

Microduplications of 16p11.2 are associated with schizophrenia

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Recurrent microdeletions and microduplications of a 600-kb genomic region of chromosome 16p11.2 have been implicated in childhood-onset developmental disorders¹⁻³. We report the association of 16p11.2 microduplications with schizophrenia in two large cohorts. The microduplication was detected in 12/1,906 (0.63%) cases and 1/3,971 (0.03%) controls ($P = 1.2 \times 10^{-5}$, OR = 25.8) from the initial cohort, and in 9/2,645 (0.34%) cases and 1/2,420 (0.04%) controls ($P = 0.022$, OR = 8.3) of the replication cohort. The 16p11.2 microduplication was associated with a 14.5-fold increased risk of schizophrenia (95% CI (3.3, 62)) in the combined sample. A meta-analysis of datasets for multiple psychiatric disorders showed a significant association of the microduplication with schizophrenia ($P = 4.8 \times 10^{-7}$), bipolar disorder ($P = 0.017$) and autism ($P = 1.9 \times 10^{-7}$). In contrast, the reciprocal microdeletion was associated only with autism and developmental disorders ($P = 2.3 \times 10^{-13}$). Head circumference was larger in patients with the microdeletion than in patients with the microduplication ($P = 0.0007$).

Rare structural mutations play an important role in schizophrenia. Recent studies have shown that the genome-wide burden of rare copy number variants (CNVs) is significantly greater in individuals with schizophrenia than in healthy controls⁴⁻⁶. In addition, multiple structural variants have been implicated in schizophrenia. Seminal examples include the recurrent microdeletion of 22q11.2 (ref. 7) and

a balanced translocation disrupting the gene *DISC1* (ref. 8). More recently, recurrent microdeletions at 1q21.1, 15q13.3 (refs. 5,9) and 15q11.2 (refs. 6,9) and copy number mutations at other genomic loci¹⁰⁻¹² have been associated with schizophrenia in large cohorts.

We previously reported two individuals with childhood-onset schizophrenia who carry a 600-kb microduplication of 16p11.2 (ref. 4). This region is a well-documented hot spot for recurrent rearrangements that are associated with autism-spectrum disorders and mental retardation^{1-3,13,14}. Genomic hot spots such as this are important candidate loci in genetic studies of schizophrenia.

We tested the hypothesis that microduplications of 16p11.2 are associated with schizophrenia by analysis of microarray intensity data in an initial cohort that included 1,906 affected individuals (cases) and 3,971 controls from several different sources. Sample collection is described in the **Supplementary Note** and **Supplementary Table 1**. Samples were analyzed with one of four microarray platforms (NimbleGen HD2, Affymetrix 6.0, Affymetrix 500K and ROMA 85K). Only the 16p11.2 region was examined. Thirteen microduplications and four microdeletions were detected in our primary sample using standard segmentation algorithms (**Fig. 1a**, **Supplementary Fig. 1a**). Microduplications at 16p11.2 were detected in 12/1,906 cases (0.63%) and 1/3,971 controls (0.03%), demonstrating a statistically significant association (**Table 1**, $P = 1.2 \times 10^{-5}$, OR = 25.8 (3.3, 199)).

In a subset of individuals evaluated at Cold Spring Harbor Laboratory (CSHL, Cold Spring Harbor, NY) consisting of 1,352 cases and 1,179 controls, CNV calls were verified by median *z*-score outlier

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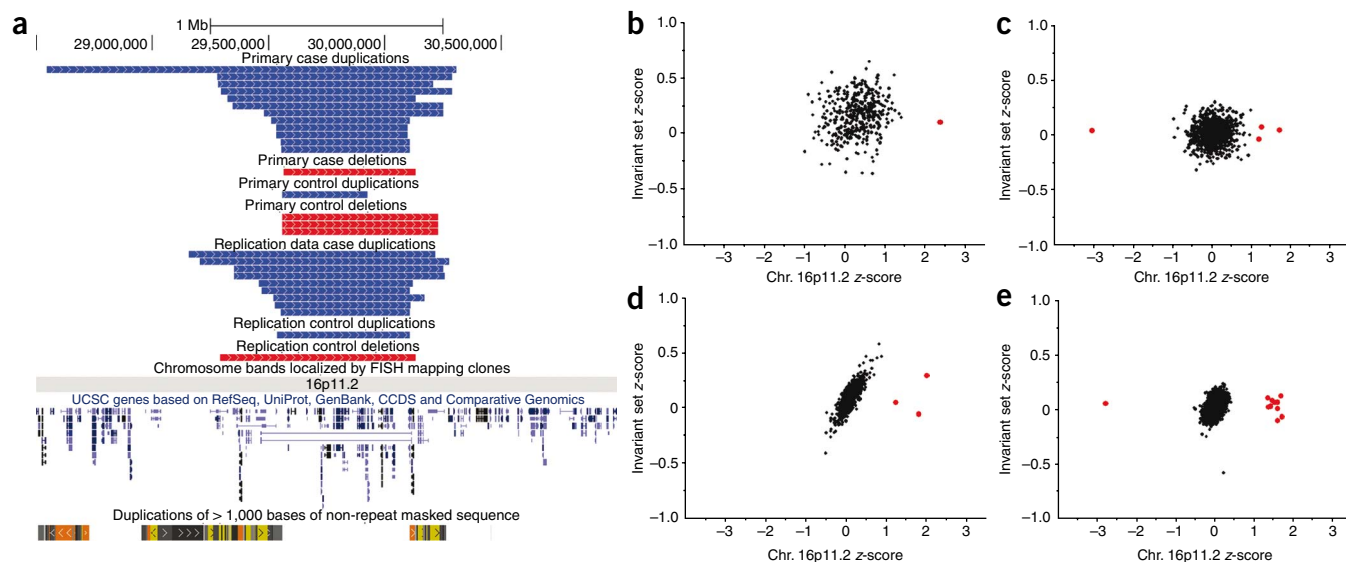


Figure 1 Microduplications and microdeletions at 16p11.2 in persons with schizophrenia and controls. (a–e) 16p11.2 rearrangements were detected in a primary sample of 1,906 cases and 3,971 controls (a–d) and a replication sample of 2,645 cases and 2,420 controls (a,e). The single microduplication and three microdeletions detected in the primary control set are presented based on the Affymetrix 500K coordinates (hg18). All other CNVs were validated in the NimbleGen HD2 platform and are illustrated based on the validation coordinates. In b–e, the median z-score for the 535-kb 16p11.2 target region is plotted on the x axis and the median z-score of flanking invariant probes is plotted on the y axis. Data are presented separately for the ROMA (b), Affymetrix500K (c), NimbleGen HD2 (d) and Affymetrix 6.0 (e) platforms. CNVs were called using thresholds of >2 s.d. for ROMA and >1 s.d. for all other platforms (red dots). MeZOD and the HMM algorithms detected the same deletions and duplications at 16p11.2.

detection (MeZOD), an independent CNV genotyping algorithm that identifies outliers in the sample based on the median probe z-score of the target region. These results are illustrated as cluster plots (Fig. 1). All microduplications and microdeletions detected in the combined sample were experimentally validated using an independent microarray platform (Supplementary Table 2).

To replicate this association, we evaluated the 16p11.2 region using microarray data (Affymetrix 6.0 platform) from an independent sample of 2,645 individuals with schizophrenia and 2,420 controls. These data were collected as part of a case-control study of schizophrenia supported by the Genetic Association Information Network (GAIN, phs000021.v2.p1). We detected ten duplications and one deletion using standard hidden Markov model (HMM) calling algorithms (Fig. 1a). The same events were also detected using MeZOD (Fig. 1e). All 16p11.2 rearrangements were validated by an independent microarray platform (Supplementary Table 2). The microduplication

was detected in 9/2,645 cases and 1/2,420 controls, demonstrating a significant association ($P = 0.022$, OR = 8.3 (1.3, 50.5)).

The odds ratios in our primary and replication datasets were not significantly different (Breslow-Day-Tarone test $P = 0.46$). Thus, our initial result was replicated in an independent sample. For the combined sample, the association of schizophrenia with microduplication at 16p11.2 was highly significant ($P = 4.3 \times 10^{-7}$, OR = 14.5 (3.3, 62)). Sex of the subject did not have a significant effect on the association (Supplementary Note).

Our present findings and those from previous studies^{1–3,13,14} suggest that mutations at 16p11.2 confer high risk for schizophrenia and for other neuropsychiatric disorders. Clinical variability associated with the 16p11.2 microduplication is evident from the heterogeneity of psychiatric diagnoses among microduplication carriers in five families in our series (Supplementary Fig. 2). In these families, ten relatives carried the microduplication found in the proband. The diagnoses of these relatives were schizophrenia ($n = 3$), bipolar disorder ($n = 1$), depression ($n = 2$), psychosis signs not otherwise specified ($n = 1$) and no mental illness ($n = 3$). We were able to determine the parent of origin for the microduplication in four families, and in all cases the microduplications were inherited from a non-schizophrenic parent. The observations in these few families suggest that penetrance of the duplication is incomplete, though substantial (perhaps 30–50%), and that expression is highly variable.

In order to more precisely define the spectrum of psychiatric phenotypes associated with rearrangements of 16p11.2, we performed a meta-analysis of data on

Table 1 Duplications and deletions at 16p11.2 among persons with schizophrenia and controls

Series	Diagnosis	Subjects <i>n</i>	Deletions		Duplications			<i>P</i> value
			<i>n</i>	%	<i>n</i>	%	OR (95% CI)	
Primary	Schizophrenia	1,906	1	0.05	12	0.63	25.8 (3.3, 199)	1.2×10^{-5}
	Controls	3,971	3	0.08	1	0.03		
Replication	Schizophrenia	2,645	0	0.00	9	0.34	8.3 (1.3, 50.5)	0.022
	Controls	2,420	1	0.04	1	0.04		
Combined	Schizophrenia	4,551	1	0.02	21	0.46	14.5 (3.3, 62.0)	4.3×10^{-5}
	Controls	6,391	4	0.06	2	0.03		

In the primary sample, which consisted of patients and controls genotyped using one of three microarray platforms, association was calculated using the Cochran-Mantel-Haenszel exact test using array type as a stratifying variable. Combined odds ratio estimates and 95% confidence intervals were calculated using a logistic regression with disease group and array-type as factors. In the replication sample, which consisted of affected individuals and controls assessed on a single microarray platform, association was calculated using a Fisher's exact test. Deletions did not show a significant association with schizophrenia or in controls.

Table 2 Meta-analysis of 16p11.2 rearrangements in schizophrenia, autism and developmental delay, and bipolar disorder

Diagnosis	Subjects <i>n</i>	Deletions				Duplications			
		<i>n</i>	%	OR (95% CI)	<i>P</i> value	<i>n</i>	%	OR (95% CI)	<i>P</i> value
Schizophrenia	8,590	3	0.03	NC ^a		26	0.30	8.4 (2.8, 25.4)	4.8×10^{-7}
Controls	28,406	9	0.03			8	0.03		
Autism or developmental delay	2,172	17	0.78	38.7 (13.4,111.8)	2.3×10^{-13}	10	0.46	20.7 (6.9,61.7)	1.9×10^{-7}
Controls	24,891	5	0.02			6	0.02		
Bipolar disorder	4,822	4	0.08	NC ^a		6	0.12	4.3 (1.3, 14.5)	0.017
Controls	25,225	6	0.02			7	0.03		

Data from four studies reporting microduplications and microdeletions of 16p11.2 in individuals with schizophrenia, autism and/or bipolar disorder were combined with data from the primary sample to assess the relative strength of the association of each variant with each disorder. Associations were calculated using the Cochran-Mantel-Haenszel exact test, using source as a stratifying variable. Combined odds ratio estimates and confidence intervals were calculated using logistic regression with disease group and source (study) as factors.

^aNot calculated (NC) because significant heterogeneity among studies was detected by the Breslow-Day-Tarone test. The partial odds ratios (95%CI) for the deletion in schizophrenia were 0.69 (0.1, 4.9), 0.3 (0.05, 2.2), 14.6 (1.9, 111.2) and 0.3 (0.03, 3.7), and those for the deletion in bipolar disorder were 0.3(0.03,3.3), 0.55(0.05,6.7) and 25(5.4,117) in this study, the GAIN study and the Weiss *et al.* studies, respectively.

schizophrenia, bipolar disorder and childhood developmental disorders (combining autism and global developmental delays). We integrated data from this study with four publicly available datasets^{1,3,5,15} to generate a combined sample of 8,590 individuals with schizophrenia, 2,172 with developmental delay or autism, 4,822 with bipolar disorder and 30,492 controls (**Supplementary Note, Supplementary Table 3**). In this combined sample, the microduplication of 16p11.2 was strongly associated with schizophrenia (**Table 2**, OR = 8.4 (2.8, 25.4), $P = 4.8 \times 10^{-7}$) and autism (OR = 20.7 (6.9, 61.7), $P = 1.9 \times 10^{-7}$). The association with bipolar disorder was also significant (OR = 4.3 (1.3, 14.5), $P = 0.017$). The reciprocal microdeletion of 16p11.2 was strongly associated with developmental delay or autism (OR = 38.7 (13.4, 111.8), $P = 2.3 \times 10^{-13}$), as reported previously¹⁻³. However, the deletion was not associated with schizophrenia or bipolar disorder (**Supplementary Note**). These results suggest that the microduplication is associated with multiple psychiatric phenotypes, whereas the reciprocal microdeletion is more specifically associated with developmental delay and autism.

We explored the association of 16p11.2 microduplications and microdeletions with two clinical measures: head circumference and height. Available data were compiled from 32 patients with 16p11.2 mutations who had a diagnosis of schizophrenia, autism-spectrum disorder or developmental delay (**Supplementary Note, Supplementary Table 4** and refs. 13,16). *Z* scores for head circumference and height were calculated using standard growth charts from the Centers for Disease Control. Head circumference was greater among 23 individuals with microdeletions than among 9 individuals with microduplications (**Supplementary Table 5**). The mean orbital frontal circumference (OFC) values of patients with microdeletions and microduplications were 1.25 and -0.28, respectively (two-tailed Wilcoxon rank sum test $P = 0.0007$). In addition, mean head circumference of the microdeletion group was significantly greater than the population mean ($P = 0.0001$), whereas the mean head circumference of the microduplication group was not significantly different from the population mean ($P = 0.29$). The association between the 16p11.2 microdeletion and larger head circumference was observed in multiple diagnostic categories and was not specifically attributable to patients with autism (**Supplementary Table 5**). The microduplication and microdeletion groups did not significantly differ from each other in height.

We report here that microduplication of 16p11.2 is associated with an 8–24-fold increased risk of schizophrenia. This region joins a growing list of genomic hot spots that confer high risk for the disorder. The odds ratios in our series for the 16p11.2 microduplication and schizophrenia

are comparable to odds ratios for deletions at other schizophrenia-associated genes and regions. Deletions of 1q21.1, 15q13.3 and *NRXN1* have reported odds ratios ranging from 7 to 18 (refs. 5,9,10).

Previous genome-wide studies of copy number variation did not find a significant association with the microduplication of 16p11.2 and schizophrenia. This microduplication event is rare, and its detection in a cohort may be influenced by several factors, including resolution of the platform, methods of analysis and chance. In the International Schizophrenia Consortium (ISC) study⁵, microduplications spanning >50% of the 16p11.2 region were detected in 5/3,391 cases and 1/3,181 controls. These results are consistent with our findings, but the association did not meet the criteria for genome-wide significance in that study. In the Schizophrenia Gene (SGENE) consortium study of schizophrenia⁹, the 16p11.2 microduplication was not selected as a candidate because the event was not observed in the initial phase of that study as a *de novo* mutation, which was the key criterion for inclusion in the association analyses.

Microduplication at 16p11.2 is associated with multiple neuropsychiatric phenotypes. Phenotypic heterogeneity has been observed for virtually all structural variants associated with schizophrenia. For example, in a large Scottish pedigree harboring a translocation disrupting *DISC1*, translocation carriers had diagnoses of schizophrenia, bipolar disorder, major depressive disorder or no mental illness⁸. Similarly, microdeletions of 1q21.1 (refs. 17,18), 15q13.3 (ref. 19), 22q11.2 (ref. 20) and *NRXN1* (refs. 10,12,21,22) are associated with adult psychiatric disorders and with autism and other pediatric neurodevelopmental disorders.

The association between the 16p11.2 microdeletion and increased head circumference is notable given that the microdeletion appears to be specific to autism and developmental delay. Several studies have found increased head circumference in patients with autism²³⁻³⁰, leading to the suggestion that early brain overgrowth may be a key neurobiological mechanism in the disorder³¹. A recent study has shown that microdeletions and microduplications of 1q21.1 are associated with microcephaly and macrocephaly, respectively¹⁸. Taken together, these studies suggest that some mutations underlying neurodevelopmental disorders may also lead to changes in brain volume.

The 16p11.2 microduplication spans a region of approximately 600 kb containing 28 genes (**Supplementary Fig. 1b**), including numerous genes with potential roles in neurodevelopment. At least 17 of the 28 genes in this region are expressed in the mammalian brain (**Supplementary Table 6**). Behavioral features have been reported in mouse *Mapk3*^{-/-} *Doc2a*^{-/-} and *Sez6l2*^{-/-} knockout models³²⁻³⁴.

Further studies are needed in order to identify the specific gene or genes in this region for which dosage effects contribute to increased risk for psychiatric and neurodevelopmental disorders.

Our findings further strengthen the evidence demonstrating a role for rare mutations in schizophrenia^{4–6,9}. Collectively, these studies demonstrate that schizophrenia is characterized by marked genetic heterogeneity. Although the 16p11.2 locus by itself may account for only a small proportion of cases, the duplication of this region confers substantial risk to the individuals who carry it. In addition, although this single mutation is rare, the collective effect of rare mutations at many different loci may account for a substantial proportion of schizophrenia-affected individuals^{4,5} and will likely influence overlapping neurobiological pathways. Characterizing these pathways will contribute substantially to our understanding of the origins of schizophrenia and suggest targets for treatment development.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

Accession code. dbGaP (genotype data): GAIN study of schizophrenia (phs000021.v2.p1) and GAIN study of bipolar disorder (phs000017.v2.p1).

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

J.S. organized and designed the study. S.E.M., J.S., S.Y., N.R.M., M.-C.K., G.K., D.G. and A.M.A. contributed to the analysis of genetic data. S.E.M., J.S., C.K.D. and D.L.L. contributed to the analysis of clinical data. S.E.M. and J.S. prepared the manuscript. All authors contributed their critical reviews of the manuscript in its preparation. The following persons contributed to the collection of samples and data: (**Schizophrenia**) G.K., D.G., N. Craddock, M.J.O., M.C.O., WTCCC, A.M.A., J.R., D.O.P., J.A.L., J.S.S., P.F.S., J.M., D.E.D., T.W., M.-C.K., E.S., O.K., V. Kraus, D.L.L., T.J.C. and L.E.D.; (**Bipolar Disorder**) J.P., M. Goodell, V.L.W., P.D., S.G., J.S., L.K., J.W., N. Chitkara, F.J.M., A.K.M., J.B.P., T.G.S., M.M.N., S.C., M.R., E.L., G.K., D.G., N. Craddock, M.J.O., M.C.O. and WTCCC; (**Autism**) V. Kustanovich, C.M.L., E.H.Z., P.K., J.G., I.D.K., N.B.S., C.H.-E., T.H.S., M. Gill, L.G., T.L., K. Puura, R.A.K., S.L.C., J.S.S. and D.S. Array-comparative genomic hybridization data collection, processing and management at CSHL were carried out by: S.E.M., D.M., V.M., S.Y., M.K., P.R., A.B., K. Pavon, B.L., A.L., J.K., Y.-H.L., L.M.L., V.V. and J.S.

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ONLINE METHODS

Cohort description. For this study, data were collected on 8,800 individuals with schizophrenia, bipolar disorder and autism (cases), and 6,391 controls analyzed on one of four microarray platforms (ROMA, NimbleGen HD2, Affymetrix500K or Affymetrix 6.0). Ascertainment of samples in the primary (1,906 schizophrenia cases and 3,971 controls), replication (2,645 cases and 2,420 controls), autism (934 cases) and bipolar disorder (3,315 cases) datasets is provided in the **Supplementary Note**. A breakdown of samples by array is provided in the **Supplementary Table 1**.

Intensity data processing. Processing of microarray data was performed at three different sites. Affymetrix 500 K data from the National Institute of Mental Health (NIMH) (83 cases) were processed at NIMH using published described methods⁴. Affymetrix 500 K data from Cardiff University and the Wellcome Trust Case Control Consortium (WTCCC) (471 schizophrenia cases, 1,697 bipolar disorder cases and 2,792 controls) were processed at Cardiff University using the same software and initial quality control parameters for CNV calls and arrays as published previously⁶; however, for these analyses, we did not remove CNVs that were detected by <10 probes on both the Affymetrix Nsp and Sty arrays.

All other data were processed at Cold Spring Harbor Laboratory (Cold Spring Harbor, NY) using different methods for dual-color intensity data (array-comparative genomic hybridization (array-CGH) platforms: ROMA 85K and NimbleGen HD2) and single-color intensity data (SNP genotyping arrays: Affymetrix 500K, Affymetrix 5.0 and Affymetrix 6.0). Processing of dual- and single-color intensity data is described in more detail in the **Supplementary Note**.

Array-CGH intensity data. Normalization of ROMA intensity data by locally weighted scatterplot smoothing (LOWESS), and geometric mean estimation of log₂ ratios has been described previously³⁵.

NimbleGen HD2 dual-color intensity data were normalized in a two-step process: first, a 'spatial' normalization of probes was performed to adjust for regional differences in intensities across the surface of the array, and second, the Cy5 and Cy3 intensities were adjusted to a fitting curve by invariant set normalization, preserving the variability in the data. The log₂ ratio for each probe was then estimated using the geometric mean of normalized and raw intensity data.

SNP genotyping data—Affymetrix 500K, Affymetrix 5.0 and Affymetrix 6.0. To analyze Affymetrix SNP array single-color intensity data, we developed a two-step process that (i) normalizes all arrays by invariant set normalization to a single reference array and (ii) calculates the ratio of intensities for each experiment in comparison to a sex-matched virtual reference genome.

GC correction of log₂ ratios. The final step of data processing involved the correction of the effects of genomic waves in log₂ ratios due to regional correlations with GC content based on the fitted linear regression model proposed by Diskin *et al.*³⁶

Chr. 16p11.2 detection by HMM segmentation. To detect 16p11.2 rearrangements in our ROMA and GC-corrected Affymetrix log₂ ratio data, we implemented the seven-state HMM algorithm described previously³⁵. We used a modified version of this HMM algorithm to identify CNVs in our higher resolution Affymetrix 5.0, 6.0 and NimbleGen HD2 GC-corrected datasets³⁷. The results of segmentation were examined for the presence of CNVs overlapping at least 50% of the 16p11.2 region (Chr. 16: 29,557,498–30,107,355 of the UCSC human genome version HG18 (NCBI Build 36.1)).

16p11.2 genotyping: rare CNV detection by outlier clustering. *Principles.* As an alternative method for genotyping rare CNVs, we developed an algorithm called median *z*-score outlier detection (MeZOD) to detect rare variants based on the probe intensity data across the population of experiments. The principles of this method are similar to other approaches that genotype common CNVs by probe intensity clustering^{38,39}; however, in most cases very few individuals carry the rare genotype. Therefore, rather than using cluster analysis to identify variants in the population, our method detects rare outliers in the standardized probe intensity distribution.

Selection of target probes and flanking probes. The 16p11.2 rearrangements were genotyped using probes selected from within the target region (Chr. 16: 29,564,890–30,100,063). Two unique sequences, one proximal (Chr. 16: 27,388,307–28,952,358) and one distal (Chr. 16: 30,304,580–31,870,683) to the 16p11.2 target region, were combined into a single set of invariant probes. The results are displayed as a scatterplot. Median *z*-scores of target probes are shown on the *x* axis, and median *z*-scores of the invariant probes are shown on the *y* axis.

To avoid patterns of common copy number polymorphism, probes were excluded if the positive or negative Pearson correlations with neighboring probes exceeded conservative maximum or minimum thresholds, respectively. Probes not exceeding these thresholds were used for genotyping. **Supplementary Table 7** contains all platform-specific probes within the target and invariant regions. The selected genotyping probes in the target and invariant regions are represented in red and green, respectively, in the UCSC human genome browser.

Median *z*-score calculation and outlier detection. Calculation of the median *z*-scores was a three-stage process involving (i) experiment-wise log₂ ratio standardization, (ii) probe-wise standardization of the genotyping probe *z*-scores and (iii), median *z*-score determination for the target and invariant region. For each probe *m* of experiment *n*, the standardized log₂ ratio *z*-score *z* is simply calculated by:

$$z(m_n) = \frac{m_n - \mu_n}{\sigma_n}$$

where μ_n and σ_n are the mean and s.d. of probe ratios for experiment *n*, respectively. The *z*-score for each genotyping probe *g* in experiment *n* was then standardized probe-wise within the population of experiments for a given platform by:

$$Z(G_n) = \frac{G_n - \mu_g}{\sigma_g}$$

where μ_g is the mean and σ_g is s.d. of genotyping probe *g*. Finally, the median for experiment *n* was calculated for the target genotyping probes and the combined proximal and distal invariant genotyping probes.

To detect rearrangements of 16p11.2, outliers of the target median *z*-score distribution were analyzed. Thresholds were set for microduplications at target median *z*-scores >2 for the ROMA array and >1 for Affymetrix500K, Affymetrix 6.0 and NimbleGen HD2 arrays, whereas the outlier threshold for microduplications on all platforms was below a target median *z*-score of –2. As noted earlier, with the exception of Affymetrix 500K data analyzed locally by the NIMH, Cardiff University and the WTCCC, all intensity data was analyzed using MeZOD at CSHL. Further assessment of 16p11.2 HMM and MeZOD genotyping is provided in the **Supplementary Note** and **Supplementary Figure 3**.

Validations of 16p11.2 rearrangements. All rearrangements of 16p11.2 detected in the primary and replication samples were validated using an additional microarray platform. Microduplications detected on the NimbleGen HD2 platform were confirmed using the Agilent 244K array. CNVs detected on other platforms, including the Cardiff schizophrenia cases, were validated on the NimbleGen HD2 array. Rearrangements detected in the WTCCC were detected independently on both Affymetrix Nsp and Sty arrays (**Supplementary Table 2**). Additional DNA was not available for WTCCC controls to perform additional fine-mapping of events detected in these samples. Of the 15 CNVs detected in additional cohorts of autism and bipolar disorder, genomic DNA was available for 12 (**Supplementary Table 2**), and all CNVs in 12 genomic DNA samples were validated.

Meta-analysis and strength of 16p11.2 associations in multiple psychiatric disorders. Data from this study were combined with data from three independent published studies^{1,3,5} to obtain a combined sample of 8,590 schizophrenia, 4,822 bipolar disorder and 2,172 autism or developmental delay cases and a combined sample of 30,492 controls. Controlling for study, the control samples used for a particular disorder were derived only from those studies contributing

cases of the same disorder. Thus, the control samples for schizophrenia, bipolar disorder and autism or developmental delay consisted of 28,406, 25,225 and 24,891 individuals, respectively. Additional information on each study included in the meta-analysis is provided in the in the **Supplementary Note**.

Statistical analysis. *Association of 16p11.2 microduplication with schizophrenia.* The primary sample consisted of data from multiple microarray platforms that varied in probe density. All had good sensitivity to detect CNVs that are 500 kb in size. However, subtle differences in sensitivity could have influenced the overall frequency of 16p11.2 microduplications when all platforms were combined into a single dataset. Therefore, we used array type as a stratifying variable when testing for association using the Cochran-Mantel-Haenszel (CMH) exact test. Logistic regression was used to estimate the combined ORs and the 95% confidence intervals and to measure the effect of array type based on the deviance in the *P* value. The Fisher's exact test was used to test the association of the 16p11.2 microduplications in the replication dataset (single array platform). The Breslow-Day-Tarone test was used to assess the homogeneity of the ORs between the primary and replication datasets. We also examined whether sex had an effect on the association by using sex as a covariate. Results of these analyses are discussed further in the **Supplementary Note**.

Meta-analysis of duplications and deletions of 16p11.2 in multiple psychiatric disorders. The association of the microduplication and microdeletion was examined independently in each disorder using the CMH exact test with source as a stratifying variable. The Breslow-Day-Tarone test was used to assess the homogeneity of the partial OR between the studies of each disorder used in the meta-analysis. A common *P* value and OR were reported from the CMH exact test and from the logistic regression, respectively, only if there was homogeneity in the ORs between the studies in the meta-analysis. Given the very small number of deletion observations in the GAIN schizophrenia study and in each of the BD studies, approximate ORs were calculated by replacing the number of deletions *n* with *n* + 0.5. Results of these analyses are discussed further in the **Supplementary Note**.

Analysis of quantitative clinical features with 16p11.2 rearrangements. Quantitative clinical data on height, weight and head circumference (occipital-frontal circumference, OFC) was collected from records on individuals carrying 16p11.2 in this study, in previously published studies (Weiss *et al.* and Ghebranious *et al.*^{1,13}) and from unpublished data on individuals carrying 16p11.2 rearrangements ascertained by referral for global developmental delay (T. Shaikh, The Children's Hospital of Philadelphia, Philadelphia, PA, personal communication). We excluded from our analysis subjects with known Hispanic, Polynesian and African American ethnicity and any subjects with documented cytogenetic abnormalities.

OFC and height measurements were converted to percentile rankings, conditioned on age and gender, using clinical growth charts from the Center for Disease Control's National Center for Health Statistics (see URLs). OFC percentile rankings were further verified using the online tool developed by SimulConsult, which is based on the same reference database (see URLs). Height and OFC percentiles were converted to *z* scores using online resources (see URLs). *Z*-scores were contrasted among 16p11.2 microdeletions versus microduplications carriers using the Wilcoxon two-sample rank sum test. We repeated the analysis using the craniofacial normative database from Farkas *et al.*^{40,41} (in individuals of European ancestry). The above analysis was also performed within subsets of samples defined by diagnoses of schizophrenia, developmental delay or autism spectrum disorders to further examine if the observed effect was present within each individual group. The results of these analyses are discussed further in the **Supplementary Note**.

Due to limited availability of data on the IQ of subject participants, we did not examine intellectual disability in microdeletion and microduplication cases. Because of the known influences of antipsychotic medication on body weight, differences in weight between individuals with microdeletions and duplications were not examined.

A description of the psychiatric symptoms in 16p11.2 carriers is provided in the **Supplementary Note** and **Supplementary Table 7**.

URLs. CDC Growth Charts, <http://www.cdc.gov/growthcharts/>; SimulConsult microcephaly calculator, <http://www.simulconsult.com/resources/ftemp20.html>; HyperStat Online normal distribution calculators, http://davidmlane.com/hyperstat/z_table.html; dbGaP, <http://www.ncbi.nlm.nih.gov/dbgap>; GAIN, <http://www.genome.gov/19518664>.

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