A polygenic burden of rare disruptive mutations in schizophrenia

Published in final edited form as:
Nature. 2014 February 13; 506(7487): 185–190. doi:10.1038/nature12975.

A polygenic burden of rare disruptive mutations in schizophrenia

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Author Contribution Statement.
Project leadership: SMP, JLM, PFS, SAM, CH, PS. Sample collection and phenotyping: AK, PKEM, PFS, KC, JLM, CH. Sample processing and data management: KC, DR, MF, JLM. Sequencing and variant calling: MDP, EB, KS, KG, TF, SG. Primary statistical analysis: DR, MF, SMP. Additional analyses: LD, ES, GG, SH, NS, PR, COD, SEB. Determination of synaptic genesets: SH, EF, MOC, NHK, JSC, SGNG. Interpretation of main findings: SMP, EMS, ESL, SH, MF, PFS, SAM, PS. Primary drafting of manuscript: SMP, PFS, SAM, ESL, PS. Production and approval of the final manuscript: all authors.

The authors declare no competing financial interests.

URLs
1000 Genomes Project : www.1000genomes.org/
ExomeChip : http://genome.sph.umich.edu/wiki/Exome_Chip_Design
Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA : http://evs.gs.washington.edu/EVS/ [accessed 5/2013]
PLINK/Seq : http://atgu.mgh.harvard.edu/plinkseq/
Summary results : http://research.mssm.edu/statgen/sweden/

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Abstract

By analyzing the exome sequences of 2,536 schizophrenia cases and 2,543 controls, we have demonstrated a polygenic burden primarily arising from rare (<1/10,000), disruptive mutations distributed across many genes. Especially enriched genesets included the voltage-gated calcium ion channel and the signaling complex formed by the activity-regulated cytoskeleton-associated (ARC) scaffold protein of the postsynaptic density (PSD), sets previously implicated by genome-wide association studies (GWAS) and copy-number variation (CNV) studies. Similar to reports in autism, targets of the fragile × mental retardation protein (FMRP, product of *FMR1*) were enriched for case mutations. No individual gene-based test achieved significance after correction for multiple testing and we did not detect any alleles of moderately low frequency (~0.5-1%) and moderately large effect. Taken together, these data suggest that population-based exome sequencing can discover risk alleles and complements established gene mapping paradigms in neuropsychiatric disease.

Genetic studies of schizophrenia (MIM: 181500) have demonstrated a substantial heritability (Sullivan et al., 2003; Lichtenstein et al., 2009) that reflects common and rare alleles at many loci. Genome-wide association studies (GWAS) continue to uncover common single nucleotide polymorphisms (SNPs) at novel loci (Ripke et al., 2013). Rare or *de novo* genic deletions and duplications (copy number variants, CNV) have been firmly established, including risk variants at 22q11.2, 15q13.3, 1q21.1 (Levinson et al, 2011; Sullivan et al., 2012). One striking outcome of these large-scale, genome-wide investigations is the degree of polygenicity, consistent with thousands of genes and non-coding loci harboring risk alleles (International Schizophrenia Consortium, 2008, 2009; Lee et al., 2012; Ripke et al, 2013; Malhotra & Sebat, 2012).

Nonetheless, progress has been made in implicating biological systems and quantifying shared genetics among related psychiatric disorders (e.g. Psychiatric Genomics Bipolar Disorder Working Group, 2011; Moreno-De-Luca et al., 2010); for example, in pointing to common variants in calcium ion channel genes impacting schizophrenia and bipolar disorder (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013) and to *de novo* CNVs impacting genes encoding members of the postsynaptic density (PSD) proteome (Kirov et al., 2012), in particular members of the neuronal ARC protein and N-methyl-D-aspartate receptor (NMDAR) postsynaptic signaling complexes.

Here we apply massively parallel short-read sequencing to assay a substantial portion of variation that previously was effectively invisible: rare coding point mutations (single nucleotide variants, SNVs) and small insertions and deletions (indels). Although previous schizophrenia studies have applied sequencing, the results have been inconclusive, reflecting limited sample sizes or a focus on small numbers of candidate genes (Need et al., 2012; Crowley et al., 2013; Takata et al., 2013; Timms et al., 2013). Exome-sequencing studies of *de novo* mutation published to date have neither demonstrated an increased rate in schizophrenia, nor conclusively implicated individual genes (Girard et al., 2011, Xu et al., 2012), although some data suggest a link with particular classes of gene, such as those with higher brain expression in early fetal life (Xu et al., 2012). *De novo* studies in intellectual disability (ID, de Ligt et al., 2012; Rauch et al., 2012) and autism (Iossifov et al., 2012; Neale et al., 2012; O'Roak et al., 2012; Sanders et al., 2012) have made significant progress in identifying large-effect alleles and the underlying gene networks, however.

In this study, we sought to identify the alleles, genes or gene networks that harbor rare coding variants of moderate or large effect on risk for schizophrenia by exome-sequencing 5,079 individuals, selected from a Swedish sample of more than 11,000 individuals. Previous analyses of the full sample (SI section 1) have demonstrated an enriched burden of rare CNVs and a polygenic common variant component (Ripke et al., 2013). We generated high coverage exome sequence to ensure sufficient sensitivity to detect and genotype alleles observed in only one heterozygous individual (singletons, implying an allele frequency of ~1/10,000 although the true population frequency will typically be rarer).

The high baseline rate of rare, neutral mutations (e.g. Keinan & Clark, 2012) makes it difficult to detect rare alleles that increase risk for common diseases. Although power can be increased by jointly testing *groups* of variants in a gene (Wu et al., 2011), association testing across all genes is likely to be under-powered at current sample sizes. Indeed, a recent application of population-based exome sequencing in autism did not identify genes (Liu et al., 2013), despite moderately large sample size and the success of the *de novo* paradigm. Furthermore, many confirmed results from candidate gene sequencing studies of non-psychiatric disease still fall short of exome-wide significance (Kiezun et al., 2012).

We therefore adopted a top-down strategy in which we studied a large set of genes with a higher likelihood of playing a role in schizophrenia, based on existing genetic evidence (SI section 7). We focused on ~2,500 genes implicated by unbiased, large-scale genome-wide screens, including GWAS, CNV and *de novo* SNV studies, testing for enrichment of rare case alleles. To prioritize individual genes, we characterized emerging signals with respect to the genes and frequency and type of mutations. We coordinated analysis with an independent trio exome sequencing study (Fromer et al., this issue) and note key points of convergence below.

After alignment and variant calling of all samples jointly, we removed 11 subjects with low quality data along with likely spurious sites and genotypes (SI sections 2 & 3). Per individual, 93% (81%) of targeted bases were covered at ≥ 10-fold (30-fold). The final dataset comprised 2,536 cases and 2,543 controls (ED Table 1a and ED Figure 2a). Cases and controls had similar technical sequencing metrics, including total coverage, proportion

of deeply covered targets, and overall proportion of non-reference alleles (ED Table 1b). We observed 635,944 coding and splice-site passing variants of which 56% were singletons. Using Sanger sequencing and ExomeChip data on these samples, we determined high specificity and sensitivity for singletons (SI section 3).

We annotated variants with respect to RefSeq and combined five *in silico* algorithms to predict missense deleteriousness (ED Table 1c and SI section 4). As expected, allelic types more likely to impact protein function showed greater constraint: 69% of nonsense variants were singletons, compared to 58% of missense and 51% of silent variants. Primary analyses tested (1) disruptive variants (nonsense, essential splice site and frameshifts, N = 15,972 alleles with MAF < 0.1%), (2) disruptive plus missense variants predicted to be damaging by all five algorithms (N = 50,369) and (3) disruptive plus missense variants predicted to be damaging by at least one algorithm (N = 233,575). These groups are labeled (where NS indicates nonsynonymous): disruptive, NS_{strict} and NS_{broad}. We also stratified most analyses by allele frequency: (1) singletons; (2) up to 0.1% (10 or fewer minor alleles); (3) up to 0.5% (50 or fewer minor alleles). In the main geneset analyses, we empirically corrected for multiple testing over the nine combinations of these factors (SI section 7).

The most significant SNV or indel association ($P = 5\times10^{-8}$) was for a common missense allele in *CCHCR1*, in the MHC, a known risk locus; this top SNP was in LD with many other schizophrenia-associated SNPs in the MHC. All $P<10^{-5}$ variants were for either common alleles or a few instances of likely aberrant variants that had escaped earlier filtering (SI section 5). We performed two series of gene-based tests: a one-sided burden test of an increased rare allele rate in cases, and the *SKAT* (Wu et al., 2011), which allows for risk and protective effects. For both tests, the distribution of gene-based statistics broadly followed a global null (ED Figure 2b).

Considering only disruptive variants, the genic test yielding the lowest nominal *P*-value was for *KYNU* (kynureninase), showing 10 variants in cases and 0 in controls (ED Table 3 and Supplementary Table 1); one novel nonsense mutation at chr2:143713804 (g.468T>A; p.Y156*) was observed in 7 cases and not present in either the Exome Variant Server or 1000 Genomes Project. Although previous studies have suggested links between the kynurenine pathway and schizophrenia (e.g. Linderholm et al., 2012), our *P*-value of 1.7×10^{-3} does not withstand correction for multiple testing, even if considering only the 246 genes with ≥ 10 rare disruptive mutations capable of achieving a nominally significant result.

A polygenic burden of rare coding variants

We evaluated a polygenic burden of rare coding variants in cases, first selecting 2,546 genes (~10% of the exome) based on prior genetic studies that we hypothesized to be enriched for schizophrenia-associated mutations (SI section 6). Sources included genome-wide CNV studies (Sullivan et al., 2012; Kirov et al., 2012), GWAS (Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011; Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Ripke et al., 2013) and exome sequencing of *de novo* mutation (Girard et al., 2011; Xu et al., 2012; Fromer et al., current issue). In our

sample, these genes had a significantly higher rate of rare (MAF < 0.1%) disruptive mutations in cases compared to controls ($P = 10^{-4}$ for 1,547 versus 1,383 mutations). The enrichment was unlikely to represent technical or ancestry-related artifact because the *P*-values controlled for potential differences in exome-wide burden in cases and controls, and because we observed no differences exome-wide ($P = 0.24$). Furthermore, enrichment *P*-values were empirically derived by permuting phenotypes within subgroups of *m:n* cases to controls, matched on exome-wide identity-by-state, experimental batch and sex; the above result withstood correction for multiple testing (Table 1). We observed similar results for rarer (singletons $P = 8\times10^{-4}$) and more frequent alleles (MAF < 0.5%, $P = 2\times10^{-4}$). We also observed case enrichment for the strictly defined set of damaging mutations (NS_{strict} P = 1.5×10-3) but not the broader set (NS_{broad} $P = 0.13$).

This enrichment suggests a polygenic burden of rare variants. Although not so marked as to be detectable at the exome-wide level given the sample size, it is relatively concentrated in genes that were found to be associated with schizophrenia by other methods. The mean allelic effect was not large: in the primary comparison, the odds ratio was 1.12 (1.04 – 1.20 95% CI) for each MAF<0.1% disruptive mutation; 46% of cases carried one or more allele in this primary set (0.62 per case) compared to 41% of controls (0.55 per control). At two extremes, the modest mean effect could represent either that a subset of mutations are fully penetrant or that every allele is associated but increases risk by only 12%, similar to common alleles from GWAS. To extract subsets of potentially stronger-effect alleles, we individually tested the constituent gene sources (Table 1, ED Figure 2c), focusing on disruptive variants as they showed the strongest omnibus enrichment. For disruptive mutations, 8 of 12 sets were nominally significant ($P < 0.05$), indicating that the initial observation was not driven by a single category.

ARC, PSD-95 and calcium ion channel genes

Three of the smaller significantly enriched sets (the ARC and PSD-95 complexes and calcium ion channel genes) had odds ratios > 5. We observed enrichment ($P = 1.6\times10^{-3}$) of disruptive mutations among the 28 ARC complex genes: 9 mutations in 9 genes (all singletons) in cases, 0 in controls yielding an odds ratio of 19.2 (2.4 – 2471 95% confidence intervals, ED Table 3). Along with the NMDAR geneset (also significantly enriched), ARC genes largely accounted for the overall PSD enrichment ($P = 4\times10^{-8}$) in Kirov et al. (2012), in which four ARC genes had one or more *de novo* CNVs. Of note, in an independent exome-sequencing study in trios, Fromer et al. (current issue) found that the ARC geneset was enriched ($P = 5\times10^{-4}$) for nonsynonymous *de novo* SNVs and indels, with four genes harboring six mutations (ED Table 8). The other PSD geneset with strong enrichment ($P = 9\times10^{-4}$, odds ratio = 5.1, 1.8 – 19.2 95% CI) was the PSD-95 complex, which contains 65 genes and overlaps with ARC. PSD genes are very highly conserved and play critical roles in excitatory neural signaling components, as well as dendrite and spine plasticity. Further categorization of neuronal genes based on subcellular localization (Kirov et al., 2012; ED Table 4a) or associated mouse and human phenotypes (Bayés et al., 2011) did not yield further enrichment.

The other subset yielding a large odds ratio of 8.4 (2.03 – 77 95% CI) was the 26 voltage-gated calcium ion channel genes (12 cases, 1 control disruptive singletons, $P=2\times10^{-3}$, although the effect is attenuated when including recurrent alleles: 15/8, $P = 0.021$, see ED Table 3). The singleton enrichment was predominantly driven by the pore-forming α_1 and auxiliary $\alpha_2\delta$ subunits; of the α_1 subunits, the Ca_V1/L-type genes carried the most case mutations, including two in *CACNA1C*, a gene implicated by GWAS of bipolar disorder and schizophrenia (PGC Bipolar Disorder Group, 2012; Ripke et al., 2013). Calcium signaling is involved in many cell functions including regulating gene expression (Dolmetsch et al., 1998) and is critical for modulating synaptic plasticity (Yasuda et al., 2003). In a secondary analysis of proteins found in the nano-environment of the calcium channel (Müller et al., 2010), we observed independent enrichment for other ion channel transporters (Supplementary Table S1), odds ratio 9.1 (2.2 – 83) for disruptive singletons ($P = 1\times10^{-3}$; 13/1 alleles).

Convergence with *de novo* studies

A line of convergence across studies was that genes carrying nonsynonymous *de novo* mutations (Girard et al., 2011; Xu et al., 2012; Fromer et al., current issue) were enriched for rare disruptive mutations ($P = 1\times10^{-3}$; Table 1 & ED Tables 7a, 7b). We observed a similar result for the smaller class of genes carrying disruptive *de novo* mutations ($P = 7\times10^{-4}$, from 47 genes in our study); these genes included *UFL1* (5/0 disruptive mutations, $P = 0.03$; 7/0 NS_{strict}, $P = 0.008$), *SYNGAP1* (4/0 NS_{strict}, $P = 0.04$), and *SZT2* (18/9 NS_{strict}, $P = 0.049$). *SYNGAP1* (Synaptic Ras GTPase Activating Protein 1) is a component of the NMDAR PSD complex (Komiyama et al, 2002) and mutations in this gene are known to cause ID and autism (Berryer et al, 2013).

Genes under previously associated CNV regions did not show significant enrichment of rare disruptive mutations, although there was an enrichment of NS_{strict} mutations ($P = 0.0044$, ED Table 5). Of the eleven CNV regions, only the 3q29 locus, that contains multiple genes including *DLG1* (Levinson et al., 2011), was significant ($P = 0.0006$) and withstood correction for multiple testing.

Autism/ID genes including targets of FMRP

We next tested, as a single set, the 2,507 genes representing autism and ID candidates (SI section 6), which yielded only nominal significance ($P < 0.05$) for disruptive and NS_{strict} variants and no test survived correction for multiple testing (Table 2). Considering the twelve constituent sets, genes from autism *de novo* studies showed no enrichment (ED Figure 2c), despite greater sample size and number of disruptive *de novo* mutations. There was no evidence for autism or ID genes curated from the literature (Betancur, 2011) or for genes in the protein-protein interaction-derived subnetworks built around autism *de novos* (O'Roak et al., 2012).

The nominal omnibus signals arose largely from the Darnell et al. (2011) list of fragile X mental retardation protein (FMRP) targets. FMRP is encoded by the gene *FMR1* (the locus of the Mendelian fragile X syndrome repeat mutation) and is an RNA-binding protein that regulates translation and is needed at synapses for normal glutamate receptor signaling and

neurogenesis (Callan & Zarnescu, 2011). Targets of FMRP are enriched for *de novos* in autism (Darnell et al., 2011; Ascano et al., 2012; Iossifov et al., 2012); here we find significant enrichment of disruptive singletons ($P = 1.4\times10^{-3}$, 289/223 case/control count, OR = 1.3). These FMRP targets overlap with PSD genes (ED Table 4b), although were still enriched independently (SI section 6). In addition, these genes were enriched in GWAS of this sample ($P < 10^{-3}$, SI section 9). Whereas the Darnell list is derived from mouse brain, a second recently reported FMRP target list (Ascano et al., 2012) was generated from cultured human embryonic kidney cells, using a different experimental approach (SI section 6). This list has relatively little overlap with Darnell targets and, in contrast to the Darnell list, does not show any enrichment for rare case mutations, for GWAS loci, or comparable overlap with PSD genes (ED Table 4b).

Our results are perhaps surprising: unlike Fromer et al., we did not observe direct evidence for overlap at the individual gene level with autism and ID, despite CNV studies showing pleiotropic effects of individual loci. Nonetheless, at the broader level of genesets, all three disorders showed enrichment for FMRP targets; autism and ID *de novos* also showed strong enrichment in several PSD complexes enriched in our study, including NMDAR and PSD-95, and (for ID) ARC (Fromer et al., current issue). At the least, our results suggest that any overlap is far from complete, although more refined analyses in larger samples will be needed before a clearer picture can emerge of which genes and pathways are shared and which are specific to one disease.

Characterizing enrichment by variant type

To further characterize the observed enrichment with respect to mutational function and frequency, we created a single “composite” set of 1,796 genes comprising all members of the most prominently enriched sets (Supplementary Table S2). Rare disruptive mutations in this set were present in 990 cases and 877 controls (for singletons, 645 to 530). Cases carrying rare disruptive mutations did not appear to be phenotypically or clinically unusual in terms of sex, ancestry, history of drug abuse, general medical conditions plausibly etiologically related to psychosis, or epilepsy, although they did have a higher rate of admissions noting co-morbid intellectual disability compared to other cases ($P = 0.009$, ED Table 9b).

Figure 1 shows composite set enrichment across a range of conditions. As this set merges other sets showing enrichment, it necessarily shows enrichment; it was not, however, due to confounding effects of ancestry, sex or experimental wave (SI section 8). It was primarily driven by singleton nonsense mutations across a large number of genes, as it was removed or greatly attenuated when either singleton or nonsense mutations were excluded. Considered alone, neither splice-site, frameshift, missense, silent nor noncoding mutations showed enrichment at $P<0.01$. Different ways of defining damaging missense mutations did not substantively impact results. Considering only nonsynonymous coding variants present on ExomeChip, we did not observe enrichment. Rather, enrichment mainly reflected novel variants (ED Table 6b), which is expected as most rare variants in our study are novel. We also took an alternative approach, whereby instead of filtering variants on frequency, we excluded genes with *any* control disruptive variants before calculating the burden of case

alleles; the composite set was still highly enriched ("case-unique" in Figure 1; see ED Table 6b & SI section 7). Finally, the enrichment could not be attributed to only a small number of variants or genes (ED Figure 9a).

These findings do not preclude potentially important effects from other classes of rare variation in specific genes or other genesets, although exploratory analyses of generic genesets (e.g. based on Gene Ontology terms) did not unambiguously identify novel signals after correction for multiple testing (SI section 7). We found preferential enrichment in genes with high brain expression, but not for genes with a prenatally-biased developmental trajectory (ED Figure 10). In fact, greater enrichment came from postnatally-biased genes. Finally, while greatly attenuated compared to disruptive mutations, other categories displayed nominal ($0.01 < P < 0.05$) enrichment in Figure 1 and strictly-defined damaging missense mutations alone showed enrichment for ARC and NMDAR genesets (32/15 for ARC, $P = 0.007$; ED Tables 6a & 8). Although rare coding alleles other than ultra-rare nonsense mutations will undoubtedly contribute to risk, it will likely prove harder still to elucidate such effects.

Rare variants, CNVs and common GWAS variants

We quantified the relative impact of common SNPs (indexed by a genome-wide polygene score from independent GWAS samples from the Schizophrenia Psychiatric Genome-Wide Association Study Consortium (2011)), rare CNVs (the burden of genic deletions) and disruptive mutations in the composite set. Considering the same 5,079 individuals, all three classes of variation were uncorrelated and significantly, independently and additively enriched in cases compared to controls. From logistic regression, the relative effect sizes (reduction in model R^2) were 5.7%, 0.2% and 0.4% for GWAS, rare CNV and rare coding variants (SI section 8). Although not a complete assessment, it indicates that for the current sets of identifiably enriched alleles, common GWAS variants account for an order-of-magnitude more heritability than this set of rare variants does. However, these estimates will be diluted to varying degrees, due to unassociated variants being included. As a consequence of this, and also the fact that true risk variants outside of composite set genes were not considered here, this estimate represents a conservative lower bound on the contribution of rare coding variation.

DISCUSSION

We have demonstrated a polygenic burden that increases risk for schizophrenia, primarily comprised of many ultra-rare nonsense mutations distributed across many genes. Implicating individual genes remains challenging, as genes that contributed to the highest-ranked sets typically had unremarkable *P*-values, often around 0.5 with the gene containing only one or two rare mutations. Nonetheless, we were able to detect several small and highly enriched sets, notably of genes related to calcium channels and the postsynaptic ARC complex. Across these ~50 genes, approximately 1% of cases carried a rare disruptive mutation likely to have a considerable impact on risk. However, reported effect sizes will have a tendency to over-estimate true population values (SI section 5).

We add to previous work that has implicated disruption of synaptic processes in schizophrenia (Kirov et al., 2012). The PSD is comprised of supramolecular multiprotein complexes that detect and discriminate patterns of neuronal activity and regulate plasticity processes responsible for learning (Migaud et al. 1998). Members of the membrane-associated guanylate kinase (MAGUK) family of scaffold proteins, such as PSD-95, play a key role in assembling ~2MDa complexes comprising calcium channels, including the glutamate-gated NMDAR, voltage-gated calcium channels and ARC (Husi et al., 2000; Husi & Grant, 2001; Fernandez et al., 2009; Müller et al., 2010). The genetic disruption of MAGUKs and their associated components result in specific cognitive impairments in mice and humans (Nithiantharajah et al., 2013). One possibility is that the genetic risk identified here reflects altered tuning in calcium-dependent signaling cascades, triggered by NMDAR (Steward & Worley, 2001) and L-type calcium channels (Waltereit et al., 2001), mediated by postsynaptic MAGUK signaling complexes driving ARC synthesis.

Although we cannot yet use rare mutations to partition patients into more homogeneous clinical subgroups, this will remain a central goal for future sequencing studies. The few population-based common disease exome sequencing studies published to date, in psychiatric (e.g. Liu et al., 2013) and non-psychiatric (e.g. Albrechtsen et al., 2012) diseases, have not been successful in finding individual genes showing significant enrichment. Our study yields similar findings for individual genes, but yields positive results when considering gene sets. These current findings likely foreshadow the definitive identification of individual genes in larger cohorts, following the trajectory of GWAS and other genetic studies of complex disease.

METHODS SUMMARY

Sample ascertainment

Cases with schizophrenia were identified via the Swedish Hospital Discharge Register (Ripke et al., 2013). Case inclusion criteria: ≥2 hospitalizations with a discharge diagnosis of schizophrenia, both parents born in Scandinavia, age ≥18 years. Case exclusion criteria: hospital register diagnosis of any disorder mitigating a confident diagnosis of schizophrenia. Controls were randomly selected from Swedish population registers. Control inclusion criteria: never hospitalized for schizophrenia or bipolar disorder, both parents born in Scandinavia, age ≥18 years. All subjects provided informed consent; institutional human subject committees approved the research.

Sequencing

The samples (2,536 cases, 2,543 controls) were sequenced using either the Agilent SureSelect Human All Exon Kit (29Mb, n=132) or the Agilent SureSelect Human All Exon v.2 Kit (33Mb). Sequencing was performed by IlluminaGAII or Illumina HiSeq2000. Sequence data were aligned and variants called by the Picard (http://picard.sourceforge.net) zBWA (Li & Durbin, 2009)/GATK (de Pristo et al., 2011) pipeline. Validation of selected variants used Sanger sequencing. Based on validation and ExomeChip data, we estimated high sensitivity and specificity of singleton calls. BAM and VCF files are available in the

dbGaP study phs000473.v1 Sweden-Schizophrenia Population-Based Case-Control Exome Sequencing.

Analysis

We used PLINK/Seq (http://atgu.mgh.harvard.edu/plinkseq/) to annotate variants according to RefSeq gene transcripts (UCSC Genome Browser, http://genome.ucsc.edu). Single site association used Fisher's exact test; primary gene-based association used a burden test and the sequence kernel association test, SKAT (Wu et al., 2011). Analyses controlled for ancestry and QC metrics. Genesets used were from recent literature. Genesets were evaluated on the empirical distribution of the sum of individual gene burden statistics, and incorporated an empirical correction for multiple testing. Odd ratios with 95% confidence intervals used penalized maximum likelihood (Firth's method) for low cell counts. See the SI for further details. Summary results are posted at http://research.mssm.edu/statgen/sweden/

Extended Data

Extended Data Table 1
Sample and detected variant properties

a. Numbers of individuals in the final dataset, after individual-level QC. Finnish ancestry was inferred by multidimensional scaling. *P*-values from Fisher's exact test. **b.** Technical metrics for the cases and controls (after individual-level QC); *P*-values for two-sided test of case/control differences (*t*-test). **c.** Properties of variants detected by exome sequencing. Counts (N) and minor allele counts (MAC) for various classes of variant in the main exome dataset, following all QC. Missense deleteriousness prediction algorithms and how they were combined described in SI section 4.

a

Case/control status by sex (*P* = 5e-11)	Status	Male	Female	Female (%)
	Control	1291	1252	49%
	Case	1520	1016	40%
Case/control status by ancestry (*P* = 2e-12)	**Ancestry**	**Control**	**Case**	**Case (%)**
	Swedish	2356	2197	48%
	Finnish	139	274	66%

b

Sample and sequencing metrics	Cases	Controls	*P* (case vs. control)
N	2536	2543	-
N (pre-QC)	2546	2545	-
Total number of reads	100,532,755	100,079,333	0.62
Filtered, unique reads aligned	68,940,753	68,339,964	0.26
Filtered, unique bases aligned	5,106,614,996	5,070,497,844	0.34
Mean target coverage	89.98	89.55	0.53
Percentage of target bases covered > 10x	92.83	92.85	0.55

b

Sample and sequencing metrics	Cases	Controls	*P* (case vs. control)
Percentage of target bases covered > 20x	87.30	87.30	0.93
Percentage of target bases covered > 30x	81.13	81.07	0.63
Percentage of targets w/out any bases covered at 2x	1.72	1.72	0.60
Mean number of non-reference genotypes per individual (unfiltered)	18772.9	18786.6	0.13
Mean number of on-target singletons per individual (unfiltered)	49.6	49.0	0.38
Mean dbSNP % per individual	98.3970%	98.3969%	1.00

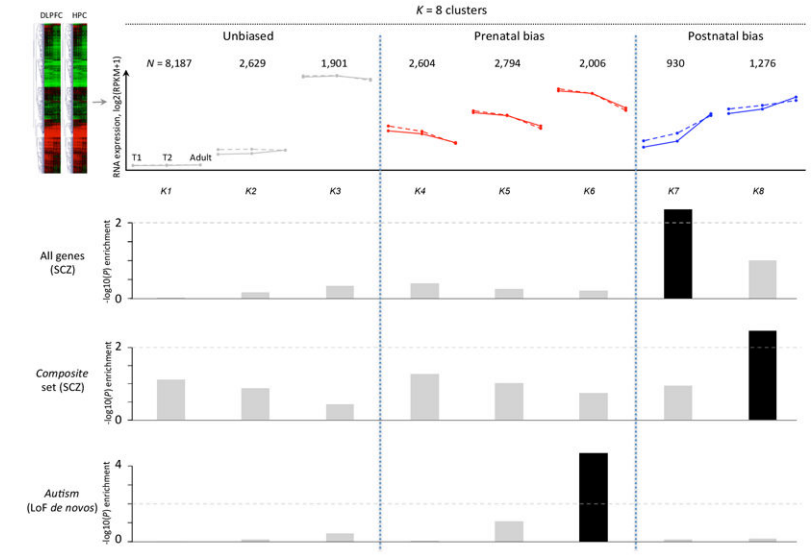
c

Property	Variant type	N	Mean MAC	% singleton
All alternate alleles		635,944	103.37	56%
Functional class				
Noncoding		61,416	142.03	53%
Silent		185,336	152.85	51%
Missense		342,561	69.52	58%
Non-essential splice site		25,450	127.04	54%
Nonsense		9,022	20.68	69%
Essential splice-site		4,394	16.18	70%
Frameshifting indel		3,461	9.46	79%
In silico annotation of missenses				
LRT		168,437	34.55	62%
Mutation Taster		167,316	19.90	63%
PolyPhen2 (HumDiv)		130,719	28.84	62%
PolyPhen2 (HumVar)		91,156	24.74	64%
SIFT		140,345	43.85	61%
Primary variant groupings for analysis				
Singletons	Gene disruptive	12,047	1.00	100%
	Nonsyn (strict)	36,542	1.00	100%
	Nonsyn (broad)	160,229	1.00	100%
<0.1% MAF (1-10 alleles)	Gene disruptive	15,972	1.56	75.4%
	Nonsyn (strict)	50,369	1.65	72.5%
	Nonsyn (broad)	233,575	1.78	68.6%
<0.5% MAF (1-50 alleles)	Gene disruptive	16,523	2.24	72.9%
	Nonsyn (strict)	52,545	2.51	69.5%
	Nonsyn (broad)	248,217	3.04	64.6%

a

		Bias: (higher in)			Prenatal			Postnatal	
	Average level:	None	Low/average	High	Low	Higher	Highest	Low	High
BrainSpan : 8-class	**Geneset**	**K1**	**K2**	**K3**	**K4**	**K5**	**K6**	**K7**	**K8**
	N genes	8,187	2,629	1,901	2,604	2,794	2,006	930	1,276
	All	0.8653	0.7511	0.8719	0.4672	0.7913	0.3518	0.1639	**0.0005**
	Composite	0.1295	0.0447	0.3896	**0.0057**	0.1395	0.4188	0.1862	**0.0014**
	PSD genes	0.9027	0.2103	0.1043	0.7012	0.4265	0.8340	0.0244	0.0210
	FMRP targets	0.1245	0.2581	0.4230	0.0351	0.5382	0.1616	0.3797	**0.0008**
	SCZ *de novos (NS)*	0.1138	0.1593	0.5044	**0.0089**	0.1855	0.2325	0.2541	0.1367
BrainSpan : 4-class		**None/low**		**High**	**Prenatal**			**Postnatal**	
	N genes	2,748		3,688	3,540			1,058	
	All genes	0.8504		**0.0020**	0.6946			0.0255	
	Composite set	0.0322		**0.0017**	0.1535			**0.0006**	
	PSD	0.3982		0.1043	0.9692			**0.0008**	
	FMRP targets	0.0257		0.0100	0.1918			0.0131	
	SCZ *de novo* (NS)	0.0500		0.0145	0.0359			0.0504	
Human Brain Transcriptome : Xu et al 3-way classifications, HPC		**Unbiased**			**Prenatal**			**Postnatal**	
	N genes	6,578			6,841			8289	
	All genes	0.6467			0.7281			0.0517	
	Composite set	0.1009			0.2876			**0.0001**	
	PSD	0.3693			0.9691			0.1043	
	FMRP targets	0.5629			0.1264			**0.0022**	
	SCZ *de novo* (NS)	0.0397			0.1395			**0.0108**	
Human Brain Transcriptome : Xu et al 3-way classifications, DLPFC		**Unbiased**			**Prenatal**			**Postnatal**	
	N genes	6,255			6,669			8,784	
	All genes	0.5076			0.7538			0.0894	
	Composite set	0.2143			0.2054			**0.0001**	
	PSD	0.5949			0.8329			0.1043	
	FMRP targets	0.3205			0.4257			**0.0005**	
	SCZ *de novo* (NS)	0.1383			0.1189			**0.0043**	

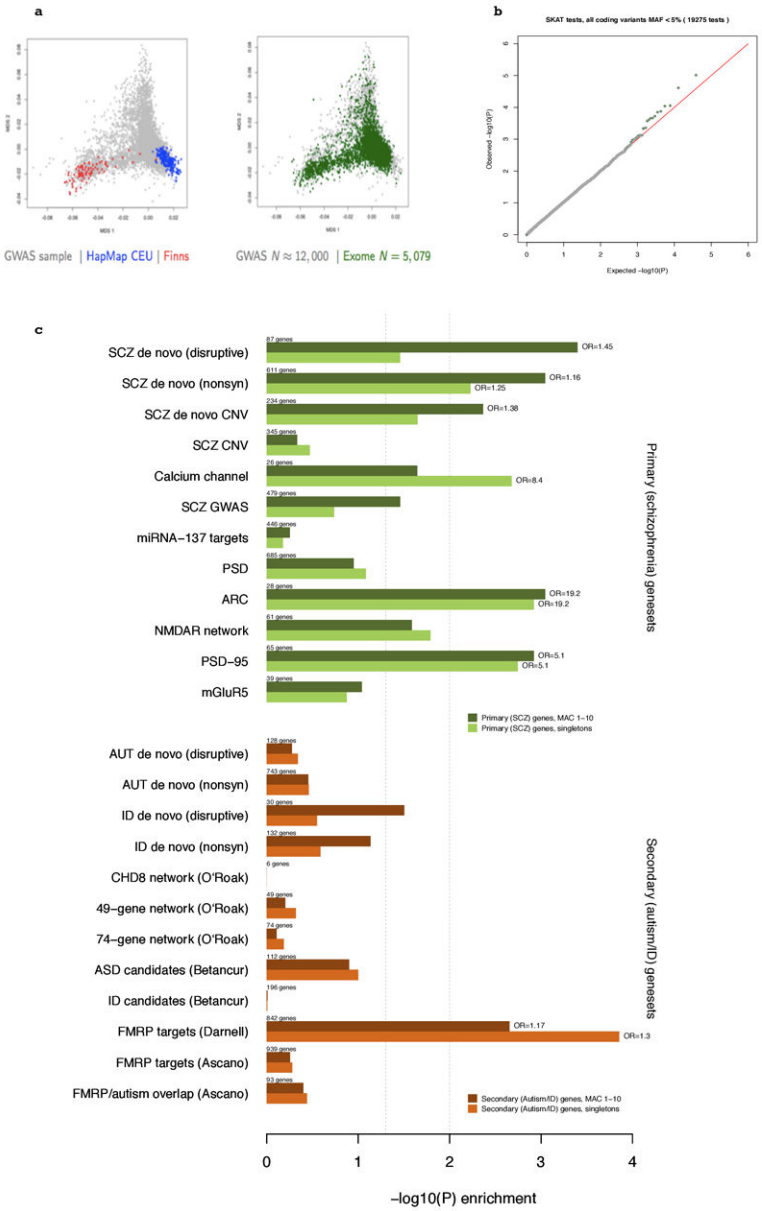
b



Extended Data Figure 10. Stratified enrichment analysis P-values by developmental trajectory of expression in brain (BrainSpan & Human Brain Transcriptome (HBT) datasets)

a. Uncorrected P-values for a set of exploratory analyses in which we stratified genes in the enrichment analyses by their developmental profile of brain expression. We used four schemes to classify genes as “brain expressed” and/or “biased” with respect to prenatal or postnatal expression (see SI section 6 and 10b below for details). We merged data on the hippocampus and dorsolateral prefrontal cortex for the BrainSpan classifications; to mirror the classification of Xu et al. (2012), we kept separate these two groupings for the HBT dataset. Results presented for MAF<0.1% disruptive variants; similar results are obtained for singletons with the exception that the “K4” prenatal enrichment signals are no longer significant. In general, the most consistent enrichment across variant classes, classification schemes and brain regions emerge for postnatally biased genes with high brain expression.

b. Analysis of exome variants by developmental expression trajectory in human brain. Genes are grouped by cluster analysis of human postmortem brain expression into eight developmental trajectories, using RNA-sequencing data from the BrainSpan project. The top row gives the number of genes per cluster and the cluster centers in log2-scaled RPKM (reads per kilobase per million) values; solid and dotted solid lines indicate dorsolateral prefrontal cortex (DLPFC) and hippocampus (HPC) respectively. The bottom two rows show enrichment in the current study, relative to the exome-wide average, for singleton disruptive mutations in cases compared to controls, either subsetting all genes by expression profile (first row), or considering only genes in the composite set (second row). In both cases, we only observed nominally ($P < 0.01$) significant enrichment for genes that are postnatally biased. In contrast, a list of genes with loss-of-function (LoF) de novo mutations (compiled and reported in Fromer et al.) shows strong enrichment for prenatal bias (see Fromer et al. for details on how de novo enrichment was calculated). Alternative approaches to classifying genes as prenatally or postnatally biased led to similar conclusions (SI section 6).



Extended Data Figure 2. Ancestry and association summaries

a. Multidimensional scaling plot of ancestry in the Swedish sample, including HapMap CEU and Finnish samples; showing sequenced individuals and the larger Swedish sample. **b**. Q-Q plot for gene-based *SKAT* results (MAF < 5% coding variants). Similar, or more conservative, profiles obtained for other subsets of variants. **c** Case enrichment of rare (MAF<0.1%) and singleton disruptive mutations for the constituent sets of the primary/ schizophrenia gene set (top panel in green) and the secondary (autism/ID) geneset (bottom panel in orange). The primary set is enriched in cases (MAF<0.1% disruptive mutations $P = 10^{-4}$; singletons $P = 8\times10^{-4}$, significant after correction for multiple testing) whereas the autism/ID shows only a modest trend ($P = 0.04$ and 0.03 for MAF<0.1% and singletons) and is not significant after correction. X-axis represents −log10(*P*); OR is odds ratio. Number of genes is for total in the set (whether or not they had a rare variant).

Extended Data Table 3

Genes prioritized as more likely to harbor large-effect alleles

Individual gene case/control counts, odds ratios and *P*-values for genes from primary genesets with odds ratios > 5, and *KYNU* (top-ranked individual gene). Odds ratios are calculated using Firth's method (penalized maximum likelihood logistic regression) and shown with 95% confidence intervals. P-values are empirical, uncorrected one-sided burden tests. FMRP target annotations are based on the Darnell et al. list only. Supplementary Table S1 lists singleton variant and genotype information for the genes listed here.

Class	Gene	Singletons	MAF < 0.1%	Notes
ARC/PSD complex				
	CYFIP1	1/0	1/0	SCZ *de novo* (CNV)
	BAIAP2	1/0	1/0	SCZ *de novo* (NS)
	DLG1	1/0	1/0	SCZ *de novo* (NS), SCZ de novo (CNV)
	SLC25A3	1/0	1/0	
	GLUD1	1/0	1/0	
	CAMK2A	1/0	1/0	FMRP target
	ATP1B1	1/0	1/0	AUT *de novo* (disruptive); FMRP target
	IQSEC2	1/0	1/0	ID *de novo* (disruptive); FMRP target
	MBP	1/0	1/0	FMRP target
	Total	9/0	9/0	
		P = 0.0016	*P* = 0.0014	
		OR = 19.2 (2.4 - 2471)	*OR* = 19.2 (2.4 - 2471)	
PSD-95 genes				
	ABLIM1	1/0	1/0	
	ACO2	1/0	1/0	FMRP target
	ANKS1B	3/1	3/1	
	ATP1B1	1/0	1/0	AUT *de novo* (disruptive); FMRP target
	ATP5A1	1/0	1/0	FMRP target
	BAIAP2	1/0	1/0	SCZ de novo (NS)
	CAMK2A	1/0	1/0	FMRP target
	CAMK2B	2/0	2/0	FMRP target
	DLG1	1/0	1/0	SCZ *de novo* (NS), SCZ de novo (CNV)
	GAPDH	1/0	1/0	
	IQSEC2	1/0	1/0	ID *de novo* (disruptive); FMRP target
	NRXN1	1/0	1/0	SCZ de novo (NS); AUT de novo (disruptive); FMRP target
	PRDX1	0/1	0/1	
	PRDX2	0/1	0/1	

Class	Gene	Singletons	MAF < 0.1%	Notes
	SUCLA2	1/0	1/0	AUT *de novo* (disruptive)
	SYNGAP1	1/0	1/0	SCZ de novo (disruptive); ID de novo (disruptive); FMRP target
	Total	17/3	17/3	
		P = 0.0017	*P* = 0.0009	
		OR = 5.1 (1.8 - 19.2)	*OR* = 5.1 (1.8 - 19.2)	
Voltage-gated calcium ion channel genes				
	CACNA1B	1/0	1/0	FMRP target
	CACNA1C	2/0	2/0	SCZ & BP GWAS hit
	CACNA1H	1/0	3/0	
	CACNA1S	2/0	2/3	SCZ & AUT *de novos* (NS)
	CACNA2D1	1/0	1/0	PSD
	CACNA2D2	3/0	3/0	
	CACNA2D3	0/0	3/0	AUT *de novo* (disruptive)
	CACNA2D4	1/0	2/4	
	CACNB2	0/1	0/1	
	CACNB4	1/0	1/0	PSD
	Total	12/1	15/8	
		P = 0.0021	*P* = 0.021	
		OR = 8.4 (2.03 - 77)	*OR* = 2.1 (0.97 - 4.9)	
Top disruptive gene-based test				
	KYNU	3/0	10/0	
		P = 0.13	*P* = 0.0017	
			OR = 21.2 (2.7 - 2725)	

Extended Data Table 4
Extended results for all PSD genesets

a. Full PSD geneset association results. For all nine (3 annotation levels by 3 frequency levels), *P*-values for enrichment of all genesets described and tested in Kirov et al. (2012). In addition to the PSD genes (top five rows), enrichment statistics for presynaptic genes, and neuronal genes clustered on the basis of subcellular location are given. Although the *P*-values presented are uncorrected, we performed this analysis correcting for all 9×17=153 tests (by considering the distribution of the minimum empirical *P*-value across tests and sets, as described in the SI). The values in **bold** are significant ($P^{corrected} < 0.05$) after correction for multiple testing. Both ARC and NMDAR network are significant after multiple test correction, for the singleton NS_{strict} category. (Note: for ARC the disruptive singleton category is, as reported in the primary test, highly significant and withstands correction for multiple testing in that context; in this broader, less focused analysis it yields $P^{corrected}$=0.17; the majority of $P^{corrected}$ values (not shown in Table) are 1.00.) **b.** PSD and FMRP-target genesets: descriptive statistics and overlap. Overlap between Darnell et al. & Ascano et al. FMRP targets and PSD genes: for example, 57% (16/28) of ARC genes are in the Darnell FMRP list. In contrast, only 7% (2/28) are in the Ascano list. There is a similar trend across the three other major PSD subsets considered here: NMDAR network, PSD-95 and mGluR5 genes. Conversely, 22% of Darnell targets are in the PSD (human core) compared to only 9% of Ascano targets.

a

		Disruptive			Nonsyn. (strict)		
Set	***N* genes**	**Singletons**	**MAF< 0.1%**	**MAF< 0.5%**	**Singletons**	**MAF< 0.1%**	**MAF< 0.5%**
PSD (human core)	685	0.0729	0.1019	0.1083	0.0058	0.1045	0.1285
ARC	28	0.0016	0.0013	0.0014	**0.0004**	0.0018	0.0047
NMDAR network	61	0.0154	0.0229	0.0225	**0.0001**	0.0007	0.0005
mGluR5	39	0.1299	0.0861	0.0862	0.0628	0.0837	0.0900
PSD-95	65	0.0015	0.0008	0.0008	0.0027	0.0204	0.0393
Pre-synapse	431	0.0187	0.0983	0.1458	0.2327	0.1811	0.3600
Pre-synaptic active zone	173	0.0518	0.0487	0.0482	0.6162	0.6641	0.7082
Synaptic vesicle	344	0.1030	0.3133	0.4151	0.1439	0.1093	0.2466
Cytoplasm	271	0.5851	0.1793	0.1034	0.8983	0.5351	0.6007
Early Endosomes	17	0.8917	0.7860	0.7826	0.2891	0.2420	0.2139
Endoplasmic Reticulum	97	0.3005	0.1882	0.2612	0.6615	0.2805	0.5036
ER/Golgi-derived vesicles	94	0.4258	0.2678	0.3644	0.3001	0.4977	0.6239
Golgi	31	0.5130	0.5493	0.5481	0.1998	0.0921	0.1338
Mitochondrion	197	0.0141	0.0259	0.0178	0.4351	0.0860	0.0671
Nucleus	167	0.1790	0.3029	0.2900	0.0626	0.1512	0.2728
Plasma membrane	50	0.7940	0.5659	0.5635	0.9416	0.8059	0.8091
Recycling Endosomes/trans-Golgi network	68	0.1502	0.0944	0.1556	0.5349	0.4514	0.5359

b

		PSD (human core)		FMRP target (Darnell)		FMRP target (Ascano)	
	N	***N***	**%**	***N***	**%**	***N***	**%**
PSD (human core)	685	-	-	170	25%	80	12%
ARC	28	-	-	16	57%	2	7%
NMDAR network	61	-	-	32	52%	5	8%
mGluR5	39	-	-	25	64%	7	18%
PSD-95	65	-	-	30	46%	4	6%
Pre-synapse	431	213	49%	87	20%	31	7%
Pre-synaptic active zone	173	121	70%	50	29%	10	6%
Synaptic vesicle	344	162	47%	72	21%	27	8%
Cytoplasm	271	77	28%	16	6%	23	8%
Early Endosomes	17	6	35%	2	12%	1	6%
Endoplasmic Reticulum	97	13	13%	5	5%	4	4%
ER/Golgi-derived vesicles	94	24	26%	7	7%	4	4%
Golgi	31	2	6%	2	6%	4	13%
Mitochondrion	197	57	29%	6	3%	12	6%
Nucleus	167	19	11%	7	4%	19	11%
Plasma membrane	50	16	32%	6	12%	5	10%
Recycling Endosomes/trans-Golgi network	68	19	28%	3	4%	7	10%
Total	1509	685	45%	170	11%	80	5%

Extended Data Table 5
Association results for individual CNV regions

Focused enrichment analysis of genes under schizophrenia-associated CNV regions. The top panel presents omnibus *P*-values testing all genes/regions (bold indicates significance after correction for the four tests, $P^{corrected} = 0.016$ for NS_{strict} MAF < 0.1% variants). This enrichment arises solely from the 3q29 locus (middle panel; bold indicates significance after correction of the 44 tests performed, $P^{corrected} = 0.024$ for 3q29). Genes and NS_{strict} case/control counts for the 3q29 region (lower panel).

		Disruptive		NS_{strict}	
Group	**Genes**	**Singletons**	**MAF < 0.1%**	**Singletons**	**MAF < 0.1%**
CNV loci	*All*	0.3279	0.4557	0.0843	**0.0044**
	1q21.1	0.4533	0.6966	0.3205	0.1832
	2p16.3	0.4775	0.3580	0.4703	0.2750
	3q29	0.1054	0.0068	0.0123	**0.0006**
	7q36.3	0.8642	0.5750	0.6411	0.2688
	7q11.23	0.7199	0.6800	0.3329	0.2207
	15q11.2	0.3208	0.1616	0.5138	0.1362
	15q13.3	0.0883	0.3976	0.3672	0.2746
	16p13.11	0.4194	0.3775	0.8346	0.4124
	16p11.2	0.1613	0.1240	0.0655	0.0974

		Disruptive		NS_{strict}	
Group	**Genes**	**Singletons**	**MAF < 0.1%**	**Singletons**	**MAF < 0.1%**
	17q12	0.7377	0.5205	0.4313	0.1385
	22q11.21	0.9386	0.9977	0.5456	0.8754

	Gene	**A/U**	**Gene name**
3q29 genes	*DLG1*	5/0	discs, large homolog 1 (Drosophila)
	RNF168	5/1	ring finger protein 168, E3 ubiquitin protein ligase
	CEP19	2/0	centrosomal protein 19kDa
	LRRC33	2/0	leucine rich repeat containing 33
	PAK2	2/0	p21 protein (Cdc42/Rac)-activated kinase 2
	PCYT1A	5/2	phosphate cytidylyltransferase 1, choline, alpha
	PIGX	6/3	phosphatidylinositol glycan anchor biosynthesis, class X
	FBXO45	1/0	F-box protein 45
	NCBP2	1/0	nuclear cap binding protein subunit 2, 20kDa
	PIGZ	1/0	phosphatidylinositol glycan anchor biosynthesis, class Z
	TFRC	1/0	transferrin receptor (p90, CD71)
	ZDHHC19	1/0	zinc finger, DHHC-type containing 19
	C3orf43	4/2	chromosome 3 open reading frame 43
	MFI2	15/12	antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5
	SLC51A	1/1	solute carrier family 51, alpha subunit
	BDH1	0/1	3-hydroxybutyrate dehydrogenase, type 1
	TCTEX1D2	0/1	Tctex1 domain containing 2
	WDR53	0/1	WD repeat domain 53

Extended Data Table 6

Further stratification of enrichment analyses by class of variant

a. Geneset analyses for damaging missense mutations only. For the primary geneset and the 12 constituent subsets, a comparison of disruptive versus (strictlydefined) damaging missenses, i.e. an independent set of variants. The omnibus result for the primary test is modest (*P*=0.04) and did not withstand correction for multiple testing: as illustrated in Figure 1 and the main text, the bulk of the enrichment signal we observe comes from (singleton) disruptive mutations. Nonetheless, specific genesets such as ARC and the NMDAR network are highly and independently enriched for missense variants. N represents the number of genes with at least one mutation of this class observed in the sample. A/U represent case/control counts of non-reference genotypes. OR represents the odds ratio (not corrected for exome-wide rates) estimated by Firth's method for sets with small cell counts. All tests are empirical and 1-sided (higher values expected in cases) as described in the main text and methods. **b.** Enrichment analyses of novel and case-unique disruptive mutations. For primary and secondary genesets (and constituent subsets) as well as the composite set: results of three alternative burden analyses. First, focusing only on genes without any control disruptive variants; no further frequency filter is imposed. Here "N genes(A/U)" indicates the number of genes with at least one disruptive variant, followed by the number of genes with case-only disruptive mutations and (for comparison) the number with control-only disruptive mutations. The "A(U)" column gives the number of case variants in the case-only genes: the test statistic is based on the empirical distribution of this count. The U in this field represents the similar quantity for controls (not explicitly used in the statistic). The second set of analyses represent standard burden/enrichment tests (i.e. as Tables 2 and 4) but stratified for novel versus known disruptive variants, according to dbSNP and the Exome Sequencing Project/Exome Variant Server (ESP/EVS) database. Novel variants show greater enrichment, although most rare variants observed in our study (both in cases and in controls) are novel, so tests of novel variants will have greater power.

a

	Disruptive singletons				Damaging missense (strict) singleton			
Primary geneset	***P***	**N**	**A/U**	**OR**	***P***	**N**	**A/U**	**OR**
All primary genes	0.0008	905	852/716	1.20	0.0393	1357	2080/2001	1.04
SCZ de novo genes								
Exome sequencing (disruptive)	0.0349	40	56/38	1.48	0.5613	53	121/120	1.01
Exome sequencing (nonsyn)	0.0059	332	384/309	1.25	0.2776	393	750/736	1.02
Copy number variants								
de novo CNV genes (Kirov et al, 2012)	0.0224	64	61/40	1.53	0.0593	90	125/112	1.12
SCZ-associated CNV genes	0.3378	72	65/55	1.19	0.0310	111	148/119	1.25
GWAS								
Voltage-gated calcium channel genes	0.0021	9	12/1	8.40	0.4629	18	37/35	1.06
Common SNPs (P < 1e-4 intervals)	0.1832	185	165/146	1.14	0.9246	268	359/395	0.91
miRNA-137 targets	0.6643	140	98/100	0.99	0.1415	263	376/361	1.05

a

	Disruptive singletons				Damaging missense (strict) singleton			
Primary geneset	***P***	**N**	**A/U**	**OR**	***P***	**N**	**A/U**	**OR**
Synaptic genes								
PSD (human core)	0.0824	219	172/145	1.19	0.0070	394	646/581	1.12
ARC	0.0012	9	9/0	19.20	0.0069	19	32/15	2.14
NMDAR network	0.0162	17	17/5	3.42	0.0003	34	76/45	1.70
PSD-95	0.0018	16	17/3	5.10	0.0218	34	44/30	1.47
mGluRS	0.1335	10	9/3	3.02	0.1715	22	52/36	1.45

b

	Case-unique burden analysis			Known variants				Novel variants			
				Singletons		MAF < 0.1%		Singletons		MAF < 0.1%	
Geneset	***P***	***N* genes(A/U)**	**A(U)**	***P***	**N**	***P***	**N**	***P***	**N**	***P***	**N**
Composite	0.0006	829(275/214)	378(297)	0.3733	145	0.2202	226	0.0002	683	0.0005	744
Primary	0.0022	1026(325/265)	440(367)	0.1003	191	0.0417	299	0.0074	831	0.0058	910
SCZ *de novo genes*											
Exome sequencing (disruptive)	0.0018	47(16/6)	29(12)	0.5514	13	0.2196	24	0.0362	35	0.0010	40
Exome sequencing (nonsyn)	0.0037	371(108/80)	159(116)	0.3647	94	0.2064	144	0.0142	302	0.0071	326
Copy number variants											
de novo CNV genes	0.1267	79(25/17)	32(24)	0.0156	13	0.0116	24	0.0819	59	0.0679	67
SCZ-associated CNV genes	0.7971	90(20/23)	24(32)	0.0355	23	0.0069	34	0.6081	63	0.9187	76
GWAS											
Voltage-gated calcium channel	0.0129	9(5/1)	10(1)	0.4922	2	0.7077	3	0.0006	7	0.0022	8
P < 1e-4 intervals	0.1079	211(65/55)	91 (78)	0.0394	44	0.0409	69	0.4425	164	0.1882	180
miRNA-137 targets	0.4498	156(52/50)	67(60)	0.9939	14	0.9972	22	0.3846	133	0.2757	147
Synaptic genes											
PSD (human core)	0.2234	244(92/79)	113(109)	0.5348	25	0.3072	40	0.1091	205	0.1629	226
ARC	0.0008	9(9/0)	9(0)		0		0	0.0016	9	0.0013	9
NMDAR network	0.0105	21(13/4)	18(4)	1.0000	1	0.6905	2	0.0075	16	0.0085	19
PSD-95	0.0137	16(13/2)	14(2)	0.1218	1	0.1559	1	0.0034	15	0.0022	15
mGluRS	0.1363	11(7/1)	8(1)		0	0.1458	1	0.1427	10	0.1826	11
Secondary (autism/ID)	0.0916	1249(348/314)	479(471)	0.1679	226	0.3543	352	0.1834	1041	0.0807	1143
De novo genes (exome sequencing)											
Autism (disruptive)	0.662	65(17/17)	20(26)	0.4463	18	0.0781	23	0.6161	50	0.7009	56
Autism (nonsyn)	0.220	407(101/96)	143(154)	0.3198	89	0.4487	133	0.6656	336	0.4960	369
ID (disruptive)	0.262	8(4/1)	4(2)	1.0000	1	0.2747	3	0.3558	8	0.0578	8
ID (nonsyn)	0.052	69(22/18)	35(28)	0.1303	14	0.0934	26	0.5331	62	0.3368	66
Neurodevelopmental candidates											
Betancur (2011), ASD candidates	0.110	37(12/6)	16(7)	0.5824	9	0.7543	14	0.0484	24	0.0429	29
Betancur (2011), ID candidates	0.994	88(14/28)	16(38)	0.6553	16	0.7056	24	0.9488	74	0.9556	82
Autism PPI networks											

b

	Case-unique burden analysis			Known variants				Novel variants			
				Singletons		MAF < 0.1%		Singletons		MAF < 0.1%	
Geneset	***P***	***N* genes(A/U)**	**A(U)**	***P***	**N**	***P***	**N**	***P***	**N**	***P***	**N**
CHD8 network	1.000	1(0/1)	0(1)		0		0	1.0000	1	1.0000	1
O'Roak et al. 49-gene network	0.796	19(3/7)	4(16)	0.4755	5	0.7326	7	0.6081	30	0.7231	33
O'Roak et al. 74-gene network	0.654	33(6/13)	10(28)	0.6438	4	0.6667	4	0.7285	17	0.8411	19
Fragile × mental retardation protein targets											
Darnell et al. targets	0.022	341(131/95)	169(133)	0.3048	39	0.3889	61	0.0007	288	0.0022	309
Ascano et al. targets	0.449	517(134/131)	187(200)	0.5571	83	0.7281	128	0.5261	439	0.4089	482
Ascano et al. FMRP/autism	0.423	33(10/6)	12(12)	0.0384	5	0.4624	11	0.6088	23	0.3954	28

Extended Data Table 7

Geneset analysis of *de novo* genes from schizophrenia exome-sequencing studies

a. Test of case enrichment of rare variants in cases compared to controls, for genes with one or more *de novos* in Fromer et al., Xu et al. and/or Girard et al. The *P*-values in **bold** are significant at $P^{corrected} < 0.05$, correcting for all 3×3×6 = 54 tests reported. **b.** Genes nominally significant (no correction) that had an observed *de novo* in one of the schizophrenia studies.

a

		Disruptive			Nonsyn. (strict)			
Set	***N* genes**	**Singletons**	**MAF < 0.1%**	**MAF < 0.5%**	**Singletons**	**MAF < 0.1%**	**MAF < 0.5%**	**Singletons**
Fromer et al. (disruptive)	63	0.1484	0.0075	0.0034	0.7401	0.7324	0.6264	0.3347
Fromer et al. (nonsyn)	464	**0.0004**	**0.0003**	0.0016	0.0341	0.0057	0.0892	0.4688
Girard & Xu (disruptive)	24	0.0342	0.0082	0.0082	0.0423	0.0412	0.0602	0.0774
Girard & Xu (nonsyn)	151	0.6916	0.4124	0.4186	0.1510	0.1326	0.1285	0.2258
Combined SCZ (disruptive)	87	0.0319	**0.0007**	**0.0003**	0.3355	0.3162	0.2692	0.1325
Combined SCZ (nonsyn)	611	0.0053	0.0011	0.0055	0.0192	0.0024	0.0379	0.3408

b

Gene	***De novo* study (type)**	**Test**	***N***	**A/U**	***P***	**Gene name**
ALDH1L2	Fromer et al. (Nonsyn)	disruptive	6	10/3	0.028	aldehyde dehydrogenase 1 family, member L2
CACNA1S	Fromer et al. (Nonsyn)	ns-strict	23	28/15	0.031	calcium channel, voltage-dependent, Ltype, alpha 1S subunit
DLG1	Fromer et al. (Nonsyn)	ns-strict	4	5/0	0.021	discs, large homolog 1 (Drosophila)
IGSF22	Fromer et al. (Nonsyn)	disruptive	5	5/0	0.043	immunoglobulin superfamily, member 22
JARID2	Fromer et al. (Nonsyn)	ns-strict	5	5/0	0.041	jumonji, AT rich interactive domain 2
LAMA4	Fromer et al. (Nonsyn)	ns-strict	8	12/4	0.041	laminin, alpha 4

b

Gene	*De novo* study (type)	Test	*N*	A/U	*P*	Gene name
NBEA	Fromer et al. (Nonsyn)	ns-strict	5	5/0	0.025	neurobeachin
POLL	Fromer et al. (Nonsyn)	disruptive	4	4/0	0.042	polymerase (DNA directed), lambda
PTK2B	Fromer et al. (Nonsyn)	ns-strict	4	4/0	0.044	PTK2B protein tyrosine kinase 2 beta
SHKBP1	Fromer et al. (Nonsyn)	ns-strict	9	15/4	0.018	SH3KBP1 binding protein 1
SULF2	Fromer et al. (Nonsyn)	ns-strict	5	7/0	0.007	sulfatase 2
SYNGAP1	Xu et al. (2012) (LoF)	ns-strict	4	4/0	0.043	synaptic Ras GTPase activating protein 1
SZT2	Xu et al. (2012) (LoF)	ns-strict	22	18/9	0.049	seizure threshold 2 homolog (mouse)
TANC1	Fromer et al. (Nonsyn)	ns-strict	14	17/4	0.002	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1
TEP1	Xu et al. (2012) (Nonsyn)	ns-strict	26	25/14	0.048	telomerase-associated protein 1
UFL1	Fromer et al. (LoF)	ns-strict	4	7/0	0.008	UFM1-specific ligase 1
UFL1	Fromer et al. (LoF)	disruptive	2	5/0	0.029	UFM1-specific ligase 1

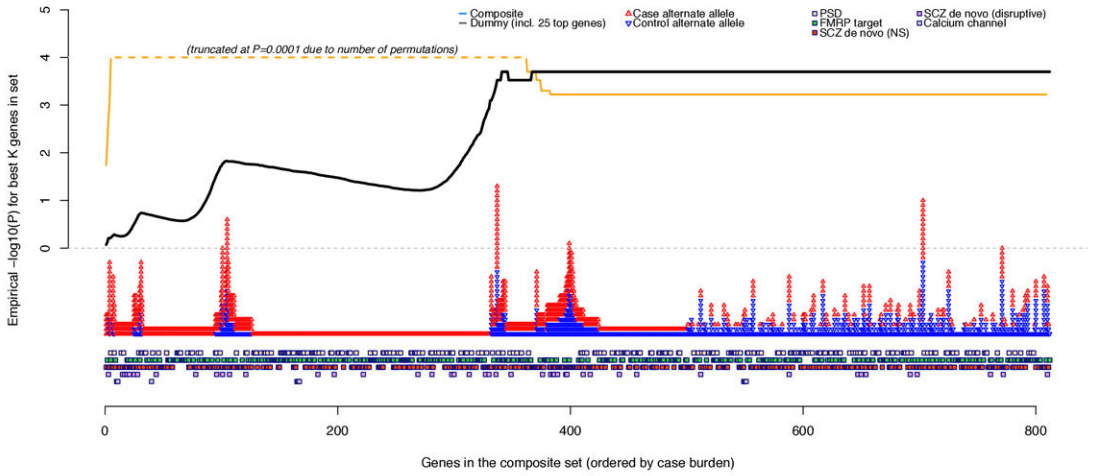
Extended Data Table 8
Summary of observed likely-deleterious variants in ARC genes across studies

For the 28 ARC genes, a summary of which genes had singleton disruptive, or damaging missense, variants in the current study, compiled alongside the genes with *de novo* CNVs or SNVs observed in Kirov et al. (2012) or Fromer et al. as well as the intellectual disability (ID) *de novo* genes (compiled in Fromer et al.). The *P*-values at the bottom indicate that in each comparison, the ARC geneset was significantly enriched.

	Current study		*de novo* CNV (Kirov et al.)	*de novo* SNV (Fromer et al.)	*de novo* SNV in ID
ARC gene (*N*=28)	Disruptive	Damaging missense (strict)			
ACTN4		3/1			
ARF5					
ATP1A1		3/0			
ATP1A3		2/1			
ATP1B1	1/0	1/0			
BAIAP2	1/0			NS(x2)	
CAMK2A	1/0	1/0			
CRMP1		1/3			
CYFIP1	1/0	4/1	2 del; 2 dup		

ARC gene (*N*=28)	Current study		*de novo* CNV (Kirov et al.)	*de novo* SNV (Fromer et al.)	*de novo* SNV in ID
	Disruptive	Damaging missense (strict)			
DLG1	1/0	2/0	1 del	NS	
DLG2		2/3	2 del	LoF	
DLG4		1/2			NS
DLGAP1		2/0	1 del		
DLGAP2		1/0			
DPYSL2		0/1			
GLUD1	1/0	1/0			
GLUL		2/0			
GRIN1		2/0			
HSPA8				LoF & NS	
IQSEC1		4/1			
IQSEC2	1/0	0/1			LoF
MBP	1/0	0/1			
PKM2					
PLP1					
SLC25A3	1/0				
SLC25A4					
SLC25A5					
STXBP1					LoF, NS(x2)
Counts:	9/0	32/15	8 SCNVs	6 SNVS	5 SNVS
***P*-value:**	0.0016	0.0069	0.00025	0.0005	0.00002

a



b

Characteristic of composite set carriers (cases only)	# admissions OR	# admissions P	>0 admissions OR	>0 admissions P	>1 admissions OR	>1 admissions P	>5 admissions OR	>5 admissions P
Hospital admissions								
Duration of hospitalization for SCZ	1.02	0.610	1.02	0.512	1.02	0.625		0.663
Total number of admissions	1.00	0.957	1.00	0.968	1.02	0.789	1.02	0.690
Year of first admission	1.00	0.861	1.00	0.884	1.00	0.895	1.00	0.924
Year of most recent admission	1.01	0.230	1.01	0.246	1.01	0.235	1.01	0.254
Drug abuse	1.00	0.401	0.96	0.675	0.90	0.400	0.77	0.122
General medical condition plausibly etiologically related to psychosis	0.99	0.596	1.12	0.421	0.96	0.831	0.85	0.664
Epilepsy	1.03	0.494	0.76	0.348	0.81	0.582	1.01	0.990
Intellectual disability	**1.05**	**0.009**	**1.41**	**0.044**	**1.59**	**0.019**	**2.03**	**0.018**
Demographics								
Male	0.89	0.187	0.90	0.199	0.90	0.222	0.90	0.230
In homogeneous subset	1.11	0.287	1.10	0.323	1.10	0.316	1.11	0.280
Finnish ancestry	0.95	0.736	0.94	0.684	0.95	0.719	0.96	0.783

Extended Data Figure 9. Genic and phenotypic subset analyses for the composite set

a. Individual gene-ranking of composite set genes. Genes are ranked by their case burden of rare disruptive mutations, from left to right, for the composite set. The squares along the bottom indicate to which sets each gene belongs. The red and blue triangles represent case and control counts for each gene. The lines above represent the statistical significance of the *best* test for this set: that is, the significance of the top *K* genes, evaluated by permutation. The black line represents results for the real data (disruptive MAF<0.1% composite set analysis). The orange line represents the *dummy* condition, in which we artificially constructed a set, where the number of genes, statistical enrichment, odds ratio and case/control counts where similar to the real composite set. However, this set included the 25 top-ranked genes from individual gene-based tests (disruptive MAF<0.1% variants), with the remainder selected at random. The profile of the *best* test line is markedly different between

the real and dummy gene sets (note: truncated at *P*=0.0001 reflecting the number of permutations performed). Whereas the *dummy P*-value climbs quickly and then drops to the final aggregate result, the true composite set line continues to climb after 200 genes, indicating that many genes with a single disruptive mutation contribute to the observed set enrichment (rather than a relatively small proportion of the 1,796 genes accounting for the majority of the signal, as in the *dummy* set). **b**. Phenotypic characteristics of cases carrying mutations. Relationship between clinical and demographic measures in schizophrenia cases in relation to carrying one or more composite set disruptive risk alleles (MAF<0.1%). Hospital Discharge Registry data (ICD9 codes) were available on 979 of the 990 case carriers. All *P*-values (uncorrected) are two-sided from a case-only joint logistic regression of carrier status (one or more risk alleles) on all admission and demographic variables including year of first and last admissions. The four pairs of columns represent analyses in which we varied the way in which the HDR admission data were represented (for drug abuse, general medication condition, epilepsy and intellectual disability). "# admissions" = independent variables are the untransformed number of admissions; ">X admissions" = independent variable is binary 0/1 variable representing whether individuals had more than *X* admissions. Of all clinical/demographic measures considered, we observed a nominally-significant increased likelihood that cases carrying a disruptive allele in the composite set have increased rates of secondary diagnoses of intellectual disability compared to other cases (based on HDR ICD9 codes).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are deeply grateful for the participation of all subjects contributing to this research, and to the collection team that worked to recruit them: Emma Flordal-Thelander, Ann-Britt Holmgren, Marie Hallin, Marie Lundin, Ann-Kristin Sundberg, Christina Pettersson, Radja Satgunanthan-Dawoud, Sonja Hassellund, Malin Rådstrom, Birgitta Ohlander, Leila Nyrén, and Isabelle Kizling. We gratefully acknowledge funding support from NIH/NIMH ARRA Grand Opportunity grant NIMH RC2 MH089905 (Purcell & Sklar), the Sylvan Herman Foundation, a philanthropic gift from Ted and Vada Stanley to the Stanley Center for Psychiatric Research, the Stanley Medical Research Institute, NIH/NHGRI grant U54HG003067 (Lander), NIH/NIMH R01 MH095088 (Haggarty), NIH/NIMH grant R01 MH091115 (Haggarty), the Tau Consortium (Haggarty), NIH/NIMH grant R01 MH099126 (Purcell), NIH/NHGRI grant R01HG005827 (Purcell), NIH/NIMH grant R01 MH077139 (Sullivan), NIH/NIMH grant R01 MH095034 (Sklar), the Friedman Brain Institute at Mount Sinai School of Medicine, the Karolinska Institutet, Karolinska University Hospital, the Swedish Research Council, an ALF grant from Swedish County Council, the Söderström Königska Foundation, the Netherlands Scientific Organization (NWO 645-000-003), the Wellcome Trust, Genes to Cognition Program, The Medical Research Council (MRC) and European Union projects GENCODYS no. 241995, EUROSPIN no. 242498 and SYNSYS no. 242167 (Fernández, Collins, Komiyama, Choudhary & Grant). Work at the Icahn School of Medicine at Mount Sinai was also supported by the Institute for Genomics and Multiscale Biology (including computational resources and staff expertise provided by the Department of Scientific Computing). The funders had no role in study design, execution, analysis, and manuscript preparation.

References

Albrechtsen A, Grarup N, Li Y, Sparsø T, Tian G, Cao H, Jiang T, Kim SY, Korneliussen T, Li Q, Nie C, Wu R, Skotte L, Morris AP, Ladenvall C, Cauchi S, Stančáková A, Andersen G, Astrup A, Banasik K, Bennett AJ, Bolund L, Charpentier G, Chen Y, Dekker JM, Doney AS, Dorkhan M, Forsen T, Frayling TM, Groves CJ, Gui Y, Hallmans G, Hattersley AT, He K, Hitman GA, Holmkvist J, Huang S, Jiang H, Jin X, Justesen JM, Kristiansen K, Kuusisto J, Lajer M, Lantieri O,

Li W, Liang H, Liao Q, Liu X, Ma T, Ma X, Manijak MP, Marre M, Mokrosiński J, Morris AD, Mu B, Nielsen AA, Nijpels G, Nilsson P, Palmer CN, Rayner NW, Renström F, Ribel-Madsen R, Robertson N, Rolandsson O, Rossing P, Schwartz TW, Slagboom PE, Sterner M, Tang M, Tarnow L, Tuomi T, Van't Riet E, van Leeuwen N, Varga TV, Vestmar MA, Walker M, Wang B, Wang Y, Wu H, Xi F, Yengo L, Yu C, Zhang X, Zhang J, Zhang Q, Zhang W, Zheng H, Zhou Y, Altshuler D, 't Hart LM, Franks PW, Balkau B, Froguel P, McCarthy MI, Laakso M, Groop L, Christensen C, Brandslund I, Lauritzen T, Witte DR, Linneberg A, Jørgensen T, Hansen T, Wang J, Nielsen R, Pedersen O. D.E.S.I.R. Study Group, the DIAGRAM Consortium. Exome sequencing-driven discovery of coding polymorphisms associated with common metabolic phenotypes. Diabetologia. 2013; 56(2):298–310. [PubMed: 23160641]

Bayés À, van de Lagemaat LN, Collins MO, Croning MDR, Whittle IR, Choudhary JS, Grant SGN. Characterisation of the proteome, diseases and evolution of the human postsynaptic density. Nature Neuroscience. 2011; 14(1):19–21.

Berryer MH, Hamdan FF, Klitten LL, Møller RS, Carmant L, Schwartzentruber J, Patry L, Dobrzeniecka S, Rochefort D, Neugnot-Cerioli M, Lacaille JC, Niu Z, Eng CM, Yang Y, Palardy S, Belhumeur C, Rouleau GA, Tommerup N, Immken L, Beauchamp MH, Patel GS, Majewski J, Tarnopolsky MA, Scheffzek K, Hjalgrim H, Michaud JL, Di Cristo G. Mutations in SYNGAP1 cause intellectual disability, autism, and a specific form of epilepsy by inducing haploinsufficiency. Hum Mutat. 2013; 34(2):385–94. [PubMed: 23161826]

Callan MA, Zarnescu DC. Heads-up: new roles for the fragile × mental retardation protein in neural stem and progenitor cells. Genesis. 2011 Jun; 49(6):424–40. [PubMed: 21404421]

Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet. 2013; 381(9875):1371–9. [PubMed: 23453885]

Crowley JJ, Hilliard CE, Kim Y, Morgan MB, Lewis LR, Muzny DM, Hawes AC, Sabo A, Wheeler DA, Lieberman JA, Sullivan PF, Gibbs RA. Deep resequencing and association analysis of schizophrenia candidate genes. Molecular Psychiatry. 2013; 18:138–140. [PubMed: 22472875]

Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraser CE, Stone EF, Chen C, Fak JJ, Chi SW, Licatalosi DD, Richter JD, Darnell RB. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell. 2011; 146(2):247–61. [PubMed: 21784246]

de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA, Vissers LE. Diagnostic exome sequencing in persons with severe intellectual disability. N Engl J Med. 2012; 367(20):1921–9. [PubMed: 23033978]

de Pristo M, Banks E, Poplin R, Garimella K, Maguire J, Hartl C, Philippakis A, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell T, Kernytsky A, Sivachenko A, Cibulskis K, Gabriel S, Altshuler D, Daly M. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature Genetics. 2011; 43:491–498. [PubMed: 21478889]

Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. Nature. 1998; 392:933–936. [PubMed: 9582075]

Fernandez E, Collins MO, Uren RT, Kopanitsa MV, Komiyama NH, Croning MD, Zografos L, Armstrong JD, Choudhary JS, Grant SG. Targeted tandem affinity purification of PSD-95 recovers core postsynaptic complexes and schizophrenia susceptibility proteins. Molecular systems biology. 2009; 5:269. [PubMed: 19455133]

Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, Georgieva L, Rees E, Palta P, Ruderfer DM, Carrera N, Humphreys I, Johnson JS, Roussos P, Barker DB, Banks E, Milanova V, Grant SGN, Hannon E, Rose SA, Chambert K, Mahajan M, Scolnick EM, Moran JL, Kirov G, Palotie A, McCarroll SA, Holmans P, Sklar P, Owen MJ, Purcell SM, O'Donovan MC. *De novo* mutations in schizophrenia implicate synaptic networks. in press.

Girard SL, Gauthier J, Noreau A, Xiong L, Zhou S, Jouan L, Dionne-Laporte A, Spiegelman D, Henrion E, Diallo O, et al. Increased exonic de novo mutation rate in individuals with schizophrenia. Nat Genetics. 2011; 43:860–863. [PubMed: 21743468]

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, et al. De novo gene disruptions in children on the autistic spectrum. Neuron. 2012; 74:285–299. [PubMed: 22542183]

International Schizophrenia Consortium. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature. 2008; 455(7210):237–41. [PubMed: 18668038]

International Schizophrenia Consortium. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature. 2009; 460(7256):748–52. [PubMed: 19571811]

Keinan A, Clark AG. Recent explosive human population growth has resulted in an excess of rare genetic variants. Science. 2012; 336:740–743. [PubMed: 22582263]

Kiezun A, Garimella K, Do R, Stitziel NO, Neale BM, McLaren PJ, Gupta N, Sklar P, Sullivan PF, Moran JL, Hultman CM, Lichtenstein P, Magnusson P, Lehner T, Shugart YY, Price AL, de Bakker PI, Purcell SM, Sunyaev SR. Exome sequencing and the genetic basis of complex traits. Nat Genet. 2012 May 29; 44(6):623–30. [PubMed: 22641211]

Kirov G, Pocklington AJ, Holmans P, Ivanov D, Ikeda M, Ruderfer D, Moran J, Chambert K, Toncheva D, Georgieva L, Grozeva D, Fjodorova M, Wollerton R, Rees E, Nikolov I, van de Lagemaat LN, Bayés A, Fernandez E, Olason PI, Böttcher Y, Komiyama NH, Collins MO, Choudhary J, Stefansson K, Stefansson H, Grant SG, Purcell S, Sklar P, O'Donovan MC, Owen MJ. De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. Mol Psychiatry. 2012; 17(2):142–53. [PubMed: 22083728]

Komiyama NH, Watabe AM, Carlisle HJ, Porter K, Charlesworth P, Monti J, Strathdee DJ, O'Carroll CM, Martin SJ, Morris RG, O'Dell TJ, Grant SGN. SynGAP regulates ERK/MAPK signaling, synaptic plasticity, and learning in the complex with postsynaptic density 95 and NMDA receptor. J Neurosci. 2002; 22(22):9721–9732. [PubMed: 12427827]

Lee SH, DeCandia TR, Ripke S, Yang J, Sullivan PF, Goddard ME, Keller MC, Visscher PM, Wray NR. Schizophrenia Psychiatric Genome-Wide Association Study Consortium (PGC-SCZ); International Schizophrenia Consortium (ISC); Molecular Genetics of Schizophrenia Collaboration (MGS). Estimating the proportion of variation in susceptibility to schizophrenia captured by common SNPs. Nature Genetics. 2012; 44(3):247–50. [PubMed: 22344220]

Levinson DF, Duan J, Oh S, Wang K, Sanders AR, Shi J, Zhang N, Mowry BJ, Olincy A, Amin F, Cloninger CR, Silverman JM, Buccola NG, Byerley WF, Black DW, Kendler KS, Freedman R, Dudbridge F, Pe'er I, Hakonarson H, Bergen SE, Fanous AH, Holmans PA, Gejman PV. Copy number variants in schizophrenia: confirmation of five previous findings and new evidence for 3q29 microdeletions and VIPR2 duplications. Am J Psychiatry. 2011; 168(3):302–16. [PubMed: 21285140]

Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics. 2009; 25:1754–60. [PubMed: 19451168]

Lichtenstein P, Yip BH, Björk C, Pawitan Y, Cannon TD, Sullivan PF, Hultman CM. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet. 2009; 373(9659):234–9. [PubMed: 19150704]

Linderholm KR, Skogh E, Olsson SK, Dahl ML, Holtze M, Engberg G, Samuelsson M, Erhardt S. Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. Schizophrenia Bulletin. 2012; 38:426–432. [PubMed: 20729465]

Liu L, Sabo A, Neale BM, Nagaswamy U, Stevens C, Lim E, Bodea CA, Muzny D, Reid JG, Banks E, Coon H, Depristo M, Dinh H, Fennel T, Flannick J, Gabriel S, Garimella K, Gross S, Hawes A, Lewis L, Makarov V, Maguire J, Newsham I, Poplin R, Ripke S, Shakir K, Samocha KE, Wu Y, Boerwinkle E, Buxbaum JD, Cook EH Jr, Devlin B, Schellenberg GD, Sutcliffe JS, Daly MJ, Gibbs RA, Roeder K. Analysis of rare, exonic variation amongst subjects with autism spectrum disorders and population controls. PLoS Genet. 2013; 9(4):e1003443. [PubMed: 23593035]

Malhotra D, Sebat J. CNVs: harbingers of a rare variant revolution in psychiatric genetics. Cell. 2012; 148(6):1223–41. [PubMed: 22424231]

Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, Grant SG. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature. 1998; 396(6710): 433–439. [PubMed: 9853749]

Moreno-De-Luca D, Mulle JG, Kaminsky EB, Sanders SJ, Myers SM, Adam MP, Pakula AT, Eisenhauer NJ, Uhas K, Weik L, Guy L, Care ME, Morel CF, Boni C, Salbert BA, Chandrareddy A, Demmer LA, Chow EW, Surti U, Aradhya S, Pickering DL, Golden DM, Sanger WG, Aston E, Brothman AR, Gliem TJ, Thorland EC, Ackley T, Iyer R, Huang S, Barber JC, Crolla JA, Warren

ST, Martin CL, Ledbetter DH. SGENE Consortium, Simons Simplex Collection Genetics Consortium, GeneSTAR. Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. Am J Hum Genet. 2010; 87(5):618–30. [PubMed: 21055719]

Müller CS, Haupt A, Bildl W, Schindler J, Knaus HG, Meissner M, Rammner B, Striessnig J, Flockerzi V, Fakler B, Schulte U. Quantitative proteomics of the Cav2 channel nano-environments in the mammalian brain. Proc Natl Acad Sci U S A. 2010; 107(34):14950–7. [PubMed: 20668236]

Need AC, McEvoy JP, Gennarelli M, Heinzen EL, Ge D, Maia JM, Shianna KV, He M, Cirulli ET, Gumbs CE, Zhao Q, Campbell CR, Hong L, Rosenquist P, Putkonen A, Hallikainen H, Repo-Tiihonen E, Tiihonen J, Levy DL, Meltzer HY, Goldstein DB. Exome Sequencing Followed by Large-Scale Genotyping Suggests a Limited Role for Moderately Rare Risk Factors of Strong Effect in Schizophrenia. American Journal of Human Genetics. 2012; 91(2):303–312. [PubMed: 22863191]

Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, Lin CF, Stevens C, Wang LS, Makarov V, Polak P, Yoon S, Maguire J, Crawford EL, Campbell NG, Geller ET, Valladares O, Schafer C, Liu H, Zhao T, Cai G, Lihm J, Dannenfelser R, Jabado O, Peralta Z, Nagaswamy U, Muzny D, Reid JG, Newsham I, Wu Y, Lewis L, Han Y, Voight BF, Lim E, Rossin E, Kirby A, Flannick J, Fromer M, Shakir K, Fennell T, Garimella K, Banks E, Poplin R, Gabriel S, DePristo M, Wimbish JR, Boone BE, Levy SE, Betancur C, Sunyaev S, Boerwinkle E, Buxbaum JD, Cook EH Jr, Devlin B, Gibbs RA, Roeder K, Schellenberg GD, Sutcliffe JS, Daly MJ. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature. 2012; 485:242–245. [PubMed: 22495311]

Nithianantharajah J, Komiyama NH, McKechanie A, Johnstone M, Blackwood DH, St Clair D, Emes RD, van de Lagemaat LN, Saksida LM, Bussey TJ, Grant SG. Synaptic scaffold evolution generated components of vertebrate cognitive complexity. Nature Neuroscience. 2013; 16(1):16–24.

O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature. 2012; 485:246–250. [PubMed: 22495309]

Psychiatric GWAS Consortium Bipolar Disorder Working Group. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nature Genetics. 2011; 43(10):977–83. [PubMed: 21926972]

Rauch A, Wieczorek D, Graf E, Wieland T, Endele S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donato N, Dufke A, Cremer K, Hempel M, Horn D, Hoyer J, Joset P, Röpke A, Moog U, Riess A, Thiel CT, Tzschach A, Wiesener A, Wohlleber E, Zweier C, Ekici AB, Zink AM, Rump A, Meisinger C, Grallert H, Sticht H, Schenck A, Engels H, Rappold G, Schröck E, Wieacker P, Riess O, Meitinger T, Reis A, Strom TM. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. Lancet. 2012; 380(9854):1674–82. [PubMed: 23020937]

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PK, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman PV, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang KY, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CC, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C,

Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CC, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF. Multicenter Genetic Studies of Schizophrenia Consortium, Psychosis Endophenotypes International Consortium, Wellcome Trust Case Control Consortium 2. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nature Genetics. 2013; 45(10):1150–9. [PubMed: 23974872]

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL, Walker MF, Ober GT, Teran NA, Song Y, El-Fishawy P, Murtha RC, Choi M, Overton JD, Bjornson RD, Carriero NJ, Meyer KA, Bilguvar K, Mane SM, Sestan N, Lifton RP, Günel M, Roeder K, Geschwind DH, Devlin B, State MW. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature. 2012; 485:237–241. [PubMed: 22495306]

Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. Nature Genetics. 2011; 43(10):969–76. [PubMed: 21926974]

Steward O, Worley PF. Selective Targeting of Newly Synthesized Arc mRNA to Active Synapses Requires NMDA Receptor Activation. Neuron. 2001; 30:227–240. [PubMed: 11343657]

Sullivan PF, Daly MJ, O'Donovan M. Genetic architectures of psychiatric disorders: the emerging picture and its implications. Nat Rev Genet. 2012; 13(8):537–51. [PubMed: 22777127]

Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 2003; 60(12):1187–92. [PubMed: 14662550]

Takata A, Iwayama Y, Fukuo Y, Ikeda M, Okochi T, Maekawa M, Toyota T, Yamada K, Hattori E, Ohnishi T, Toyoshima M, Ujike H, Inada T, Kunugi H, Ozaki N, Nanko S, Nakamura K, Mori N, Kanba S, Iwata N, Kato T, Yoshikawa T. A population-specific uncommon variant in GRIN3A associated with schizophrenia. Biol Psychiatry. 2013; 73(6):532–9. [PubMed: 23237318]

Timms AE, Dorschner MO, Wechsler J, Choi KY, Kirkwood R, Girirajan S, Baker C, Eichler EE, Korvatska O, Roche KW, Horwitz MS, Tsuang DW. Support for the N -Methyl-D-Aspartate Receptor Hypofunction Hypothesis of Schizophrenia From Exome Sequencing in Multiplex Families. JAMA Psychiatry. 2013; 3:1–9.

Waltereit R, Dammermann B, Wulff P, Scafidi J, Staubli U, Kauselmann G, Bundman M, Kuhl D. Arg3.1/Arc mRNA induction by Ca2+ and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. J Neurosci. 2001; 21(15):5484–93. [PubMed: 11466419]

Wu MC, Lee S, Cai T, Li Y, Boehnke M, Lin X. Rare-variant association testing for sequencing data with the sequence kernel association test. American Journal of Human Genetics. 2011; 89(1):82–93. [PubMed: 21737059]

Xu B, Ionita-Laza I, Roos JL, Boone B, Woodrick S, Sun Y, Levy S, Gogos JA, Karayiorgou M. De novo gene mutations highlight patterns of genetic and neural complexity in schizophrenia. Nature Genetics. 2012; 44:1365–1369. [PubMed: 23042115]

Yasuda R, Sabatini BL, Svoboda K. Plasticity of calcium channels in dendritic spines. Nat Neurosci. 2003 Sep; 6(9):948–55. [PubMed: 12937422]

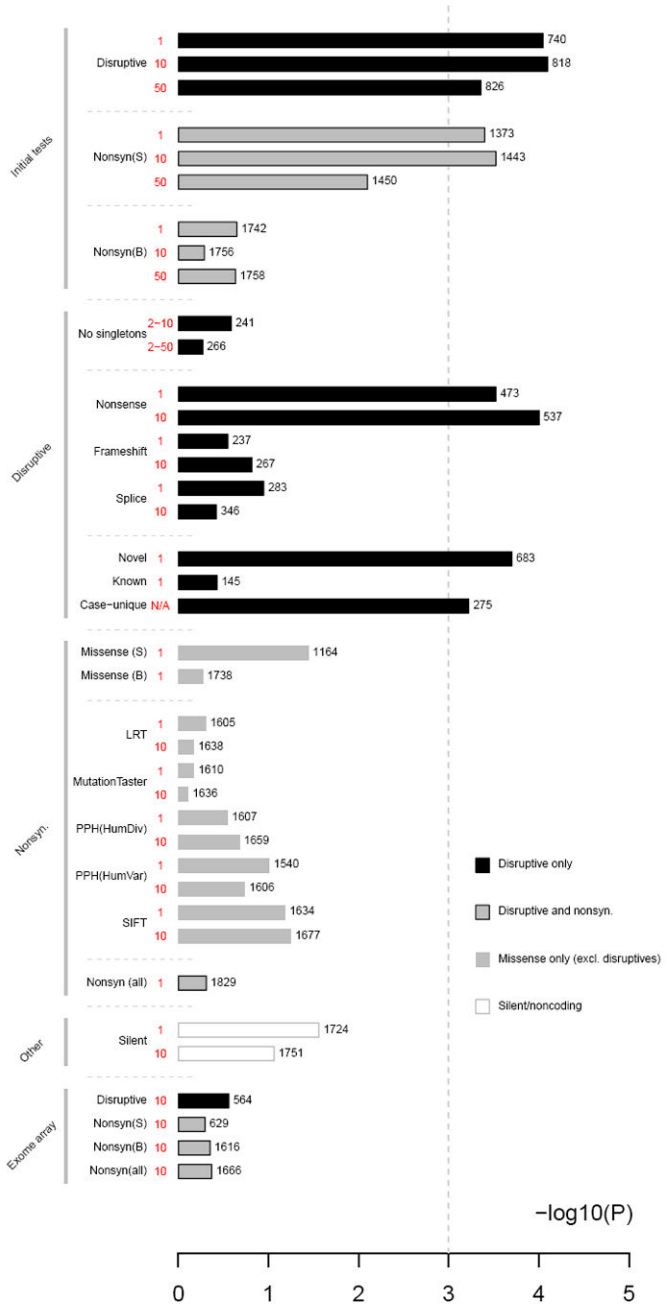


Figure 1. Composite set geneset analysis, stratified by mutation type

Statistical significance (x-axis) for the composite gene set stratified by type and frequency of mutation and other variables. Numbers to the right of each bar represent the number of genes with at least one mutation in that category for the composite set. (S) represents strictly-defined damaging missenses; (B) broadly-defined group. For the exome array contrasts (in which ExomeChip sites were tested using the exome sequence calls), D represents disruptive mutations, NS all nonsynonymous mutations.

Table 1
Geneset analysis of primary schizophrenia candidate genesets

Enrichment test empirical *P*-values for rare (singleton; minor allele frequency (MAF) <0.1%; MAF < 0.5%) variants from disruptive, NS_{strict} and NS_{broad} sets. *P*-values represent the relative case enrichment compared to average exome-wide case/control difference. **Bolded** values are significant at $P^{corrected}<0.05$. Initial comparison corrects (based on the empirical distribution of minimum *P*-values) for the 9 correlated tests (top panel). The lower panel focuses on the 12 subsets of the primary geneset, for disruptive variants only as they showed the greatest enrichment for the entire primary set. Again, **bold** values are significant after correcting for the 36 tests performed.

Variant type	Geneset/subset	*N* genes	Singletons	MAF < 0.1%	MAF < 0.5%
Disruptive			**0.0008**	**0.0001**	**0.0002**
Nonsyn (strict)	Primary	2,546	0.0059	**0.0015**	0.0110
Nonsyn (broad)			0.0986	0.1295	0.1126
Disruptive	*SCZ de novo genes*				
	Exome sequencing (disruptive)	87	0.0319	**0.0007**	**0.0003**
	Exome sequencing (nonsyn)	611	0.0053	**0.0011**	0.0055
	Copy number variants				
	de novo CNV genes (Kirov et al, 2012)	234	0.0234	0.0039	0.0124
	SCZ-associated CNV genes	345	0.3308	0.4596	0.4376
	GWAS				
	Voltage-gated calcium channel genes	26	**0.0019**	0.0214	0.0212
	Common SNPs (P < 1e-4 intervals)	479	0.1794	0.0368	0.0037
	miRNA-137 targets	446	0.6573	0.5609	0.4747
	Synaptic genes				
	PSD (human core)	685	0.0808	0.1154	0.1256
	ARC	28	**0.0016**	**0.0014**	**0.0014**
	NMDAR network	61	0.0158	0.0251	0.0252
	PSD-95	65	**0.0017**	**0.0009**	**0.0010**
	mGluRS	39	0.1327	0.0900	0.0902

Table 2

Geneset analysis of secondary autism/ID candidate genesets

Enrichment test empirical *P*-values for the secondary (autism/ID) geneset. As in Table 2, the top panel shows uncorrected P-values; tests significant after multiple test correction are in bold (i.e. all $P^{corrected}$ >0.05). Because no class of variant is significant after multiple test correction for the omnibus test (top panel), we applied and corrected for all 108 tests (9 conditions by 12 subsets) in the lower panel. The single category FMRP targets (Darnell et al.) mainly reflects disruptive and NS_{strict} singleton enrichment.

Variant type	Geneset/subset	*N* genes	Singletons	MAF < 0.1%	MAF < 0.5%
Disruptive			0.029	0.043	0.049
Nonsyn (strict)	Autism/ID	2,507	0.052	0.008	0.013
Nonsyn (broad)			0.532	0.619	0.287
		***N* genes**	**Min. $p^{corrected}$ (for 9×12=108 tests)**		
Disruptive, nonsyn(strict), & nonsyn(broad)	*De novo genes (exome sequencing)*				
	Autism (disruptive)	128	1.000		
	Autism (nonsyn)	743	1.000		
	ID (disruptive)	30	0.070		
	ID (nonsyn)	132	0.995		
	Neurodevelopmental candidates				
	Betancur (2011), ASD candidates	112	1.000		
	Betancur (2011), ID candidates	196	1.000		
	Autism PPI networks				
	O'Roak et al (2012), CHD8 network	6	1.000		
	O'Roak et al (2011), 49-gene network	49	1.000		
	O'Roak et al (2012), 74-gene network	74	1.000		
	Fragile × mental retardation protein targets				
	Darnell et al. (2012) targets	788	**0.010**		
	Ascano et al. (2012) targets	939	0.997		
	Ascano et al. (2012) FMRP/autism overlap	93	0.993		