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Rare Copy Number Variation in Treatment-Resistant Major Depressive Disorder

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Abstract

Background—While antidepressant treatment response appears to be partially heritable, no consistent genetic associations have been identified. Large, rare copy number variants (CNVs) play a role in other neuropsychiatric diseases, so we assessed their association with treatment-resistant depression (TRD).

Methods—We analyzed data from two genome-wide association studies comprising 1263 Caucasian patients with major depressive disorder. One was drawn from a large health system by applying natural language processing to electronic health records (i2b2 cohort). The second consisted of a multicenter study of sequential antidepressant treatments, Sequenced Treatment Alternatives to Relieve Depression. The Birdsuite package was used to identify rare deletions and duplications. Individuals without symptomatic remission, despite two antidepressant treatment trials, were contrasted with those who remitted with a first treatment trial.

Results—CNV data were derived for 778 subjects in the i2b2 cohort, including 300 subjects (37%) with TRD, and 485 subjects in Sequenced Treatment Alternatives to Relieve Depression cohort, including 152 (31%) with TRD. CNV burden analyses identified modest enrichment of duplications in cases (empirical $p = .04$ for duplications of 100–200 kilobase) and a particular deletion region spanning gene *PABPC4L* (empirical $p = .02$, 6 cases: 0 controls). Pathway analysis suggested enrichment of CNVs intersecting genes regulating actin cytoskeleton. However, none of these associations survived genome-wide correction.

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Conclusions—Contribution of rare CNVs to TRD appears to be modest, individually or in aggregate. The electronic health record-based methodology demonstrated here should facilitate collection of larger TRD cohorts necessary to further characterize these effects.

Keywords

Antidepressant; copy number; deletion; duplication; pharmacogenetic; pharmacogenomic; rare genetic variation

A third or more of individuals treated for major depressive disorder (MDD) do not reach symptomatic remission despite multiple adequate antidepressant treatment trials (1). Treatment-resistant depression (TRD), defined as failure to remit despite two or more treatment trials, contributes substantially to the morbidity associated with MDD, increasing health care costs, as well as functional impairment (2), suicide liability, and increased risk of relapse even following remission (1). Despite its clinical importance, little is known of the underlying neurobiology, likely because identification of TRD cohorts requires multiple treatment trials so few such cohorts exist. Identifying genetic associations with TRD could facilitate risk stratification and development of novel interventions for this patient population (3).

Prior genetic studies of antidepressant response have focused on common variation in individuals receiving a single treatment(4-6), while rarer copy number variants (CNVs) (i.e., deletions and duplications) have not been examined, despite a burgeoning body of evidence implicating them in neuropsychiatric disorders (7-11), including major depressive disorder (12-14). These data suggest that common phenotypes may still be associated with rare variants. In particular, a recent in silico investigation of genes coding for known drug targets suggests the possibility that copy number variation is likely to have large effects on treatment response (15). An alternate hypothesis, also examined here, is that a small subset of individuals with treatment-resistant depression fail to respond to treatment because of phenotypic overlap with another neuropsychiatric disorder mediated by CNVs that may be less responsive to antidepressant treatment; in particular, we anticipated that we might observe an increased frequency of CNVs previously implicated in schizophrenia, autism, or related disorders.

To examine these hypotheses, data were identified from a novel treatment-response cohort drawn from electronic health records (EHR) (16,17), referred to as the i2b2 cohort, as well as from the largest prospective investigation of treatment resistance to date, the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study (1). The former cohort represents one of the first applications in psychiatry of EHR data for genetic investigation, an approach that may be particularly useful for studying rare or otherwise difficult to ascertain clinical phenotypes.

Methods and Materials

Subjects

For the i2b2 cohort, TRD and selective serotonin reuptake inhibitor responsive phenotypes were defined using a previously validated natural language processing tool (16), which

classifies clinical status cross-sectionally using the adaptive lasso approach to regression, then determines longitudinal outcome with a rules-based classifier. Individuals were defined as treatment-resistant if they had received two or more antidepressants during a period of depression or received electroconvulsive therapy following at least one documented antidepressant treatment trial, who would have been referred because of prior documented treatment failures. Individuals were defined as antidepressant-responsive if they achieved remission with the initial documented antidepressant treatment trial. Notably, we have previously demonstrated that similar treatment effects can be observed in analyses of clinical data from both i2b2 and STAR*D (17), suggesting the relative comparability of these two data sets despite the different means of ascertainment. Because the investigators did not interact with any individuals for the ascertainment of data or samples and samples were de-identified before receipt by investigators, the Massachusetts General Hospital Institutional Review Board elected to waive the requirement of seeking informed consent as detailed by Code of Federal Regulations, Title 45, Part 46, Section 116 (46.116). The sample collection utilizes a one-way hash to ensure that, once matched with phenotypic data, all identifiers are stripped.

For replication, subjects were drawn from the STAR*D cohort (18). Assessment of outcomes has been previously described (19). Treatment resistance was defined for primary analyses as Quick Inventory of Depressive Symptomatology-Self-Report of 10 or greater after two antidepressant trials as defined in the STAR*D protocol (i.e., guideline-based antidepressant treatment according to dosing parameters at levels 1 and 2). Selective serotonin reuptake inhibitor responsiveness was defined as Quick Inventory of Depressive Symptomatology-Self-Report of 5 or less after one or two antidepressant treatment trials. As the present analysis targeted pharmacologic treatment response, subjects who received cognitive therapy at level 2 of STAR*D were excluded from the analysis. The STAR*D clinical and genetics protocols were approved by institutional review boards at participating sites.

Genotyping and Quality Control

For the i2b2 discovery cohort, DNA was extracted from discarded blood samples. Genotyping for the two waves of this cohort utilized the Illumina Omni 1 MM (n = 453) or Omni Express (n = 488) array (Illumina Inc., San Diego, California) at the Broad Institute of Massachusetts Institute of Technology and Harvard University; all analyses were therefore stratified by array type. We included only samples with genotyping call rates ≥95%, non-outliers on multidimensional scaling measures of ancestry, and no evidence of substantial relatedness by pi-hat; resulting BeadStudio call rates exceeded 99%. Copy number variants were detected using a hidden Markov model as previously described, using the Birdsuite package (20), which performs well in comparisons with other CNV-calling tools (21). Subjects who failed to pass standard single nucleotide polymorphism (SNP) quality control and those with >20 total CNVs or >10 Mb of total CNV area were excluded. These thresholds were selected based on manual inspection of distributions within each cohort and genotyping platform. Consistent with prior reports (11), CNVs with frequency greater than 1% in any individual data set, those spanning centromeres or other genomic gaps, those overlapping with common CNVs in HapMap, those overlapping events of frequency >1% in

the database of genomic variants, those with less than 10 probes/SNPs spanning the event, and those with size <100 kilobase (kb) were excluded.

Details of genotyping for STAR*D are presented elsewhere (4). The STAR*D cohort was originally genotyped on the Affymetrix 500k and 5.0 arrays (Affymetrix, Santa Clara, California); only the latter contains copy number variation probes, but SNP probe intensity may also be applied to identify CNVs, albeit more indirectly and with less precision. We obtained raw intensity data from both platforms from the investigator (S.P.H.) and utilized this data to call CNVs using the Birdsuite package (20). As with the i2b2 cohort, analyses were stratified by array type. The same quality control thresholds and methodology were applied as for the i2b2 cohort.

Analysis

Using an approach consistent with prior CNV analyses (11), we evaluated overall CNV burden for deletions and duplications considered separately, then for tranches of CNV frequencies (occurring once in the data set, or between two and six times) as well as tranches of CNV sizes (100–200 kb, 200–500 kb, and >500 kb). To compare CNV burden between cases and control subjects, one-sided tests were utilized, with 10,000 permutations used to evaluate statistical significance (22). The same approach, in which burden was examined for all duplications or deletions considered together, then for individual tranches, was used to compare proportion of genes intersected by CNVs in cases and control subjects. We also used permutation to identify individual loci where the proportion of CNVs observed in cases versus control subjects exceeded that expected by chance. Loci with specific CNVs that have previously been associated with schizophrenia or autism in a recent meta-analysis (10), as well as those associated with MDD (12-14), were examined to determine whether any were present in the TRD cases versus control subjects.

Finally, we examined curated pathways in Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) to examine whether individual pathways were enriched for duplications or deletions, using a test for gene-set enrichment described in Raychaudhuri *et al.* (23) implemented in PLINK (24). Such analyses may point to relevant biology even when individual variants fail to meet standard thresholds for statistical significance.

Results

Copy number variation data were derived for 778 Caucasian subjects in the i2b2 cohort (300 cases, 478 control subjects; 246 male and 532 female subjects) and 485 Caucasian subjects in STAR*D (152 cases, 333 control subjects; 199 male and 286 female subjects). The overall burden (count) of CNVs is listed in Table 1 for stratified analysis of the two cohorts, with initial analysis of all deletions or duplications followed by tranches corresponding to frequency of observation (top) or CNV size (bottom). For the combined cohorts, 100 kb to 200 kb duplications intersecting genes were nominally enriched among cases versus control subjects (case/control ratio 1.43; permuted $p = .04$). Table S1 in Supplement 1 presents results for each cohort individually.

Next, we examined whether there was statistical evidence for CNV association in any particular region (Table 2). We identified nominally significant regions of deletions spanning a single gene *PABPC4L* (empirical $p = .02$, uncorrected; 6 cases: 0 control subjects) (Figure 1) and in 9p23, a region without annotated genes (empirical $p = .03$, uncorrected; 11 cases: 3 control subjects). We found no nominally significant duplication regions. In addition, there existed no evidence of enrichment for individual schizophrenia or autism-associated CNVs among the TRD cases (Table S2 in Supplement 1). Among individual CNVs previously associated with MDD, we observed three cases and no control subjects with deletions in 7p21.3, and two cases and no control subjects with duplications in 15q26.3. At 16p11.2, deletions were observed in four cases and three control subjects; for duplications, five in cases and two in control subjects. No duplications at the SLIT3 locus on 5q35.1 were observed.

Finally, we analyzed annotated Kyoto Encyclopedia of Genes and Genomes pathways for enrichment of CNVs in TRD cases compared with selective serotonin reuptake inhibitor responsive control subjects (Table 3). For these analyses, duplications and deletions were initially considered together, reasoning that either form of variation could disrupt a given pathway. As this test does not incorporate stratification, we analyzed the larger i2b2 cohort first, then the STAR*D cohort. Among the 61 pathways that included at least one CNV, one pathway, Regulation of Actin Cytoskeleton, reached a nominal threshold for significance in i2b2 with CNVs intersecting 8 of 191 genes (beta = 1.292, $p = .04$ uncorrected). This result appeared to be driven primarily by duplications (6 of 8 events, including *ITGA8*, *ITGA10*, *RAK3*, *CYFIP1*, and *CRKL*). No pathways survived a Bonferroni correction for multiple comparisons. In STAR*D, significant enrichment in this pathway was not observed ($p = .23$).

Discussion

We explored whether rare deletions and duplications were associated with treatment resistance among 1263 individuals with MDD, including a novel cohort of subjects drawn from a large health care system using natural language processing of EHR data. While rarely investigated at a biological level, this phenotype has a profound impact on morbidity and health care costs(1,2). Our results do not provide strong support for the contribution of rare CNVs to TRD. While no single locus was strongly implicated, the deletion with greatest nominal evidence of association spans one gene, *PABPC4L*, expressed in brain and multiple other tissues, though little else is known about its function (25).

We also tested the hypothesis that schizophrenia or autism loci would be enriched in TRD compared with control subjects, representing either pleiotropy of the underlying loci, as has been observed with common neuropsychiatric disease variants (26), or phenotypic overlap with disorders less responsive to standard antidepressants. Our results exclude a large contribution of these variants to TRD based on the CNVs curated by Malhotra and Sebat (10), but given the rarity of these events, larger cohorts will be required to assess the true impact they have on TRD. Likewise, we do not observe strong evidence of association with CNVs previously associated with MDD itself (12-14).

Further analysis of biological pathways in our results suggests a possible enrichment of duplications in genes related to actin cytoskeleton, although this result must be interpreted with caution in light of multiple hypotheses examined and failure to replicate in the STAR*D cohort. Still, the finding is intriguing in light of a recent report of interaction between serotonin transporter, the proximal site of action of many modern antidepressants, and actin cytoskeleton (27). More generally, several studies demonstrate the importance of actin cytoskeleton in dendritic spine morphology and possibly in other neuropsychiatric disorders such as autism (28) or depression (29).

Although we did not identify strong and consistent effects between the two cohorts, we note that phenotypic heterogeneity may have obscured a true effect. While both cohorts define cases as failure of two or more antidepressant strategies, the measurement of outcome and the study population were rather different: while STAR*D utilized standard rating scales and thresholds for remission, the i2b2 cohort relied on extraction of outcomes from electronic health records. On the other hand, in a prior clinical investigation using the two cohorts, we demonstrated that both groups showed effects of nonsteroidal anti-inflammatory drugs on outcomes, including similar effects of confounding clinical features (30). While inclusion of data from randomized, controlled trials might be optimal, over 5 years of efforts by the senior author, no additional large TRD cohorts with genome-wide associated studies data could be identified. Most such cohorts are smaller than those presented here and closely held by manufacturers of Food and Drug Administration-approved medications. Both a large-scale effort to pool such resources and the application of informatics approaches, such as those we have proposed to leverage large clinical cohorts, may be required to reliably identify variation associated with anti-depressant treatment response and resistance. A further caveat is that our results do not address individuals treated with emerging antidepressant strategies, such as glutamatergic interventions. Finally, while a prior investigation of the STAR*D cohort failed to identify associations with cytochrome p450 variation (31), consideration of these genes may still be useful in future investigations.

In light of the inability of genome-wide association studies of MDD or antidepressant response to identify loci with strong evidence of association (6,32), in marked contrast to other psychiatric disorders such as bipolar disorder or schizophrenia(23,33), the use of subphenotypes or more extreme phenotypes may facilitate the genetic dissection of depression-related phenotypes. The present results suggest the need for larger cohorts to investigate rare structural variation in treatment response. They further indicate the feasibility and potential utility of efforts using electronic health records to more efficiently characterize treatment response in large cohorts. For phenotypes that are difficult to study but have a profound impact on clinical outcomes, EHR-based strategies may provide an alternate means of conducting biological or translational investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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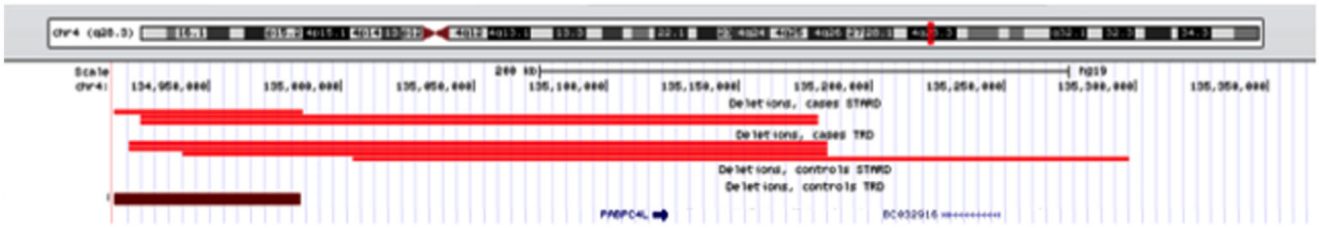

Figure 1.

Deletion with strongest evidence of association with treatment resistance across a cohort drawn from electronic health records, referred to as the i2b2 cohort, and the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) cohort. The figure illustrates the physical position and size of deletions observed in the i2b2 and STAR*D cohorts, relative to *PABPC4L*. bp, base pair; kb, kilobase; TRD, treatment-resistant depression; tRNA, transfer ribonucleic acid.

Table 1

Burden of Deletions and Duplications in Individuals with Treatment-Resistant Major Depressive Disorder and Control Subjects

		CNV Burden (Number)		CNV Brden (Gene Count)	
	Total	***P***	**Case/Control Ratio**	***P***	**Case/Control Ratio**
Deletions					
All Frequency	547	.984	.768	1.000	.475
1	203	.844	.789	.951	.496
2–6	243	.927	.786	.997	.429
Size (kb)					
100–200	359	.994	.732	.996	.512
200–500	150	.793	.871	.997	.382
500+	38	.849	.731	.831	.523
Duplications					
All Frequency	780	.235	1.033	.074	1.197
1	286	.260	1.073	.550	.934
2–6	345	.483	.969	.149	1.208
Size (kb)					
100–200	390	.692	.950	.036	1.433
200–500	265	.410	1.036	.615	.943
500+	125	.080	1.321	.205	1.295

CNV, copy number variant; kb, kilobase.

Table 2

Individual Loci with Overrepresentation of Deletions or Duplications Among Individuals with Treatment-Resistant Major Depreive Disorder

				Case: Control Ratio		
CHR	**Position**	**EMP1**	**EMP2**	**i2b2**	**STAR*D**	**Genes**
Duplications						
21q22.11	chr21:35773931	.058694	.869313	3:0	0:0	*KCNE2, FAM165B, KC*
15q11.2	chr15:22299434	.054795	.869313	3:0	10:4	*LOC727924, OR4N4*
7q11.21	chr7:62561413	.062894	.869313	3:0	1:2	(none)
17q21.31	chr17:44248225	.093391	.938406	5:2	5:6	*KIAA1267*
Deletions						
4q28.3	chr4:135183337	.023998	.318768	4:0	2:0	*PABPC4L*
9p23	chr9:12003806	.029697	.347565	5:1	6:2	(none)

CHR, chromosome; EMP1, empirical *P* value (two-tailed); EMP2, empirical *P* value (genome-wide, 2-tailed); i2b2, a cohort drawn from electronic health records; STAR*D, Sequenced Treatment Alternatives to Relieve Depression.

Table 3

Pathway-Based Analysis of Genic Duplications and Deletions in i2b2 Cohort ($p < .3$)

Pathway Name	Genes in Pathway	Intersections (Cases)	Intersections (Control/Subjects)	Beta	Permuted *p* Value
Duplications and Deletions, Analyzed Jointly ($n = 61$ Pathways with at Least One Intersected Gene)					
Regulation of Actin Cytoskeleton	191	8	4	1.18	.04491
1,1,1TRICHLORO2,2BIS4CHLOROPHENYLETHANE DDT Degradation	1556	37	28	.3072	.09482
Fatty Acid Metabolism	186	4	3	1.463	.0997
Fluorene Degradation	24	6	2	1.077	.1035
Butanoate Metabolism	957	33	28	.3181	.1438
Pantothenate and Coa Biosynthesis	25	3	3	1.269	.2104
Blood Group Glycolipid Biosynthesisneolactoseries	74	3	3	1.175	.2127
Alanine and Aspartate Metabolism	25	2	1	1.503	.2264
Glutamate Metabolism	30	2	1	1.503	.2264
Glycerophospholipid Metabolism	77	2	2	1.46	.2382
Duplications Only ($n = 45$ Pathways with at Least One Intersected Gene)					
Regulation of Actin Cytoskeleton	191	6	4	1.009	.1314
1,1,1TRICHLORO2,2BIS4CHLOROPHENYLETHANE DDT Degradation	1556	27	21	.288	.1867
Pantothenate and Coa Biosynthesis	25	3	3	1.193	.2603
Gap Junction	87	2	2	1.347	.2767
Bisphenol A Degradation	133	3	3	1.016	.2828
Deletions Only ($n = 22$ Pathways with at Least One Intersected Gene)					
Regulation of Actin Cytoskeleton	191	2	1	1.33	.2988

i2b2, a cohort drown from electronic health records.