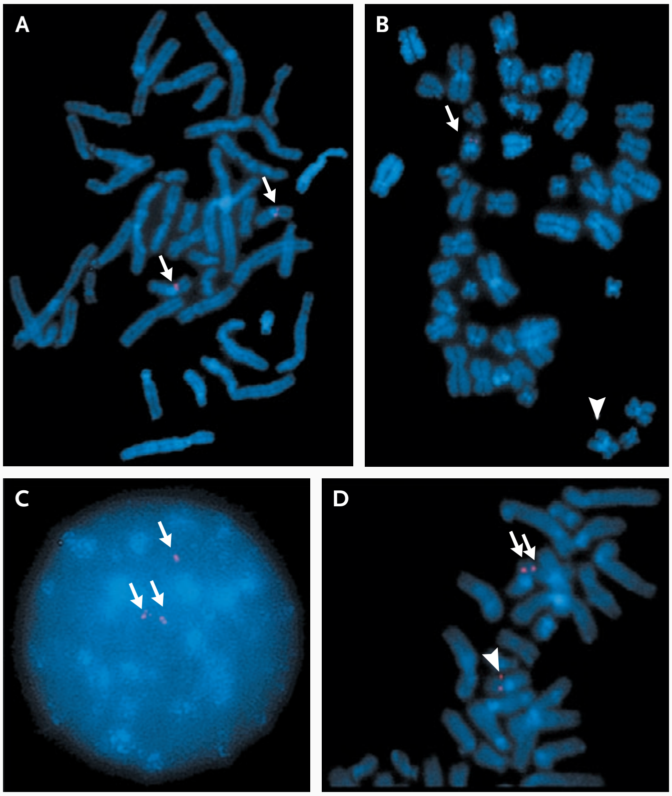

Figure 2. Analysis with Fluorescence in Situ Hybridization (FISH).

BAC clone RP11-504I2 was used as a probe for FISH analysis. Panel A represents a cell in metaphase from a normal control in which FISH signals (arrows) were detected on a pair of chromosome 16. Panel B represents a cell in metaphase from a sample with a 16p11.2 deletion in which a FISH signal was seen on one chromosome 16 (arrow) but not on the other chromosome 16 (arrowhead). Panel C shows a cell in interphase from a sample with a 16p11.2 duplication in which three FISH signals (arrows) were detected. Panel D shows a cell in metaphase from a sample with a 16p11.2 duplication in which FISH signals appear to be stronger on one chromosome 16 (arrows) than on the other chromosome 16 (arrowhead).

tion (as might well be expected from an allele that increases the risk of autism by as much as a factor of 100), given how often it arises de novo in a single generation.

Although multiplex families are expected to have fewer causal de novo mutations (and these do not contribute to the heritability of the disorder, except in cases arising from germ-line mosaicism or in monozygotic twins), the size and resolution of this study have enabled the identification of a recurrent de novo event that has a major effect in autism. Our observation of the same event at very low prevalence in control samples and enriched in samples from Icelandic subjects with a psychiatric or language disorder suggests that rather than having 100% mendelian penetrance for a strictly defined form of autism, this region resembles other microdeletion or duplication syndromes that are high-penetrance causes of developmental abnormalities but show a range of phenotypic severity across entire populations. The four AGRE families whose members had deletions had three affected children without this event, which suggests intrafamilial heterogeneity within some multiplex families with autism, a finding that is not unexpected given the high rate of autism spectrum disorder.

Neither the specific deletion nor duplication of this region was found in the Autism Chromosome Rearrangement Database (http://projects.tcag.ca/autism). In independently published studies, one observation of this microdeletion on 16p11.2 was made in a patient with autism[6] and in an adult twin pair with multiple congenital anomalies, including mild developmental delay.[13] Another recent study[14] showed the association between a larger microdeletion on 16p and a syndrome that included developmental delay, distinct facial appearance, and other variable features. In this study, four deletions are described, three of which are adjacent to, but do not appear to overlap with, the 16p11.2 deletion; the fourth deletion is even larger and encompasses the 16p11.2 deletion. Additional case reports of deletions on 16p11.2 with undetermined boundaries included a de novo deletion in a neonate with multiple congenital malformations[15] and a deletion of unknown inheritance in a female subject with mild mental retardation, severe speech delay, and facial dysmorphism.[16]

In our study, duplication of the 16p11.2 region cosegregated with autism in two families (six of six affected offspring) and occurred as a de novo event in a third family. Duplication in this region was also observed in four clinical samples referred for diagnosis of developmental delay, although no autistic features were reported in three of these subjects. A larger duplication including this region was reported in two patients with autistic behavior and cognitive impairment.[17] We observed this duplication in none of the additional 2814 samples that were screened and at a very low rate in the Icelandic population, indicating that duplication of the same region similarly influences susceptibility to developmental delay with variable features of autism and that one or more genes in the region may be particularly dosage sensitive (with significant developmental manifestations when the region is either duplicated or deleted).

A comparison of our findings with those of other recent studies that have reported de novo copy-number variations in autism does not add compelling evidence in favor of susceptibility to autism conferred by other recently highlighted genomic regions. Furthermore, our data show little evidence of similar large, highly recurrent regions of de novo abnormality outside the 16p11.2 region and the frequently noted 15q11–13 duplications.

In conclusion, our data indicate that a region of chromosome 16p11.2 influences susceptibility to autism when it is either deleted or duplicated. Deletion and duplication events were observed in nearly 1% of multiplex families with autism, in 1% of subjects with autism in Iceland, and in more than 1.5% of clinical samples from subjects with developmental delay. These events are either as frequent as or more frequent than the most common known cause of autism (duplication of the Prader–Willi/Angelman region), which is estimated to occur in 1% of subjects with autism[2] and was present in 0.35% of subjects in our sample. We detected no other recurrent events of note, which suggests that large de novo copy-number mutations may explain only a fraction of familial idiopathic autism.

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APPENDIX

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