Genome-wide Burden of Rare Short Deletions Is Enriched in Major Depressive Disorder in Four Cohorts


ABSTRACT
BACKGROUND: Major depressive disorder (MDD) is moderately heritable, with a high prevalence and a presumed high heterogeneity. Copy number variants (CNVs) could contribute to the heritable component of risk, but the two previous genome-wide association studies of rare CNVs did not report significant findings.

METHODS: In this meta-analysis of four cohorts (5780 patients and 6626 control subjects), we analyzed the association of MDD to 1) genome-wide burden of rare deletions and duplications, partitioned by length (<100 kb or >100 kb) and other characteristics, and 2) individual rare exonic CNVs and CNV regions.

RESULTS: Patients with MDD carried significantly more short deletions than control subjects (p = .0059) but not long deletions or short or long duplications. The confidence interval for long deletions overlapped with that for short deletions, but long deletions were 70% less frequent genome-wide, reducing the power to detect increased burden. The increased burden of short deletions was primarily in intergenic regions. Short deletions in cases were also modestly enriched for high-confidence enhancer regions. No individual CNV achieved thresholds for suggestive or significant association after genome-wide correction. p values < .01 were observed for 15q11.2 duplications (TUBGCP5, CYFIP1, NIPA1, and NIPA2), deletions in or near PRKN or MSR1, and exonic duplications of ATG5.

CONCLUSIONS: The increased burden of short deletions in patients with MDD suggests that rare CNVs increase the risk of MDD by disrupting regulatory regions. Results for longer deletions were less clear, but no large effects were observed for long multigenic CNVs (as seen in schizophrenia and autism). Further studies with larger sample sizes are warranted.

Keywords: Copy number variation, Genetics, Genome-wide association study, Major depressive disorder, Meta-analysis, Neuroscience

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Major depressive disorder (MDD) is a common psychiatric disorder with a lifetime prevalence of 10% to 20% (1). It was the third leading cause of global disability in 2015 (2). Heritability is approximately 37%, lower than that of several other psychiatric disorders (3). The genome-wide contribution of common single nucleotide polymorphisms (SNPs) to MDD risk is approximately 20% (4). Consistent with the moderate heritability and high population prevalence, it has required more than 100,000 MDD cases to detect large numbers of genome-wide significant SNP associations, e.g., 15 loci in 121,380 cases plus 338,101 control subjects (5) and 44 loci in a partially overlapping sample (135,458 cases plus 344,901 control subjects) (6).

Rare copy number variants (CNVs) could be contributing to the unexplained portion of genetic risk and provide information about disease mechanisms. Two previous MDD studies of longer CNVs reported no significant genome-wide burden in patients with MDD (7,8). Here, to achieve a larger sample size, we performed a meta-analysis of the association of MDD to rare CNVs in 5780 patients and 6626 control subjects from four cohorts. A significant increase of rare, shorter deletions (<100,000 base pairs) was observed in
patients with MDD, and this was driven by CNVs in intergenic regions.

METHODS AND MATERIALS

Samples

We studied four European ancestry cohorts as shown in Table 1. All participants gave signed informed consent under protocols approved by the relevant institutional review boards.

RADIANT Cohort. The RADIANT cohort (7) included patients from three studies of recurrent MDD and two control subject cohorts (458 control subjects who were screened for lifetime absence of psychiatric disorder and 2699 control subjects from phase 2 of the National Blood Service Wellcome Trust Case Control Consortium subcohort). Patients were interviewed with the Schedules for Clinical Assessment in Neuropsychiatry (9) and diagnosed using ICD-10 or DSM-IV criteria. Exclusion criteria were any history or family history of schizophrenia or bipolar disorder or any history of mood disorder secondary to alcohol/substance misuse or of mood-incongruent psychosis (7).

Netherlands Study of Depression and Anxiety/Netherlands Twin Register. Patients with MDD and control subjects were drawn from the Netherlands Twin Register (NTR) (10) and the Netherlands Study of Depression and Anxiety (NESDA) (11). Cases had DSM-IV MDD diagnoses as assessed with the Composite Interview Diagnostic Instrument (12).

Genetics of Recurrent Early-Onset Depression.

Patients with MDD and control subjects were drawn from the Genetics of Recurrent Early-Onset Depression (GenRED) cohort (13,14). Patients had a consensus DSM-IV MDD diagnosis based on a Diagnostic Interview for Genetic Studies interview and other information, with recurrence or chronicity (an episode lasting $\geq$3 years), onset before 31 years of age, one or more siblings or parents with recurrent MDD and onset before 41 years of age, MDD independent of substance dependence, no bipolar, schizo-affective disorder or schizophrenia diagnosis, and no parent or sibling with suspected bipolar disorder I. The control subjects ($n = 1345$) from the Molecular Genetics of Schizophrenia cohort (15) (denied by online screen) ever meeting DSM-IV MDD gate criteria (no 2-week period of depressed mood or anhedonia most of the day, nearly every day), whereas the published GenRED genome-wide association study (GWAS) (13) included control subjects who never met full MDD criteria by online screen (16).

GenRED II. Patients with MDD were from the second GenRED GWAS wave (same criteria as GenRED). Control subjects were drawn from the Genomic Psychiatry Cohort (17), Depression Genes and Networks (18), and the Mayo Clinic (19). Control subjects were drawn from the Genomic Psychiatry Cohort (17) (screened for lifetime depression with a self-report questionnaire), Depression Genes and Networks (18) (screened with a Structured Clinical Interview for DSM-IV), and the Mayo Clinic (19) (screened based on diagnoses in the electronic medical record over an extended period).

Genotyping

Patients with MDD in the RADIANT cohort and screened control subjects were genotyped with the HumanHap 610-Quad beadchip (Illumina, Inc., San Diego, CA), and the unscreened National Blood Service samples were genotyped with Illumina Infinium 1M beadchips (hg18 for both) (7). The NTR/NESDA (20) and GenRED cohorts were genotyped with the Affymetrix Human Genome-Wide SNP 6.0 Array (Affymetrix, Santa Clara, CA) (hg18) (14), and GenRED II patients and control subjects were genotyped with the Illumina Omni1-Quad beadchip (hg19) (21).

Selection of CNV Calling Algorithms

CNVs were called with PennCNV (22), QuantiSNP (23) and iPattern (24) in the RADIANT dataset (using 562,329 probes common to the two platforms), with Birdsuite (25) and PennCNV (22) in the NTR/NESDA dataset, with Birdsuite (25) in the GenRED dataset, and with QuantiSNP (23) and PennCNV (22) in the GenRED II dataset. There is no consensus “optimal” calling algorithm for each platform. Various authors use a single calling method (8,26), agreement between two methods (20,27), or more complex approaches (28,29).

We conducted a preliminary analysis of CNV call concordance for duplicate genotypes for 115 Affymetrix 6.0 samples and 20 Illumina Human610-Quad samples. For Affymetrix, we compared CNVision (28), QuantiSNP (23), PennCNV (22), and Birdsuite (25) and each pair of algorithms, plus the addition of CNVision’s pCNV parameter (estimating the probability of a true CNV, based on per-SNP variability of log R ratio [LRR]) and the number of SNPs consistent with a CNV based on B allele

| Table 1. Cohorts and Sample Sizes Before and After Quality Control Filtering |
|------------------|------------------|------------------|------------------|------------------|
|                 | Pre–Quality Control Sample Size | Post–Quality Control Sample Size |
| **Cohort**       | Patients With MDD (Male/Female) | Control Subjects (Male/Female) | Patients With MDD (Male/Female) | Control Subjects (Male/Female) |
| RADIANT          | 3087 (908/2179)  | 3157 (1522/1635) | 2460 (724/1736) | 2587 (1240/1347) |
| NESDA/NTR       | 1637 (509/1128) | 2030 (765/1265) | 1568 (488/1080) | 1913 (719/1194) |
| GenRED I         | 1089 (319/770)  | 1345 (784/561)  | 941 (271/670)   | 1264 (743/521)   |
| GenRED II        | 831 (144/687)   | 944 (418/526)   | 811 (139/672)   | 862 (384/478)    |
| **Total**        | 6644 (1880/4764) | 7476 (3489/3987) | 5780 (1622/4158) | 6626 (3086/3540) |

GenRED, Genetics of Recurrent Early-Onset Depression; MDD, major depressive disorder; NESDA, Netherlands Study of Depression and Anxiety; NTR, Netherlands Twin Register.
Rare Deletions in Major Depressive Disorder

frequency [BAF]. For Illumina we compared all algorithms (except Birdsuite) and pairs plus the addition of pCNV. We also conducted the analyses for short (<100 kb) and long (>100 kb) CNVs separately.

For Affymetrix, Birdsuite had the highest concordance rate (deletions and duplications), whereas combining it with any other method slightly increased concordance but excluded >40% of calls (Supplemental Table S1). We therefore used Birdsuite alone for Affymetrix data. For Illumina data, QuantiSNP alone had the best concordance for deletions (Supplemental Table S2). For duplications, concordance was highest for QuantiSNP alone; calls made by both PennCNV and QuantiSNP showed improved concordance but excluded >30% of calls. We used QuantiSNP for primary analyses, plus a secondary “narrow” QuantiSNP + PennCNV analysis. For both platforms, concordance was similar for shorter and longer CNVs (Supplemental Tables S3–S6).

Quality Control of Samples and CNV Calls

Exclusion criteria for samples were applied to each cohort separately. For NTR/NESDA (20) and GenRED (14), exclusions were applied to samples retained by the original studies, using the previous Birdsuite calls: 1) probe intensity variances were applied to samples retained by the original studies, using Quality Control of Samples and CNV Calls conducted the analyses for short (except Birdsuite) and pairs plus the addition of pCNV. We also

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Celebrate Psychiatry: Biological thresholds for significant association (expected by chance once in 20 genome-wide studies) and suggestive association (expected once per study). For all 994 CNV regions, the 329 deletions were considered artifacts or were the edges of a common CNV region, we recomputed the CMH tests. We computed a proportion test across the four cohorts for each gene region and excluded those with significant heterogeneity ($p < 3.53 \times 10^{-5}$ to correct for multiple tests, see below). Supplemental Table S10 lists the inspected regions and reasons for all exclusions.

Additional exploratory analyses (permutation tests) considered each transcript (http://genome.ucsc.edu), Encyclopedia of DNA Elements regulatory region, Roadmap Project putative enhancer, promoter and dyadic region, and in aggregate for lists of CNVs with reported associations to psychiatric disorders (29,37) or developmental delay (32).

We used a previously described method (30) to estimate thresholds for significant association (expected by chance once in 20 genome-wide studies) and suggestive association (expected once per study). For all 994 CNV regions, the 329 deletion regions intersected with 487 genes, and 665 duplication regions intersected with 1475 genes (a total of 1962 genic tests). However, tests of genes within a region are somewhat correlated, and each region contained 4.64 genes on average. The 1962 genic tests represented 423 independent tests (~1962/4.64). We corrected for 1417 tests (994 regions and 423 genes)—a conservative estimate, because some regions were partially overlapping, and many genes were in more than one region, resulting in a $p$ value threshold for significant association of $3.53 \times 10^{-5}$ ($0.05/1417$) and for suggestive association of $7.06 \times 10^{-4}$ ($1.0/1417$).

**Power Analysis**

Power analyses were conducted for detection of specific CNVs (Supplemental Figure S1). For the ranges of allele frequencies and genotypic relative risks that were observed in this study, power was good to excellent to detect associations at $p = .01$, but the detection of suggestive or significant association would have required larger relative risks than were observed here.

**Enrichment Analysis of Functional Pathways**

To detect gene sets associated with MDD, we downloaded pathways from the Kyoto Encyclopedia of Genes and Genomes (http://rest.kegg.jp/list/pathway) and Gene Ontology (http://geneontology.org/page/download-annotations). Gene-set enrichment methods (38) were used to test for the enrichment of CNVs (separately for all or exonic CNVs) in all the genes of each pathway relative to all genic CNVs using “-cnv-enrichment-test” in PLINK. Permutation tests of enrichment in cases were also performed by adding “-mperm 10000” in PLINK, with batch and sex as covariates. A set of schizophrenia-associated genes (39) was also tested.

We also evaluated whether case CNVs were enriched in high-confidence DNasel regions (~log10 $p \geq 10$) from the Encyclopedia of DNA Elements (40) or the Roadmap Epigenomics Project (41) (https://personal.broadinstitute.org/meuleman/reg2map/HoneyBadger2_release). Separately for promoter, enhancer, and dyadic regions, we analyzed all tissues together (i.e., whether more case short deletions intersected with at least one high-confidence regulatory sequence from any tissue) and then each tissue separately (counting high-confidence sequences for that tissue). For intergenic short deletions, averaged across tissues, the proportion of CNVs that overlap high-confidence regulatory regions was 1.3% for promoter regions, 2.0% for dyadic regions, and 14.9% for enhancer regions.

**RESULTS**

Of 14,429 samples, 12,406 passed QC (5780 patients with MDD and 6626 control subjects) (Table 1). The total numbers of rare deletion and duplication calls are shown in Supplemental Table S11.

**Genome-wide Burden**

Cases had more CNVs per subject for rare, short (<100 kb) deletions ($p = .00592$, OR = 1.0483), driven by intergenic deletions ($p = .00714$, OR = 1.0716) (Table 2) and by cohort in Supplemental Table S12. Similar results were observed by the primary logistic regression tests (Supplemental Table S13), meta-analysis of cohort-specific logistic regressions (Supplemental Table S14), stratified permutation tests (Supplemental Table S7), and the down-sampled dataset (Supplemental Table S9). Short deletions across the 10- to 100-kb range contributed to the case-control difference (Supplemental Table S15 and Supplemental Figures S2–S3). No significant differences were observed for duplications or long deletions, but the OR for long deletions was positive (1.03), the confidence interval overlapped with that for short deletions (Table 2), and a secondary analysis of all rare deletions was significant (OR = 1.044; 95% confidence interval = 1.013–1.075; $p = .0046$) (Supplemental Table S16). No significant effect was observed for singleton or very long (>500 kb, >1000 kb) deletions or duplications. There was no evidence of strong heterogeneity by cohort for short deletions (Cochran’s Q test; $p = .31$) or short intergenic deletions ($p = .14$) (Supplemental Table S14 and Supplemental Table S12 and Supplemental Figure S4 for results by cohort). The excess of short deletions in cases became more significant when CNVs with frequency $>1\%$ in each cohort separately were excluded (rather than $>1\%$ in any cohort) (Supplemental Table S17) or when QuantiSNP + PennCNV calls were required for Illumina data (Supplemental Table S18). Burden results did not change after excluding nominally significant CNV regions that failed manual checks (Supplemental Table S19).
Table 2. Genome-wide Burden Analyses of Long and Short Deletions and Duplications (CNVs/Subject)

<table>
<thead>
<tr>
<th>CNV Type</th>
<th>Patients With MDD</th>
<th>Control Subjects</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;100 kb—All</td>
<td>0.324</td>
<td>0.318</td>
<td>1.0296 (0.9658–1.0975)</td>
<td>3.71 × 10⁻¹</td>
</tr>
<tr>
<td>Intergenic</td>
<td>0.134</td>
<td>0.138</td>
<td>0.9881 (0.8956–1.0899)</td>
<td>8.11 × 10⁻¹</td>
</tr>
<tr>
<td>Genic</td>
<td>0.191</td>
<td>0.181</td>
<td>1.0606 (0.9754–1.1531)</td>
<td>1.68 × 10⁻¹</td>
</tr>
<tr>
<td>Exonic</td>
<td>0.175</td>
<td>0.168</td>
<td>1.0521 (0.9646–1.1475)</td>
<td>2.51 × 10⁻¹</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>0.015</td>
<td>0.012</td>
<td>1.1672 (0.8591–1.5876)</td>
<td>3.23 × 10⁻¹</td>
</tr>
<tr>
<td>&lt;100 kb—All</td>
<td>1.015</td>
<td>0.978</td>
<td>1.0483 (1.0139–1.0843)</td>
<td>5.92 × 10⁻³</td>
</tr>
<tr>
<td>Intergenic</td>
<td>0.506</td>
<td>0.483</td>
<td>1.0716 (1.0190–1.1270)</td>
<td>7.14 × 10⁻³</td>
</tr>
<tr>
<td>Genic</td>
<td>0.509</td>
<td>0.495</td>
<td>1.0343 (0.9877–1.0842)</td>
<td>1.56 × 10⁻¹</td>
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<tr>
<td>Exonic</td>
<td>0.330</td>
<td>0.310</td>
<td>1.0552 (0.9965–1.1192)</td>
<td>6.95 × 10⁻²</td>
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<tr>
<td>Intrinsic</td>
<td>0.179</td>
<td>0.185</td>
<td>0.9952 (0.9149–1.0825)</td>
<td>9.11 × 10⁻¹</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Duplications</th>
<th></th>
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<tbody>
<tr>
<td>&gt;100 kb—All</td>
<td>0.496</td>
<td>0.476</td>
<td>1.0268 (0.9837–1.0725)</td>
<td>2.29 × 10⁻¹</td>
</tr>
<tr>
<td>Intergenic</td>
<td>0.087</td>
<td>0.079</td>
<td>1.0912 (0.9654–1.2333)</td>
<td>1.62 × 10⁻¹</td>
</tr>
<tr>
<td>Genic</td>
<td>0.409</td>
<td>0.397</td>
<td>1.0187 (0.9723–1.0677)</td>
<td>4.37 × 10⁻¹</td>
</tr>
<tr>
<td>Exonic</td>
<td>0.406</td>
<td>0.395</td>
<td>1.0166 (0.9702–1.0657)</td>
<td>4.89 × 10⁻¹</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>0.004</td>
<td>0.002</td>
<td>1.5254 (0.7880–3.0095)</td>
<td>2.13 × 10⁻¹</td>
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<tr>
<td>&lt;100 kb—All</td>
<td>0.670</td>
<td>0.702</td>
<td>0.9850 (0.9512–1.0194)</td>
<td>3.90 × 10⁻¹</td>
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<tr>
<td>Intergenic</td>
<td>0.252</td>
<td>0.266</td>
<td>0.9788 (0.9166–1.0449)</td>
<td>5.21 × 10⁻¹</td>
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<td>Genic</td>
<td>0.418</td>
<td>0.436</td>
<td>0.9845 (0.9410–1.0296)</td>
<td>4.96 × 10⁻¹</td>
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<tr>
<td>Exonic</td>
<td>0.345</td>
<td>0.365</td>
<td>0.9730 (0.9253–1.0225)</td>
<td>2.83 × 10⁻¹</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>0.073</td>
<td>0.072</td>
<td>1.0586 (0.9285–1.2066)</td>
<td>3.94 × 10⁻¹</td>
</tr>
</tbody>
</table>

For rare CNVs (carried by <1% of control subjects in each cohort), we defined four primary case-control tests of CNV subsets: deletions and duplications, and within each type, long (>100 kb) and short (<100 kb). For each subset, the case-control difference in CNVs per subject was tested by logistic regression, stratified for cohort and sex (Bonferroni-corrected threshold of significance p = .05/4, or .0125). Further exploration then considered genomic location: only intergenic, genic (exonic and/or intronic impact), exonic (subset of genic), and only intronic (subset of genic). See Supplemental Table S8 for complete results.

CI, confidence interval; CNV, copy number variant; MDD, major depressive disorder; OR, odds ratio.

*Significant result.

We considered two possible within-cohort confounding factors: DNA source and genotyping platforms. In GenRED II, there were two DNA sources: blood (137 patients and all control subjects) or lymphoblastic cell lines (674 patients) (Supplemental Table S20). CNV burden did not significantly differ between blood and lymphoblastic cell line case DNAs for any category, with a trend for more long deletions in lymphoblastic cell line DNA (Supplemental Table S21). RADIANT CNV calls used probes common to Illumina 610-Quad (assayed in patients and screened control subjects) and Illumina 1M (unscreened control subjects). Burden results were similar for patients versus screened or unscreened control subjects, except that patients had more short deletions than screened control subjects (assayed with the same array) (Supplemental Table S22). Thus, neither factor accounted for the main finding.

Exonic CNVs and CNV Regions

After all QC, no gene or region met the criteria for significant or suggestive association (Supplemental Table S10). Results with p < .01 are shown in Table 3. These represent four independent loci. Duplications in 15q11.2 achieved p = .00076 (OR = 3.88). These duplications are reciprocal to a well-known deletion region (see Discussion), consistently impacting four genes. Less consistent results are observed in surrounding genes in segmental duplication regions (Supplemental Table S10). Exonic deletions in MSR1 achieve p = .0019 (OR = 1.96); the region test includes several intronic deletions, with a similar result (p = .00075; OR = 2.05). A CNV region containing exonic and intronic deletions in PRKN (formerly PARK2) produced p = .00097 (OR = 1.92); the exonic test for PRKN had p < .01. Finally, there were six duplications, all in patients with MDD, in 6q21 (p = .0059; OR = ∞), including five exonic duplications in ATG5 that overlapped with one upstream duplication. LRR/BAF plots of CNVs shown in Table 3 are provided in Supplemental Figure S5.

Pathway Enrichment Analysis

After correction for multiple testing, no Kyoto Encyclopedia of Genes and Genomes or Gene Ontology pathway was enriched with short deletions in patients with MDD.

Regulatory Regions

Enhancer regions were modestly enriched in patients with MDD for all tissues combined as defined above (p = .024), and in 5 of 127 specific tissues (p < .05) (Supplemental Table S23).

Known Loci Associated With Psychiatric Disorders or Developmental Delay

Permutation tests did not demonstrate case enrichment of CNVs in loci associated with psychiatric disorders.
**Celebrating 50 Years**

Psychiatry: Biological

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**Table 3. Copy Number Variant Genes and Regions**

<table>
<thead>
<tr>
<th>Gene or Region</th>
<th>Chromosome</th>
<th>Start</th>
<th>End</th>
<th>All Ca</th>
<th>Co</th>
<th>CMH Test OR</th>
<th>p Value</th>
<th>RAD Ca</th>
<th>Co</th>
<th>GR2 Ca</th>
<th>Co</th>
<th>GR1 Ca</th>
<th>Co</th>
<th>Neth Ca</th>
<th>Co</th>
<th>Annotation</th>
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<tbody>
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<td><strong>Genes (Exonic)</strong></td>
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<tr>
<td>Deletion</td>
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<tr>
<td>MSR1</td>
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<td>15,965,386</td>
<td>16,050,300</td>
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<td>1.96</td>
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<td>6</td>
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<td>11</td>
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<td><strong>Duplication</strong></td>
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<tr>
<td>TUBGCP5</td>
<td>15</td>
<td>22,833,394</td>
<td>22,873,891</td>
<td>24</td>
<td>7</td>
<td>3.88</td>
<td>7.6 x 10^-4</td>
<td>7</td>
<td>3</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>15q11.2 (reciprocal to well-known deletion region)</td>
</tr>
<tr>
<td>CYFIP1</td>
<td>15</td>
<td>22,892,648</td>
<td>23,003,603</td>
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<tr>
<td>NIPA2</td>
<td>15</td>
<td>23,004,683</td>
<td>23,034,427</td>
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<tr>
<td>NIPA1</td>
<td>15</td>
<td>23,043,278</td>
<td>23,086,843</td>
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<tr>
<td><strong>Regions (Genic and/or Intergenic)</strong></td>
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<td>Deletion</td>
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<tr>
<td>6q26</td>
<td>6</td>
<td>162,136,159</td>
<td>163,489,668</td>
<td>65</td>
<td>40</td>
<td>1.92</td>
<td>9.7 x 10^-4</td>
<td>33</td>
<td>17</td>
<td>10</td>
<td>11</td>
<td>9</td>
<td>5</td>
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<td>8p22</td>
<td>8</td>
<td>15,817,196</td>
<td>16,092,656</td>
<td>59</td>
<td>33</td>
<td>2.05</td>
<td>7.5 x 10^-4</td>
<td>24</td>
<td>10</td>
<td>18</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>11</td>
<td>9</td>
<td>MSR1</td>
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<tr>
<td>6q21</td>
<td>6</td>
<td>106,549,398</td>
<td>107,026,323</td>
<td>6</td>
<td>0</td>
<td>Inf</td>
<td>5.9 x 10^-3</td>
<td>2</td>
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<td>1</td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
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<tr>
<td>15q11.2</td>
<td>12</td>
<td>22,652,330</td>
<td>23,309,294</td>
<td>24</td>
<td>7</td>
<td>3.88</td>
<td>7.6 x 10^-4</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>TUBGCP5, CYFIP1, NIPA1, and NIPA2</td>
</tr>
</tbody>
</table>

Shown are the numbers of cases (patients with major depressive disorder) (out of 5780) and of control subjects (out of 6626) carrying each CNV with post–quality control p < .01. Start and end are genomic positions in base pairs (build hg19) either for the gene for which one or more exons was impacted by each CNV or for the region within which CNVs were counted.

Ca, cases; CMH, Cochran-Mantel-Haenszel; CNV, copy number variant; Co, control subjects; GR1, Genetics of Recurrent Early-Onset Depression (genotyped with Affymetrix); GR2, Genetics of Recurrent Early-Onset Depression II (genotyped with Illumina); Neth, Netherlands Study of Depression and Anxiety and Netherlands Twin Register (genotyped with Affymetrix); OR, CMH odds ratio; Inf, infinite; RAD, RADIANT (genotyped with Illumina).

*p < .01 case-control difference.

(Supplemental Table S24) or developmental delay (Supplemental Table S25). There was no overlap between the CNVs reported in Table 3 and significant MDD GWAS loci (6,42).

**DISCUSSION**

This is the largest genome-wide study to date of the association of MDD with rare CNVs. An excess of long CNVs (>100 kb) was initially reported in an analysis of the RADIANT cohort that included additional controls (43), but a subsequent reanalysis (without the extra controls and with stricter QC, producing a substantial reduction in number of CNVs per subject similar to that reported here) detected no significant excess (7). Another study of longer CNVs in 452 patients with treatment-resistant depression and 811 control subjects also reported no significant differences (8). For schizophrenia, evidence for association of several long CNVs with large effects on risk could be detected with samples comparable in size to RADIANT (44). There were no such findings for single CNVs in the present, larger study. It appears that long, multigene CNVs are less likely to have large effects on the risk of MDD.

**Global Burden of Short Deletions**

We observed enrichment of short deletions (<100 kb) in patients with MDD, and particularly intergenic deletions. This suggests that the effect on MDD risk is due to the deletion of regulatory elements, consistent with the (modest) enrichment of high-confidence enhancer regions in short deletions in patients with MDD. This is consistent with the extensive analyses of the Psychiatric Genomics Consortium’s meta-analysis of depression GWAS data (6) that detected 44 significant associations primarily in nonexonic SNPs, including several in genes that are involved with extensive regulatory networks (RBFOX1, RBFOX2, RBFOX3, and CELF4), as well as genome-wide enrichment of highly conserved regions, open chromatin in human brain and an epigenetic mark of active enhancers (H3K4me1).

One might expect an increased burden of longer CNVs as well, because they contain more genes and regulatory elements. We analyzed short and long deletions separately because longer CNVs have been more frequently implicated in disease risk. Similar ORs were observed for burden of short and long deletions in patients with MDD, and their confidence intervals overlapped, but we had less power to detect an excess of long deletions because they were 70% less frequent than short deletions. Thus, an increased burden of longer deletions might be observed in larger meta-analyses. We also suspect that the ascertainment methods of most MDD studies are biased against individuals with long multigene CNVs, whose carriers are at higher risk of disorders such as schizophrenia, autism, and intellectual disability. Individuals with these phenotypes have an increased risk of depression (45,46), but they are often excluded from MDD cohorts and are often not specifically diagnosed with, or treated for, depression—resulting in exclusion even from registry-based cohorts. Thus, both short and long rare deletions could impact the risk of MDD, but the current results are significant only for shorter deletions (10–100 kb), and larger cohorts will be needed to resolve the issue.
Rare Deletions in Major Depressive Disorder

Individual Genes and Regions
No significant or suggestive associations were detected for individual exonic CNVs or for CNV regions, after conservative correction for genome-wide testing. Larger datasets will be needed to identify true positive findings. Nominal association was observed in several regions ($p < 0.01$ but not achieving suggestive or significant thresholds). The first region was 15q11.2 duplications encompassing the small, nonimprinted BP1/BP2 segment of the Prader-Willi/Angelman region. Deletions of this segment are weakly associated with risk of schizophrenia (29,37) and have been reported to be associated with dyslexia and dyscalculia (with deletions and duplications associated with reductions or increases, respectively, in the size and activity of the left fusiform gyrus) (47). Second, deletions in exons of MSR1 (or all deletions in that region) have been implicated in atherosclerosis, Alzheimer’s disease, and host defense. Third, deletions in 6q26 impact introns or exons of PRKN, where recessive mutations cause early-onset Parkinson’s disease (type 2), but heterozygous variants are not associated with Parkinson’s disease (48), although Parkinson’s disease is associated with increased depressive symptoms (49). The final region included duplications in exons of, or upstream sequence near, ATG5, which has multiple immune functions, including negative regulation of the type I interferon production pathway—this is of note because reduced white blood cell expression of interferon-I response genes was reported (18) but not replicated (50) in studies of MDD.

Limitations
The sample size is larger than previous CNV studies of MDD but remains underpowered. Combining CNV cohorts presents challenges including differences in clinical methods (inclusion criteria, ascertainment, and assessments) and genotyping (platforms that differ in genome coverage and signal to noise ratio). Also, the present cohorts are not ideal for testing whether the long, multi-ascertainment, and assessments) and genotyping (platforms that remains underpowered. Combining CNV cohorts presents challenges including differences in clinical methods (inclusion criteria, ascertainment, and assessments) and genotyping (platforms that differ in genome coverage and signal to noise ratio). Also, the present cohorts are not ideal for testing whether the long, multigenic “neuropsychiatric” CNVs are also predisposing for depression: the psychiatric and neurological features of these CNVs may be considered exclusion criteria from MDD studies; and the associated cognitive impairments reduce the probability of being recruited into MDD cohorts because individual carriers are less likely to volunteer or to be treated in the targeted clinical settings. On the other hand, the cohorts are broadly representative of the current concept of clinically significant MDD.

In conclusion, we found significant evidence for an increased global burden of shorter rare deletions that was mainly driven by intergenic deletions in patients with MDD from four cohorts. The evidence regarding longer deletions was inconclusive: They were not significantly increased in patients with MDD, but the confidence intervals overlapped with the case-control ORs for shorter and longer deletions, and there was less power to detect a difference because longer deletions are less frequent. Overall, the results suggest that the effects of CNVs on regulatory elements, primarily in intergenic regions, play a role in predisposition to MDD.

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GenRED and GenRED II: These projects were supported by National Institute of Mental Health (NIMH) R01 Grants MH061686 (to DFL), MH059542 (to WH Coryell), MH075131 (to WB Lawson), MH059552 (to JBP), MH059541 (to WA Scheftner) and MH060912 (to MMW). The NIMH Cell Repository at Rutgers University and the NIMH Center for Collaborative Genetic Studies on Mental Disorders made essential contributions to this project. Genotyping was carried out by the Broad Institute Center for Genotyping and Analysis with support from Grant U54 RR020278 (which partially subsidized the genotyping of the GenRED cases). Collection and quality control analyses of the control data set were supported by grants from NIMH and the National Alliance for Research on Schizophrenia and Depression.

For the Molecular Genetics of Schizophrenia (MGS) control cohort from which GenRED-I controls were drawn: This work was supported primarily by the National Institutes of Health (Grant Nos. R01MH067257 [to NS Buccola], R01MH059589 [to BJ Mowry], R01MH059571 [to PVS], R01MH059569 to [R Freedman], R01MH059587 [to F Amin], R01MH060870 [to WF Byerley], R01MH059568 [to DW Black], R01MH059586 [to JM Silverman], R01MH061675 [to DFL], R01MH060879 [to CR Cloninger], R01MH081800 [to PVG], U01MH046276 to [to CR Cloninger], U01MH046289 [to CK Kaufmann], U01MH046318 [to MT Tsuang], U01MH079469 [to PVG], and U01MH079470 to [DFL], the Genetic Association Information Network (GAIN, for genotyping of half of the EA sample), and The Paul Michael Donavan Charitable Foundation. Genotyping was carried out by the Center for Genotyping and Analysis at the Broad Institute of Harvard and MIT (to S Gabriel and DB Mirel), supported by NIH Grant No. U54RR020278. We are grateful to Knowledge Networks (Menlo Park, CA) for assistance in collecting the control data set.

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Biological Psychiatry: Celebrating 60 Years
Availability of Data and Biomaterials: Biomaterials and clinical data are available from the NIMH repository (https://nimhgenetics.org) for the GenRED cases (the GenRED1 cohort includes the family-based linkage cohort and part of the subsequent case collection; the GenRED2 cohort includes the remainder of the case collection); for the MGS controls; and for Genomic Psychiatry Cohort controls, including the Mayo Clinic controls. The authors report no biomedical financial interests or potential conflicts of interest.

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