

# Microdeletions of 3q29 Confer High Risk for Schizophrenia

Jennifer Gladys Mulle,<sup>1,\*</sup> Anne F. Dodd,<sup>1</sup> John A. McGrath,<sup>2</sup> Paula S. Wolyniec,<sup>2</sup> Adele A. Mitchell,<sup>3</sup> Amol C. Shetty,<sup>1</sup> Nara L. Sobreira,<sup>4</sup> David Valle,<sup>4</sup> M. Katharine Rudd,<sup>1</sup> Glen Satten,<sup>1,5</sup> David J. Cutler,<sup>1</sup> Ann E. Pulver,<sup>2,6</sup> and Stephen T. Warren<sup>1,7,\*</sup>

Schizophrenia (SZ) is a severe psychiatric illness that affects ~1% of the population and has a strong genetic underpinning. Recently, genome-wide analysis of copy-number variation (CNV) has implicated rare and de novo events as important in SZ. Here, we report a genome-wide analysis of 245 SZ cases and 490 controls, all of Ashkenazi Jewish descent. Because many studies have found an excess burden of large, rare deletions in cases, we limited our analysis to deletions over 500 kb in size. We observed seven large, rare deletions in cases, with 57% of these being de novo. We focused on one 836 kb de novo deletion at chromosome 3q29 that falls within a 1.3–1.6 Mb deletion previously identified in children with intellectual disability (ID) and autism, because increasing evidence suggests an overlap of specific rare copy-number variants (CNVs) between autism and SZ. By combining our data with prior CNV studies of SZ and analysis of the data of the Genetic Association Information Network (GAIN), we identified six 3q29 deletions among 7545 schizophrenic subjects and one among 39,748 controls, resulting in a statistically significant association with SZ ( $p = 0.02$ ) and an odds ratio estimate of 17 (95% confidence interval: 1.36–1198.4). Moreover, this 3q29 deletion region contains two linkage peaks from prior SZ family studies, and the minimal deletion interval implicates 20 annotated genes, including *PAK2* and *DLG1*, both paralogous to X-linked ID genes and now strong candidates for SZ susceptibility.

## Introduction

Substantial evidence from both twin and family studies has demonstrated a strong genetic component for the development of schizophrenia (SZ [MIM 181500]), one of the most frequent and devastating psychiatric diseases in the world.<sup>1,2</sup> Genome-wide association studies (GWAS) with unprecedented sample sizes have yielded only a small number of candidate loci with odds ratio estimates of 1.2–1.4,<sup>3–5</sup> leaving a substantial component of the genetic variance unexplained. This missing heritability has led to the view that selection against common susceptibility loci may be greater than previously believed and has driven the search for rare and de novo variation as contributors to predisposition, which could account for both the substantial heritability estimates and the absence of significant association with common genome variation.

Copy-number variation (CNV) has been the most widely considered source of rare variation in complex disorders such as SZ, fueled by the advent of technology that could rapidly and reliably identify submicroscopic copy-number variants (CNVs). Indeed, several studies have documented rare and often de novo deletions and duplications in SZ, with significantly more such events in cases versus controls.<sup>6–10</sup> This increased CNV burden contains a minority of CNV risk loci that are shared among SZ cases; the majority of the excess is driven by variants

that are observed only once.<sup>2</sup> Walsh et al. reported that SZ cases were four times more likely than controls to carry gene-containing CNVs > 100 kb.<sup>9</sup> The International Schizophrenia Consortium reported a 1.67-fold excess of deletions > 500 kb in cases.<sup>6</sup> Need et al. found an excess of deletions > 2 Mb in cases.<sup>7</sup> Kirov and colleagues reported that CNVs > 1 Mb were 2.6-fold more common in cases, with deletions specifically being 4.53-fold more common in cases.<sup>11</sup> In addition, Xu et al. reported an 8-fold increase in de novo CNV in SZ trios, lending further support to the rare-variant hypothesis for SZ susceptibility.<sup>10</sup> Despite these many reports, only a small number of specific CNVs are found to be enriched in SZ cases, including deletions at 1q21, 15q11, 15q13, 22q11, and in the *NRXN* gene, as well as duplication at 16p11.<sup>6,8,11,12</sup>

We sought to examine the burden of large rare CNVs in a cohort of 245 unrelated SZ cases and 490 unaffected controls, all of whom were of Ashkenazi Jewish descent. The Ashkenazim are an ethnically and genetically distinct population,<sup>13,14</sup> although their prevalence of SZ appears similar to the general population. For ease of comparison with other studies, we limited our analysis to deletions larger than 500 kb. Available parents of 182 cases were also genotyped for the assessment of inherited or de novo status. We found a 2.8-fold excess of large rare deletions in SZ cases, which is fully consistent with other studies. Two deletions in our sample, on chromosomes

<sup>1</sup>Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322, USA; <sup>2</sup>Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA; <sup>3</sup>Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY 10029, USA; <sup>4</sup>McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD 21231, USA; <sup>5</sup>National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA; <sup>6</sup>Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21231, USA; <sup>7</sup>Departments of Biochemistry and Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA

\*Correspondence: [jmulle@emory.edu](mailto:jmulle@emory.edu) (J.G.M.), [swarren@emory.edu](mailto:swarren@emory.edu) (S.T.W.)

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3q29 and 22q11, are also found in other SZ populations and show a statistically significant enrichment in SZ cases, with a 17-fold increase in risk for SZ associated with the 3q29 variant.

## Subjects and Methods

### Study Subjects

Ashkenazi Jewish individuals affected with SZ were recruited nationally over a 6 yr period through advertisements in newspapers and Jewish newsletters, talks to community organizations, letters to leaders of the Jewish community, and a study website (Johns Hopkins Epidemiology-Genetics Program in Psychiatry). Case-parent trios were eligible for inclusion in these analyses if the proband met DSM-IV criteria for a SZ diagnosis and all four grandparents were of Ashkenazi Jewish descent. When available, DNA from parents of probands was also collected. This recruitment effort led to 300 eligible families for analysis. Seventy-two percent of affected individuals in our study were male, and the mean age of onset for SZ was 19.3 yrs (Table S1, available online). Recruitment of this population has been previously described.<sup>15</sup> All recruitment methods and protocols for collection of clinical data and blood samples were approved by the Johns Hopkins institutional review board, and informed consent was obtained from all individuals.

Probands were assessed for psychiatric illness according to established procedure, as follows: a trained clinical examiner (doctoral-level clinical psychologist) interviewed each study subject in person (usually in their home) using the Diagnostic Interview for Genetic Studies (DIGS), a standard, semistructured instrument widely used for phenotypic classification of individuals for psychiatric genetic studies. These interviews were tape recorded. For each study subject, medical records were also obtained, and collateral interviews with at least one additional informant were conducted. The examiner then completed a written diagnostic workup, including relevant clinical features and course of illness. All collected clinical information (tape-recorded interviews, DIGS interview booklets, medical records, collateral interview information, and written diagnostic workup) was forwarded to two independent, trained clinicians, who reached a consensus diagnosis of SZ before an individual was included for study. Complete details about clinical methods are available in Fallin et al.<sup>16</sup>

### Ascertainment of Controls

Control subjects were selected from two cohorts: one from a study of Crohn disease (CD) in the Ashkenazim, and one from a study of neuromuscular disease (Parkinson disease [PD] and dystonia) in the Ashkenazim. The Mount Sinai and/or Beth Israel institutional review boards approved all recruitment methods and protocols for DNA collection, and informed consent was obtained from all individuals. Control subjects were not screened for psychiatric disease. Population substructure analysis using noncorrelated SNPs showed cases and controls to be a homogeneous population.

#### *Crohn Disease Cohort*

Ashkenazi Jewish individuals with CD were recruited over a 3 yr period through New York metropolitan area physicians participating in the New York Crohn's Study Group, which is based at The Mount Sinai Medical Center. Patients were phenotyped by a gastroenterologist and were included in the study if they met the definition of CD according to the Montreal classification and if all four grandparents were of Ashkenazi Jewish descent.

#### *Neuromuscular Cohort*

Ashkenazi Jewish individuals with PD or dystonia were recruited from the Department of Neurology at Beth Israel Medical Center and through research solicitation. Each study participant was consented, and histories, exams, and blood samples were obtained. Determination of Ashkenazi Jewish ancestry was based on patient report of one or more Ashkenazi grandparents.

### Genotyping and Identification of CNVs

DNA was extracted with the Gentra Puregene Kit or the QIAGEN DNeasy Blood and Tissue Kit. All DNA used for this study was extracted from blood (no cell-line DNA was used). Samples were genotyped with the Affymetrix Human Genome-Wide SNP Array 6.0 at Emory University. Genotyping was performed with the use of the Birdseed algorithm, as implemented in Affymetrix Power Tools software. Individual genotypes with confidence scores < 0.9 were excluded. SNPs with completion rates < 90% were also excluded. This rendered 814,263 autosomal SNPs.

For CNV analysis, normalization and log ratio data calculation was achieved with the use of the Affymetrix Power Tools software (version 1.10.0). Log<sub>2</sub> ratio data for autosomes were extracted and analyzed with the use of three algorithms: GLAD,<sup>17</sup> GADA,<sup>18</sup> and BEAST (G.A.S., unpublished data). CNVs called by only a single algorithm were removed from analysis. When algorithms disagreed on endpoint determination, the smallest startpoint and the largest endpoint (i.e., the largest interval) were considered. Putative deletion intervals were filtered by size (> 500 Kb), the number of SNPs in the deletion interval (> 20 SNPs), and SNP homozygosity rate (> 90%).

Samples were excluded (1) if they failed the manufacturer's recommended cutoffs for chip quality control; (2) patterns of SNP genotypes revealed inconsistent family structure (trios only) or unknown duplicates or if the heterozygosity was greater than three standard deviations (SD) above the mean and the missing data rate was > 3 SD above the mean (indicating potential sample mixing); (3) if patterns of SNP genotypes revealed cryptic relatedness determined by pairwise identity-by-state analysis (first- and second-degree relationships were removed, controls preferentially removed when possible); or (4) if the number of detected CNVs were > 3 SD from the mean number of CNVs in the overall sample (Table S2).

### Validation of Variants and Endpoint Mapping

The 3q29 variant was validated by TaqMan Assay (ID Hs03476335\_cn). Assays were performed in quadruplicate in 384-well format with 10 ng of DNA per reaction. Copy number was determined with the use of CopyCaller software version 1.0 (Applied Biosystems). Fine mapping of deletion endpoints was accomplished with the use of a custom-designed Agilent array with 244,000 probes, of which 27,661 are located in the terminal 6.2 Mb of the long arm of chromosome 3. Patient and reference genomes were labeled and hybridized according to the manufacturer's instructions. Arrays were scanned with an Axon GenePix 4000B scanner, and data were extracted and analyzed with the Feature Extraction and DNA Analytics programs, respectively.

The 10q21 variant was validated by long PCR across the deletion endpoints and sequencing of the PCR product to map the precise breakpoints. The 16p11 deletion and both 22q11 deletions were validated by Illumina HumanOmni1-Quad array, according to the manufacturer's instructions. Deletions were called with the use of cnvPartition 2.4.4 from within GenomeStudio v2009.2. Deletions were detected with confidence scores of 2249.5 (16p11), 1730.5 (22q11), and 2891.3 (22q11).

**Table 1. Rare Deletions > 500 kb in Cases and Controls**

Chromosomal Region	Start	Size (kb)	No. of Cases	No. of Controls	No. of Genes	Inheritance in Case
3p26.3	1302426	525	2	1	1	inherited/ unknown
3q29	197412253	836	1	0	19	de novo
10q11.23-q21.1	53181991	4266	1	0	4	inherited
16p11.2-p12.1	26581888	2980	1	0	41	de novo
22q11.21	17030967	3139	2	0	66	de novo/ de novo
3q12.3	102522271	672	0	1	11	-
5p15.2	12868768	622	0	1	0	-
9p21.1	31487560	788	0	1	0	-
10q11.21-q11.23	45576362	5912	0	1	52	-

### Statistical Analysis

All *p* values and odds ratios were calculated with Fisher's exact test or the Cochran-Mantel-Haenszel exact test, stratified by study, in the R statistical package.<sup>19–21</sup> For multiple-test correction by permutation, one million randomized data sets were created for all four deletions. The observed *p* value for each deletion was compared to the lowest *p* value in the randomized set of four *p* values.<sup>22</sup> For the permutation analysis, all test statistics were Cochran-Mantel-Haenszel chi-squared tests with continuity correction (exact tests being too computationally inefficient for permutation analysis).

### Results

In our population of 245 SZ cases and 490 controls, we identified a total of 192 deletions, mapping to 22 genomic locations (Table S3). After excluding variants that overlapped  $\geq 60\%$  of their length with a deletion reported in the Database of Genomic Variants, 12 variants at nine loci remained (Table 1), with seven of these variants in cases (0.028 per person) and five in controls (0.01 per person). This 2.8-fold excess of rare variants in cases, although not statistically significant, nevertheless reiterates what has been observed in many studies of CNV and SZ.<sup>6–10,23</sup> Variants were also larger in cases (case average [median]: 2150 kb [2791 kb] versus 1629 kb [672 kb] in controls) and contained more genes overall (132 genes at case loci versus 64 genes in control loci), as well as more genes per locus (18.8 genes per case locus versus 12.8 genes per control locus). Of the seven case deletions, two were inherited, four were de novo, and one could not be determined.

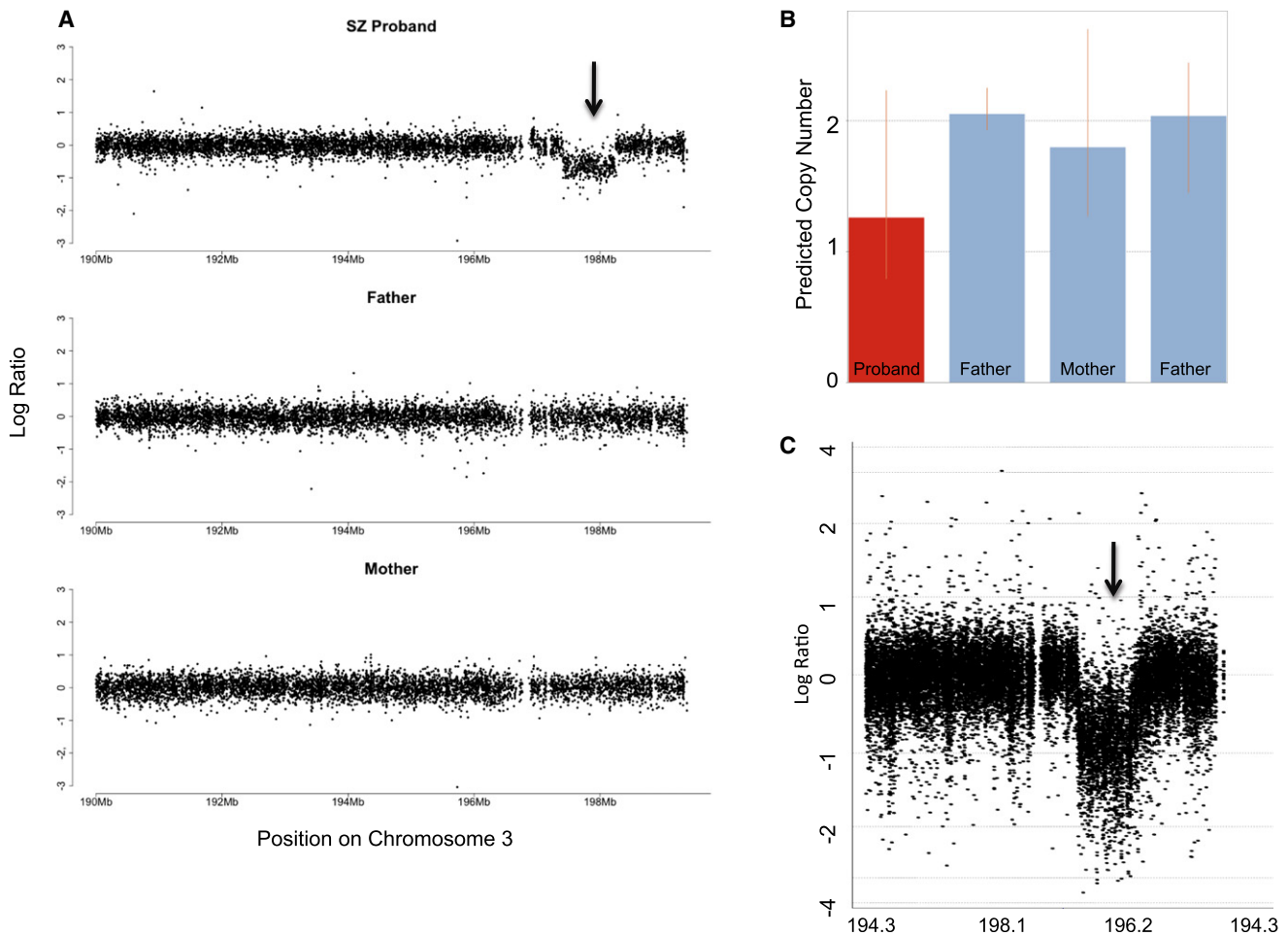
The four case-only loci (at 3q29, 10q11.23-q21.1, 16p11.2-p12.1, and 22q11) were investigated for determining whether these deletions were reported in three previous CNV studies of SZ.<sup>6,9,10</sup> In addition, we also obtained and extracted intensities from the raw data of 2169 SZ cases and 2419 controls from the Genetic Association Information Network (GAIN) study.<sup>4</sup> We found a statistically significant excess of deletions in cases at 3q29 and 22q11 (Figure 1 and Table 2). In total, we identi-

fied six deletions at the 3q29 locus in 6107 cases and none in 6502 controls ( $p = 0.0074$ , odds ratio  $\infty$ , 95% confidence interval [CI]: 1.50– $\infty$ ). Whereas the 3q29 deletion discovered in our study is 836 kb, in all other studies the interval is a larger 1.3–1.6 Mb, completely encompassing the 836 Kb de novo event (Figure 2). We also identified 29 22q11 deletions in 6107 cases and none in 6502 controls ( $p = 4.7 \times 10^{-10}$ , 95% CI: 8.19– $\infty$ ). This is consistent with studies showing the 22q11 deletion to be the single strongest known genetic influence on SZ susceptibility.

We pursued the 3q29 locus further by examining data from an Icelandic cohort of 1438 SZ cases and 33,246 controls.<sup>8</sup> In this sample, a 378 kb region, completely within the large 1.6 Mb deletion, had been interrogated for copy number with the use of 49 SNPs. Of these, 36 SNPs fall within our smaller 836 kb deletion (Figure 2). Among these data, only one additional deletion was found in a control individual. In aggregate, we identified six 3q29 deletions in a total of 7545 cases, compared to one deletion in 39,748 controls. We note that Itsara et al. had also identified three of these deletions from the literature, but in their study this observation failed to reach statistical significance.<sup>23</sup> Our combined meta-analysis refines the odds ratio estimate to 16.98 ( $p = 0.0097$ , 95% CI: 1.36–1198.4). By permutation testing we calculate a multiple-test-adjusted *p* value of 0.02 (correcting for four deletions). The frequency of the 3q29 deletion appears to be consistent among populations, justifying the combining of data across populations to generate an overall odds ratio of 31.5 (uncorrected  $p = 0.0001$ , 95% CI: 3.83–1440.6). Even at the lowest end of this broad CI, this odds ratio rivals that of any GWAS of SZ and suggests that the 3q29 deletion confers a significant risk for this severe psychiatric phenotype.

### Discussion

A recurrent 1.6 Mb microdeletion of 3q29 mediated by recombination between flanking segmental duplications



**Figure 1. Raw Data, Validation, and Fine Mapping of the Chromosome 3q29 Variant**

(A) Raw log ratio data from the Affymetrix Human Genome-Wide SNP Array 6.0 is shown for chromosome 3, 190 Mb–3qtel, for the SZ proband and both parents. The 3q29 de novo deletion is denoted with an arrow.

(B) TaqMan validation data for the 3q29 variant for the SZ proband and both parents (the father was assayed in replicate). Results of ABI CopyCaller software are shown; predicted copy number is indicated on the y axis.

(C) Raw log ratio data for the SZ proband from a custom Agilent array with 27,661 probes in the terminal 6.2 Mb of chromosome 3q. Position on chromosome 3 is plotted on the x axis (194.3–200.1 Mb shown), log ratio data on the y axis. The deletion region is indicated with an arrow.

has been described (MIM 609425).<sup>24–26</sup> This 3q29 microdeletion is most often encountered in children with mild to moderate intellectual disability (ID) that can be associated with microcephaly, dysmorphology, and autistic features.<sup>25,26</sup> Like other deletions, such as those found at 1q21, 15q13, and 22q11, the same CNVs can be identified in children with ID and/or autism (MIM 209850) as well as in adults with SZ.<sup>27–31</sup> Indeed, Quintero-Rivera et al. reported a 10-yr-old girl with 3q29 deletion syndrome and a history of both SZ and autism (Quintero-Rivera and Martinez-Agosto, 2009, *Am. Soc. Hum. Genet.*, abstract). Moreover, the schizophrenic individual we report here with the 836 kb deletion has a childhood history of mild learning disability and impaired social interaction. Our result therefore strengthens the emerging link between SZ and childhood autism or ID.<sup>12,32–34</sup>

The data for the 3q29 deletion are similar to what has been observed at additional loci, including 1q21, 15q11,

15q13, and 16p11, although these copy-number changes are approximately an order of magnitude more frequent than the 3q29 deletion, in both cases and controls. The low frequency of the 3q29 deletion is likely why it was not detected as a risk variant in any one study; even within the combined meta-analysis of over 7000 cases that we present here, only modest statistical significance is achieved. There are no clear relationships between the genes in the 3q29 interval and other SZ-associated intervals, though detailed functional studies may reveal interactions that are as yet unknown.

Additional support for the 3q29 interval comes from two linkage studies. Bailer et al. reported linkage to 3q29 in eight pedigrees<sup>35</sup> (five SZ families, three bipolar disorder families), and two follow-up studies define a linkage peak at 197.09 Mb, within 400 kb of the de novo event we observe at 3q29.<sup>36,37</sup> Furthermore, Devlin et al. reported a pedigree showing a LOD score of 2.6 with marker



**Table 2. Aggregate Data for Deletions at Four Loci, in Independent SZ CNV Studies**

Study	Cases Tested	Controls Tested	3q29		10q11.23		16p12.1		22q11	
			Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Present study	245	490	1	0	1	0	1	0	2	0
Walsh	150	256	1	0	0	0	0	0	0	0
Xu	152	156	0	0	0	0	0	0	3	0
ISC	3391	3181	2	0	0	0	0	0	13 <sup>a</sup>	0
GAIN EA	1215	1440	1	0	0	0	1 <sup>b</sup>	0	10	0
GAIN AA	952	978	1	0	0	0	0	0	1	0
<i>Subtotal</i>	<i>6107</i>	<i>6502</i>	<i>6</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>2</i>	<i>0</i>	<i>29</i>	<i>0</i>
deCODE	1438	33246	0	1	nt	nt	nt	nt	nt	nt
<i>Total</i>	<i>7545</i>	<i>39748</i>	<i>6</i>	<i>1</i>						
OR (95% CI)			16.98 (1.36–1198.4)		$\infty$ (0.051– $\infty$ )		$\infty$ (0.29– $\infty$ )		$\infty$ (8.19– $\infty$ )	
p value			0.0097		0.33		0.15		$4.7 \times 10^{-10}$	

Italics indicate total deletions across samples. EA, European American; AA, African American; nt, not tested.

<sup>a</sup> Includes two “atypical” deletions reported by the ISC.

<sup>b</sup> Deletion is 941 kb, completely contained within the 2980 kb de novo deletion discovered in the present sample.

D3S1311, which maps within our 3q29 deletion region.<sup>38</sup> Finally, nominal association was reported in an SZ case-control study using 23 SNPs immediately adjacent to the distal breakpoint of the 836 kb deletion.<sup>28</sup> These data imply that additional variants at the 3q29 locus, such as single-base mutations in protein-coding or regulatory sequence, may be contributing to SZ susceptibility.

Within the 836 kb 3q29 deletion interval, there are 19 annotated genes (Table S5). Of these, p21-activated kinase 2 (*PAK2*) appears particularly interesting because it has a highly similar paralog that, when mutated, causes X-linked ID (*PAK3*) and is strongly expressed in the brain.<sup>25</sup> *PAK2* has been shown to attenuate the inhibitory interaction between RhoGDI and Rac1,<sup>39</sup> the latter being a prominent regulator of spine morphology and plasticity that was also recently reported to be regulated by the “schizophrenia-related” protein, DISC1.<sup>40</sup> We also note that the telomeric breakpoint of the minimal deletion is 4.7 kb from the transcriptional stop of the *DLG1* gene. The *DLG1* gene has been implicated in SZ by both case-control SNP association and protein-based assays in postmortem brain tissue.<sup>41,42</sup> Furthermore, *DLG1* (also called *SAP97*) has been shown to bind to neuroligin (an autism-susceptibility gene) and the AMPA receptor subunit GluR1.<sup>43–45</sup> There is mounting evidence to implicate perturbation of the glutamate system in SZ pathology.<sup>46</sup> Although the remaining genes in this interval cannot be confidently excluded as being functionally relevant to this disorder, this study, along with the cumulative prior evidence, strongly implicates *PAK2* and *DLG1* as candidate genes for SZ susceptibility.

Here, we assessed CNV in SZ using a genetic isolate, the Ashkenazi Jewish population. We hypothesized that if rare variants contribute to risk, we might identify a risk variant

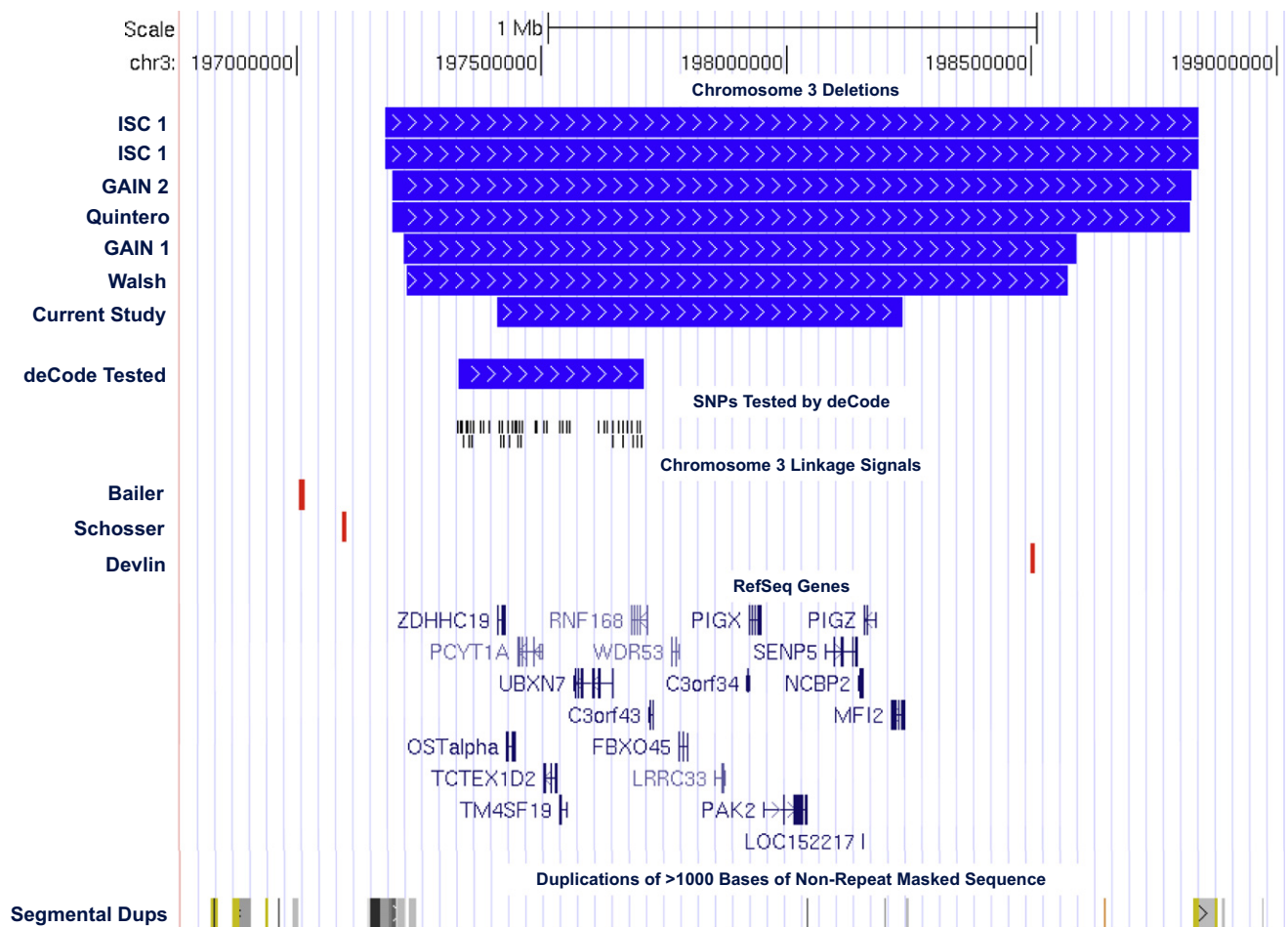
that, although rare, was shared by multiple cases in this population. Instead, we identified mostly de novo events, including SZ-related deletions at 16p11.2-p12.1 and 22q11. However, we did find that 3q29 deletions are highly associated with SZ, regardless of ethnicity. This observation provides an additional example of a microdeletion conferring either childhood-onset ID or adult-onset SZ, strengthening the emerging overlap between these two disparate phenotypes. Our data imply that 3q29 deletions may impart a genetic susceptibility to SZ that approaches the strength of the well known 22q11 deletion. Exploring such rare deletions may prove to be the single most fruitful approach to begin to unravel the mechanisms of SZ, not because these deletions themselves are a frequent cause of SZ, but rather because they illuminate genes, such as *PAK2* and *DLG1* described here, that provide the substrate for further study.

### Supplemental Data

Supplemental Data include five tables and one figure and can be found with this article online at <http://www.cell.com/AJHG/>.

### Acknowledgments

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**Figure 2. Chromosome 3, Coordinates 196.5–199 Mb Shown**

The deletion detected in the current study is shown, along with other 3q29 deletions reported or detected in SZ cohorts (top track: ISC, International Schizophrenia Consortium; GAIN, Genetic Analysis Information Network cohort; Walsh, reference<sup>5</sup>; Quintero, reference<sup>24</sup>). The 378 kb region tested by deCode is indicated, along with the positions of 49 SNPs used by deCode to determine deletion status of 1438 SZ cases and 33,246 controls (tracks 2 and 3). Locations of reported linkage peaks in prior studies are shown (track 4: study indicated by first author of publication). Refseq genes and segmental duplications, which likely mediate nonhomologous recombination events, are also shown (tracks 5 and 6).

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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## Web Resources

The URLs for data presented herein are as follows:

Database of Genomic Variants, <http://projects.tcag.ca/variation/>  
 database of Genotypes and Phenotypes (dbGAP), <http://www.ncbi.nlm.nih.gov/gap>  
 Gene Expression Omnibus (GEO), <http://www.ncbi.nlm.nih.gov/geo>  
 Johns Hopkins Epidemiology-Genetics Program in Psychiatry, <http://www.hopkinsmedicine.org/epigen>  
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>  
 R project for statistical computing, <http://www.R-project.org>  
 UCSC Genome Browser, <http://genome.ucsc.edu/>

## Accession Numbers

The GEO accession number for the microarray data reported in this paper is GSE23201.

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