The contribution of rare variants to risk of schizophrenia in individuals with and without intellectual disability

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Abstract

By meta-analyzing rare coding variants in whole-exome sequences of 4,133 schizophrenia cases and 9,274 controls, de novo mutations in 1,077 trios, and copy number variants from 6,882 cases and 11,255 controls, we show that individuals with schizophrenia carry a significant burden of rare damaging variants in 3,488 genes previously identified as having a near-complete

depletion of loss-of-function variants. In schizophrenia patients who also have intellectual disability, this burden is concentrated in risk genes associated with neurodevelopmental disorders. After excluding known neurodevelopmental disorder risk genes, a significant rare variant burden persists in other loss-of-function intolerant genes, and while this effect is notably stronger in schizophrenia patients with intellectual disability, it is also seen in patients who do not have intellectual disability. Together, our results show that rare damaging variants contribute to the risk of schizophrenia both with and without intellectual disability, and support an overlap of genetic risk between schizophrenia and other neurodevelopmental disorders.

Introduction

Schizophrenia is a common and debilitating psychiatric illness characterized by positive symptoms (hallucinations, delusions, disorganized speech and behaviour), negative symptoms (social withdrawal and diminished emotional expression), and cognitive impairment that result in social and occupational dysfunction[1,2]. Operational diagnostic criteria for the disorder as described in the DSM-V require the presence of at least two of the core symptoms over a period of six months with at least one month of active symptoms[3]. It is increasingly recognized that current categorical psychiatric classifications have a number of shortcomings, in particular that they overlook the increasing evidence for etiological and mechanistic overlap between psychiatric disorders[4].

A diverse range of pathophysiological processes may contribute to the clinical features of schizophrenia[5]. Indeed, previous studies have suggested a number of hypotheses about schizophrenia pathogenesis, including abnormal pre-synaptic dopaminergic activity[6], postsynaptic mechanisms involved in synaptic plasticity[7], dysregulation of synaptic pruning[8], and disruption to early brain development[9,10]. This complexity is underpinned by the varied nature of genetic contributions to risk of schizophrenia. Genome-wide association studies have identified over 100 independent loci defined by common (minor allele frequency [MAF] > 1%) single nucleotide variants (SNVs)[11], and a recent analysis determined that more than 71% of all one-megabase regions in the genome contain at least one common risk allele[12]. The modest effects of these variants (median odds ratio [OR] = 1.08) combine to produce a polygenic contribution that explains only a fraction (h_g^2 = 0.274) of the overall liability[12]. In addition, a number of rare variants have been identified that have far larger effects on individual risk. These are best exemplified by eleven large, rare recurrent copy number variants (CNVs) but evidence from whole-exome sequencing studies implies that many other rare coding SNVs and *de novo* mutations also confer substantial individual risk[13–17]. There is growing evidence that some of the same genes and pathways are affected by both common and rare variants[7,18]. Pathway analyses of common variants and hypothesis-driven gene set analyses of rare variants have begun to enumerate some of these specific biological processes, including histone methylation, transmission at glutamatergic synapses, calcium channel signaling, synaptic plasticity, and translational regulation by the fragile X mental retardation protein (FMRP)[11,13,14,19,20].

In addition to exploring the biological mechanisms underlying schizophrenia, genetic analyses can also be used to understand its relationship to other neuropsychiatric and neurodevelopmental disorders. For instance, schizophrenia, bipolar disorder, and autism (ASD) show substantial sharing of common risk variants[21,22]. Sequencing studies of neurodevelopmental disorders suggest that this sharing of genetic risk may extend to rare variants of large effect. In the largest sequencing study of ASD to date, 20 of the 46 genes and all six CNVs implicated (false discovery rate [FDR] < 5%) had been previously described as dominant causes of developmental disorders[23]. Furthermore, an analysis of 60,706 whole exomes led by the ExAC consortium identified 3,230 genes with near-complete depletion of protein-truncating variants, and *de novo* loss-of-function (LoF) mutations identified in individuals with ASD or developmental disorders were concentrated in this set of "LoF intolerant" genes[23–25]. Similarly, evidence from rare variants for a broader shared genetic etiology between schizophrenia and neurodevelopmental disorders has begun to emerge. Analyses of whole-exome data provided support for an enrichment of schizophrenia rare variants in intellectual disability genes, and schizophrenia cases were also found to have a higher concentration of ultra-rare disruptive SNVs in the ExAC LoF intolerant genes compared to controls[13,17,26].

However, the contribution of these rare variants to risk in the wider population of individuals diagnosed with schizophrenia, including those without intellectual disability, remains unclear. Intriguingly, the 11 rare CNVs found to be highly penetrant for schizophrenia also increased risk for intellectual disability and other congenital defects[16,27], and more recently, a meta-analysis of whole-exome sequence data showed that LoF variants in *SETD1A* conferred substantial risk for both schizophrenia and neurodevelopmental disorders[18]. Concurrent analyses of autism whole-exome data found that *de novo* loss-of-function (LoF) mutations identified in ASD probands, particularly those that disrupt genes associated with neurodevelopmental disorders, were disproportionately found in individuals with intellectual disability[23,28]. These emerging results raise the possibility that rare schizophrenia risk variants may be concentrated in a subset of schizophrenia patients with co-morbid intellectual disability. Here, we present the one of the largest accumulation of schizophrenia rare variant data to date, which we jointly analyze with phenotype data on cognitive function. Using this data set, we attempt to identify groups of genes disrupted by schizophrenia rare risk variants, and determine if a subset of patients disproportionately carry these damaging alleles.

Results

Study design

To maximize our power to detect enrichment of damaging variants in schizophrenia cases in groups of genes, we performed a meta-analysis of three different types of rare coding variant studies: (1) high-quality SNV calls from whole-exome sequences of 4,133 schizophrenia cases and 9,274 matched controls, (2) *de novo* mutations identified in 1,077 schizophrenia parent-proband

143 trios (Figure 1), and (3) CNV calls from genotyping array data of 6,882 cases and
144 11,255 controls. The ascertainment of these samples, data production, and
145 quality control were described previously[18,29]. All *de novo* mutations included in
146 our analysis had been validated through Sanger sequencing, and stringent
147 quality control steps were performed on the case-control data to ensure that
148 sample ancestry and batch were closely matched between cases and controls
149 (Online Methods).
150
151 For each data type, we used appropriate methods to test for an excess of
152 rare variants (Figure 1, Online Methods). In analyses of case-control SNV data,
153 we applied an extension of the variant threshold burden test that corrected for
154 exome-wide differences between cases and controls[30]. We tested all allele
155 frequency thresholds below 0.1% observed in our data, and assessed statistical
156 significance by permutation testing. In analyses of *de novo* SNV data, we
157 compared the observed number of *de novo* mutations to random samples from
158 an expected distribution based on a gene-specific mutation rate model to
159 calculate an empirical *P*-value. For both types of whole-exome sequencing data,
160 we restricted our analyses to loss-of-function variants. Finally, in analyses of
161 case-control CNV data, we used a logistic regression framework that compares
162 the rate of CNVs overlapping a specific gene set while correcting for differences
163 in CNV size and number of genes disrupted[7,19,31]. To ensure our model was well
164 calibrated, we restricted our analyses to small deletions and duplications
165 overlapping fewer than seven genes with MAF < 0.1% (Supplementary Figure 1,
166 Online Methods).
167
168 We tested for an excess of rare damaging variants in schizophrenia
169 patients in 1,766 gene sets (Online Methods, Supplementary Table 1, and
170 detailed results below). Gene set *P*-values were computed using the three
171 methods and variant definitions described above, and then meta-analyzed using
172 Fisher's Method to provide a single *P*-value for each gene set. Because we gave
173 each data type equal weight, gene sets achieving significance typically show at
174 least some signal in all three types of data. We observed a marked inflation in the
175 quantile-quantile (Q-Q) plot of gene set *P*-values (Supplementary Figure 2), so
176 we conducted two analyses to ensure our results were robust and not biased due
177 to methodological or technical artifacts. First, we observed no inflation of *P*-
178 values when testing for enrichment of synonymous variants in our case-control
179 and *de novo* analyses (Supplementary Figure 2). Second, we created random
180 gene sets by sampling uniformly across the genome, and observed null
181 distributions in Q-Q plots regardless of variant class and analytical method
182 (Supplementary Figure 3). These findings suggested that our methods
183 sufficiently corrected for known genome-wide differences in LoF and CNV
184 burden between cases and controls, and other technical confounders like batch
185 and ancestry.
186
187 **Rare, damaging schizophrenia variants are concentrated in LoF intolerant genes**
188
189 We first tested whether rare schizophrenia risk variants were
190 consistently concentrated in genes defined loss-of-function intolerant across
191 study design and variant type. Because some of our schizophrenia exome data

192 was included in the ExAC database, we focused on the subset of 45,376 ExAC
193 exomes without a known psychiatric diagnosis and that were not present in our
194 study. From this subset, 3,488 genes were found to have near-complete
195 depletion of such variants, which we defined as the LoF intolerant gene set. We
196 found that rare damaging variants in schizophrenia cases were enriched in LoF
197 intolerant genes ($P < 3.6 \times 10^{-10}$, Table 1, Figure 2), with support in case-control
198 SNVs ($P < 5 \times 10^{-7}$; OR 1.24, 1.16-1.31, 95% CI), case-control CNVs ($P =$
199 2.6×10^{-4}; OR 1.21, 1.15 – 1.28, 95% CI), and *de novo* mutations ($P = 6.7 \times 10^{-3}$;
200 OR 1.36, 1.1 – 1.68, 95% CI). While this result was consistent with observations
201 in intellectual disability and ASD[24,32] the absolute effect size is smaller (e.g. *de*
202 *novos*, Supplementary Figure 4 and 5). We observed no excess burden of rare
203 damaging variants in the remaining 14,753 genes (Figure 2, Supplementary
204 Figure 5). Furthermore, this signal was spread among many different LoF
205 intolerant genes: if we rank genes by decreasing significance, the enrichment
206 disappears in the case-control SNV analysis ($P > 0.05$) only after the exclusion of
207 the top 50 genes. This suggests that the contribution of damaging rare variants in
208 schizophrenia is not concentrated in just a handful of genes, but instead spread
209 across many genes.
210
211 **Schizophrenia risk genes are shared with other neurodevelopmental disorders**
212
213 Given the significant enrichment of rare damaging variants in LoF
214 intolerant genes in developmental disorders, autism and schizophrenia, we next
215 asked whether these variants affected the same genes. We found that autism
216 risk genes identified from exome sequencing meta-analyses[23] and genes in which
217 LoF variants are known causes of severe developmental disorders as defined by
218 the DDD study[33,34] were significantly enriched for rare variants in individuals
219 with schizophrenia ($P_{ASD} = 9.5\times 10^{-6}$; $P_{DD} = 2.3\times 10^{-6}$; Table 1, Online Methods).
220 Previous analyses have shown an enrichment of rare damaging variants in genes
221 whose mRNA are bound by FMRP in both schizophrenia and autism[35,13,32], so we
222 sought to identify further shared biology by testing targets of neural regulatory
223 genes previously implicated in autism[32,36]. We observed enrichment of both
224 such sets: promoter targets of *CHD8* ($P = 1.1\times 10^{-6}$) and splice targets of *RBFOX*
225 ($P = 1.3\times 10^{-5}$) (Table 1). We noted that some published gene lists attributed to
226 same biological process differed due to choices of assay, cell type, method of
227 sample extraction, and threshold of statistical significance, leading to distinct
228 results in our gene set analyses. For example, we observed a significant
229 enrichment in the published FMRP binding gene set based on mouse brain
230 data[37], but with no signal in one based on a human kidney cell line[38].
231
232 We also tested an additional 1,759 gene sets from databases of biological
233 pathways with at least 100 genes, as we lacked power to detect weak
234 enrichments in smaller sets (Online Methods). We observed enrichment of
235 damaging rare variants in schizophrenia cases at FDR $q < 0.05$ in 35 of these
236 gene sets (Supplementary Table 1, 2). These included previously implicated gene
237 sets, like the NMDA receptor and ARC complexes[13,14,35,37], as well as novel gene
238 sets, such as genes involved in cytoskeleton (GO: 0007010), chromatin
239 modification (GO:0016568), and chromatin organization (GO: 0006325).
240 Furthermore, the gene sets most significantly enriched (FDR $q < 0.01$) for

241 schizophrenia rare variants (Table 1) had all been previously linked to autism,
242 intellectual disability, and severe developmental disorders[23,32,33]. Our
243 enrichment results matched some of the findings from a pathway analysis of
244 common risk variants in psychiatric disorders, which also implicated neuronal
245 and chromatin gene sets[20]. However, unlike that study, we found no enrichment
246 of rare variants in immune-related gene sets.
247
248 We noticed that the 1,759 gene sets we tested were collectively enriched
249 with LoF intolerant genes when compared to a random sampling of genes from
250 the genome (Supplementary Figure 6 and 7). For some of the gene sets
251 associated with schizophrenia, this over-representation was quite substantial:
252 67% of the gene targets of FMRP and 74% of the genes associated with severe
253 neurodevelopmental disorders are LoF intolerant. To better understand the
254 consequences of this overlap on our results, we extended the gene set
255 enrichment methods (Online Methods) to condition on LoF intolerance and
256 brain-expression for the 35 gene sets with FDR $q < 0.05$ in the previous analysis
257 (Supplementary Table 2). We first observed that 22 of the 35 gene sets remained
258 significant even after conditioning on brain expression (Supplementary Tables 3,
259 Online Methods), suggesting they represent more specific biological processes
260 involved in schizophrenia. However, only known autism risk genes (P =
261 4.4×10^{-4}) and neurodevelopmental disorder genes ($P = 3\times 10^{-5}$) had an excess
262 of rare coding variants above the enrichment already observed in LoF intolerant
263 genes (Supplementary Table 3). Thus, in addition to biological pathways
264 implicated specifically in schizophrenia, at least a portion of the schizophrenia
265 risk conferred by rare variants of large effect is shared with childhood onset
266 disorders of neurodevelopment.
267
268 **Schizophrenia patients with intellectual disability have a greater burden of rare**
269 **damaging variants**
270
271 In autism spectrum disorders, the observed excess of rare damaging
272 variants has been shown to be greater in individuals with intellectual disability
273 than those with normal levels of cognitive function[28]. We observed a similar
274 phenomenon in schizophrenia cases carrying *SETD1A* LoF variants[18], so next
275 sought to explore whether this pattern is consistent in gene sets implicated in
276 schizophrenia. We acquired relevant cognitive phenotype data for 2,971 of the
277 4,131 schizophrenia patients with whole-exome sequencing data
278 (Supplementary Figure 8). Of these individuals, 279 were clinically diagnosed
279 with intellectual disability in addition to fulfilling the full diagnostic criteria for
280 schizophrenia (SCZ-ID subgroup, Online Methods). We also identified 1,165
281 individuals for whom we could rule out cognitive impairment (by excluding pre-
282 morbid IQ < 85, fewer than 12 years of schooling or lowest decile of composite
283 cognitive measures, depending on available data, Online Methods). Finally, we
284 identified 1,527 individuals who were not diagnosed with intellectual disability,
285 but in whom some cognitive impairment could not be excluded.
286
287 When stratifying into these three groups (intellectual disability, no
288 intellectual disability but cognitive impairment not excluded, no cognitive
289 impairment), we observed that the burden of rare damaging variants in LoF

290 intolerant genes was significantly greater in the SCZ-ID subgroup than in the
291 remaining schizophrenia cases ($P = 2.6 \times 10^{-4}$; OR 1.3, 1.12– 1.51, 95% CI) or
292 controls ($P < 5 \times 10^{-7}$; OR 1.61, 1.37 – 1.89, 95% CI; Figure 3). In the LoF
293 intolerant gene set, 0.27 (0.2 – 0.35, 95% CI) extra singleton (defined as having
294 an allele count of one in our data set) LoF variants were observed per exome in
295 SCZ-ID cases compared to controls, while 0.10 (0.065 – 0.13, 95% CI) extra
296 singleton LoF variants per exome were observed in the remaining schizophrenia
297 cases compared to controls (Online Methods). Furthermore, SCZ-ID individuals
298 had significant enrichment of rare LoF variants in developmental disorder genes
299 compared to the other cases ($P = 9 \times 10^{-4}$; OR 2.36, 1.41– 3.92, 95% CI) or to
300 controls ($P = 9.5 \times 10^{-6}$; OR 3.43, 2.01– 5.86, 95% CI; Figure 4). Compared to
301 controls, the SCZ-ID individuals carried 0.045 (0.03 – 0.06, 95% CI) extra
302 singleton LoF variants in developmental disorder genes per exome, suggesting
303 that around 4% of these cases had a LoF variant that is relevant to their clinical
304 presentation. No enrichment in neurodevelopmental disorder genes was
305 observed in schizophrenia patients without intellectual disability, suggesting
306 that these genes were relevant only for that subset of schizophrenia patients
307 (Figure 4, Supplementary Table 4). Notably, even after excluding known
308 developmental disorder genes from the set of LoF intolerant genes, we still
309 observed an enrichment of rare variants in SCZ-ID patients compared to the
310 remaining cases ($P = 1 \times 10^{-3}$; 1.26, 1.08 – 1.47, 95% CI) or to controls (P
311 $< 5 \times 10^{-7}$; OR 1.54, 1.31– 1.81, 95% CI; Supplementary Figure 9). Rare variation
312 in these genes contributes more to disease risk in the subset of patients with
313 both schizophrenia and intellectual disability.
314
315 **Rare variants confer risk for schizophrenia in individuals without intellectual**
316 **disability**
317
318 While rare damaging variants in LoF intolerant genes were most enriched
319 in the subset of schizophrenia patients with intellectual disability, we still
320 observed a weaker but significant enrichment in individuals with schizophrenia
321 for whom we could confirm do not have intellectual disability ($P = 5.5 \times 10^{-4}$;
322 1.16, 1.05 – 1.27, 95% CI; Figure 3). Therefore, rare risk variants for
323 schizophrenia follow the pattern previously described in autism: concentrated in
324 individuals with intellectual disability, but not exclusive to that group. To
325 produce a more accurate estimate of the effect of damaging rare variants on
326 schizophrenia conditional on their effects on overall cognition, we recalculated
327 the enrichment of rare variants in LoF intolerant genes in a subset of 2,161
328 schizophrenia cases and 2,398 controls for which data on years of education was
329 available and for whom intellectual disability could be excluded (Supplementary
330 Figure 8). After controlling for differences in educational attainment (Online
331 Methods), individuals with schizophrenia have a 1.26-fold excess of rare variants
332 in LoF intolerant genes ($P = 2 \times 10^{-6}$; 1.14 – 1.38, 95% CI). This increase in our
333 observed odds ratio is consistent with previous accounts that rare damaging
334 variants also affect educational attainment in controls[39], thus biasing our
335 unconditional estimate.

336 **Discussion**
337

338 Our integrated analysis of thousands of whole-exome sequences
339 demonstrates that rare damaging variants increase risk of schizophrenia both
340 with and without co-morbid intellectual disability. While the identification of
341 individual genes remains difficult at current samples sizes, we show that the
342 burden of damaging *de novo* mutations, rare SNVs and CNVs in schizophrenia is
343 not scattered across the genome but is primarily concentrated in 3,488 genes
344 intolerant of loss-of-function variants. This observation is shared with autism,
345 intellectual disability, and severe neurodevelopmental disorders[32,40]. We
346 recapitulate enrichment in previously published gene sets, including
347 transmission at glutamatergic synapses and translational regulation by FMRP,
348 and implicate other gene sets previously linked to autism, intellectual disability,
349 and severe developmental disorders. However, we find that all of these gene sets
350 share a large number of underlying genes, and are especially enriched with the
351 3,488 genes intolerant of LoF variants. These overlaps among gene sets
352 originating from very different analyses, as well as the subtleties of how they are
353 defined, suggest caution in interpreting biological explanations from observed
354 enrichments.
355
356 We jointly analyzed the case-control SNV data with information on
357 cognitive function for 2,971 patients, and find that LoF variants disrupting genes
358 associated with severe developmental disorders are disproportionately found in
359 individuals with schizophrenia with co-morbid intellectual disability, with 4% of
360 these cases having a single LoF variant that is relevant to their clinical
361 presentation. Even after excluding variants in known developmental disorder
362 genes, rare variants contribute a greater degree to schizophrenia risk in the SCZ-
363 ID subgroup of patients than the remaining schizophrenia population. These
364 results show that some of these genetic perturbations have clear manifestations
365 in childhood, and that rare risk variants in schizophrenia are particularly
366 associated with co-morbid intellectual disability. Our observations are consistent
367 with results in autism in which rare risk variants are associated with intellectual
368 disability[22,23,28]. Notably, a weaker but still significant rare variant burden was
369 observed in schizophrenia patients without cognitive impairment, and this signal
370 persists even after controlling for educational attainment. Together, these results
371 demonstrate that rare variants have different contributions to schizophrenia risk
372 depending on the degree of cognitive impairment. Importantly, they do not
373 simply confer risk for a small subset of patients but contribute to disease
374 pathogenesis more broadly.
375
376 Our study supports the observation that genetic risk factors for
377 psychiatric and neurodevelopmental disorders do not follow clear diagnostic
378 boundaries. Coding variants disrupting the same genes, and quite possibly, the
379 same biological processes, increase risk for a range of phenotypic manifestation.
380 This clinically variable presentation is reminiscent of LoF variants in *SETD1A*
381 and 11 large copy number variant syndromes, previously shown to confer risk
382 for schizophrenia in addition to other prominent developmental defects[16,18]. It is
383 possible that these genes contain an allelic series of variants conferring
384 gradations of risk. A recent schizophrenia GWAS meta-analysis demonstrated
385 that the common variant association signal was similarly enriched in LoF
386 intolerant genes[41], suggesting that schizophrenia risk genes may be perturbed by

common variants of subtle effects and disrupted by rare variants of high penetrance in the population. This possibility is also supported by the overlap in at least some of the pathways affected by both rare and common variation, such as chromatin remodeling. However, the most common deletion in the 22q11.2 locus and a recurrent two base deletion in *SETD1A* are associated with both schizophrenia and more severe neurodevelopmental disorders, suggesting the same variants can also confer risk for a range of clinical features[18,42,43]. Ultimately, it may prove difficult to clearly partition patients genetically into subtypes with similar clinical features, especially if genes and variants previously thought to cause well-characterized Mendelian disorders can have such varied outcomes. This pattern is consistent with the hypothesis that LoF variants in genes under genic constraint result in a spectrum of neurodevelopmental outcomes with the burden of mutations highest in intellectual disability and least in schizophrenia, corresponding to a gradient of neurodevelopmental pathology indexed by the degree of cognitive impairment, age of onset, and severity[4].

Despite the complex nature of genetic contributions to risk of schizophrenia, it is notable that across study design (trio or case-control) and variant class (SNVs or CNVs), risk loci of large effect are concentrated in a small subset of genes. Previous rare variant analyses in other neurodevelopmental disorders, such as autism, have successfully integrated information across *de novo* SNVs and CNVs to identify novel risk loci[23]. As sample sizes increase, meta-analyses leveraging the shared genetic risk across study designs and variant types, including those we did not consider here, such as classical recessive inheritance, will be similarly well powered to identify additional risk genes in schizophrenia.

Acknowledgements

We gratefully thank all participants in these studies. We thank Timi Touloupoulou, Marco Picchioni, Chiara Nosarti, Fiona Gaughran, and Oliver Howes for contributing clinical data used in this study. The UK10K project was funded by Wellcome Trust grant WT091310. The INTERVAL sequencing studies are funded by Wellcome Trust grant WT098051. T.S. is supported by the Williams College Dr. Herchel Smith Fellowship. A.P. is supported by Academy of Finland grants 251704 and 286500, NIMH U01MH105666 and the Sigrid Juselius Foundation. The work at Cardiff University was funded by Medical Research Council (MRC) Centre (G0801418) and Program Grants (G0800509). P.F.S. gratefully acknowledges support from the Swedish Research Council (Vetenskapsrådet, award D0886501). Creation of the Sweden schizophrenia study data was supported by NIMH R01 MH077139 and the Stanley Center of the Broad Institute. Participants in INTERVAL were recruited with the active collaboration of NHS Blood and Transplant England, which has supported fieldwork and other elements of the trial. DNA extraction and genotyping was funded by the National Institute of Health Research (NIHR), the NIHR BioResource and the NIHR Cambridge Biomedical Research Centre. The academic coordinating centre for INTERVAL was supported by core funding from: NIHR Blood and Transplant Research Unit in Donor Health and Genomics,

UK Medical Research Council (G0800270), and British Heart Foundation (SP/09/002). We would like to acknowledge the contribution of data from outside sources: (i) Genetic Architecture of Smoking and Smoking Cessation accessed through dbGAP: Study Accession: phs000404.v1.p1. Funding support for genotyping, which was performed at the Center for Inherited Disease Research (CIDR), was provided by 1 X01 HG005274-01. CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C. Assistance with genotype cleaning, as well as with general study coordination, was provided by the Gene Environment Association Studies (GENEVA) Coordinating Center (U01 HG004446). Funding support for collection of datasets and samples was provided by the Collaborative Genetic Study of Nicotine Dependence (COGEND; P01 CA089392) and the University of Wisconsin Transdisciplinary Tobacco Use Research Center (P50 DA019706, P50 CA084724). (ii). High-Density SNP Association Analysis of Melanoma: Case–Control and Outcomes Investigation, dbGaP Study Accession: phs000187.v1.p1. Research support to collect data and develop an application to support this project was provided by 3P50CA093459, 5P50CA097007, 5R01ES011740 and 5R01CA133996. (iii) Genetic Epidemiology of Refractive Error in the KORA Study, dbGaP Study Accession: phs000303.v1.p1. Principal investigators: Dwight Stambolian, University of Pennsylvania, Philadelphia, PA, USA; H. Erich Wichmann, Institut für Humangenetik, Helmholtz-Zentrum München, Germany, National Eye Institute, National Institutes of Health, Bethesda, MD, USA. Funded by R01 EY020483, National Institutes of Health, Bethesda, MD, USA. (iv) WTCCC2 study: Samples were downloaded from https://www.ebi.ac.uk/ega/ and include samples from the National Blood Donors Cohort, EGAD00000000024 and samples from the 1958 British Birth Cohort, EGAD00000000022. Funding for these projects was provided by the Wellcome Trust Case Control Consortium 2 project (085475/B/08/Z and 085475/Z/08/Z), the Wellcome Trust (072894/Z/03/Z, 090532/Z/09/Z and 075491/Z/04/B) and NIMH grants (MH 41953 and MH083094).

Author contributions

T.S., J.C.B conceived and designed the experiments.
T.S performed the statistical analysis.
T.S., J.T.R.W., M.J., D.C., J.S., M.T., E.R., P.F.S analysed the data.
T.S., J.T.R.W., M.J., J.S., M.T., E.R., C.I., D.B., A.M.M., G.K., D.G., R.M.M., M.D.F., E.B., M.G., C.M.H., P.S., A.P., M.C.O., M.J.O., J.C.B contributed reagents/materials/analysis tools.
T.S., D.C., M.J.O., J.C.B wrote the paper

Competing financial interests statement

We have no competing financial interests to declare.

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589 **Figure captions**

590

591 **Figure 1:** Analysis workflow. Data sets are shown in blue, statistical methods
592 and analysis steps are shown in green, and results (figures and tables) from the
593 analysis are shown in orange. **A:** Enrichment analyses in 1,766 gene sets using
594 the entire rare variant data set. **B:** Enrichment analyses in LoF intolerant and
595 developmental disorder genes in the subset of cases with information on
596 cognitive function. ID: intellectual disability; SCZ: schizophrenia; SCZ-ID:
597 schizophrenia patients with intellectual disability.

598 **Figure 2:** Enrichment of schizophrenia rare variants in genes intolerant of loss-
599 of-function variants. **A:** Schizophrenia cases compared to controls for rare SNVs
600 and indels; **B:** Rates of *de novo* mutations in schizophrenia probands compared
601 to control probands; **C:** Case-control CNVs. *P*-values shown were from the test of
602 LoF enrichment in **A**, LoF enrichment in **B**, and all CNVs enrichment in **C**. Error
603 bars represent the 95% CI of the point estimate. LoF intolerant: 3,448 genes with
604 near-complete depletion of truncating variants in the ExAC database; Rest: the
605 remaining genes in the genome with pLI < 0.9; Damaging missense: missense
606 variants with CADD phred > 15. Asterisk: $P < 1 \times 10^{-3}$.
607

608 **Figure 3:** Enrichment of rare loss-of-function variants in LoF intolerant genes in
609 schizophrenia cases stratified by information on cognitive function compared to
610 controls. The *P*-values shown were calculated using the variant threshold
611 method comparing LoF burden between the corresponding cases and controls.
612 Error bars represent the 95% CI of the point estimate. Damaging missense:
613 missense variants with CADD phred > 15.
614

615 **Figure 4:** Enrichment of rare loss-of-function variants in known severe
616 developmental disorder genes in schizophrenia cases stratified by information
617 on cognitive function compared to controls. The *P*-values shown were calculated
618 using the variant threshold method comparing LoF burden between the
619 corresponding cases and controls. Error bars represent the 95% CI of the point
620 estimate. Damaging missense: missense variants with CADD phred > 15.
621

Name	N_{genes}	Est_{SNV}	95% CI of Est_{SNV}	P_{SNV}	Est_{DNM}	95% CI of Est_{DNM}	P_{DNM}	Est_{CNV}	95% CI of Est_{CNV}	P_{CNV}	P_{meta}	Q_{meta}
ExAC LoF intolerant genes (pLI > 0.9)	3488	1.24	1.16-1.31	$< 5.0 \times 10^{-7}$	1.36	1.1-1.68	0.0067	1.21	1.15-1.28	0.00026	$< 3.60 \times 10^{-10}$	4.30×10^{-7}
Dominant, diagnostic DDG2P genes, in which LoF variants result in developmental disorders with brain abnormalities	156	1.42	1.07-1.88	0.011	4.18	2.21-8.03	0.00073	1.92	1.54-2.39	0.0016	2.30×10^{-6}	0.00067
Sanders *et al.* autism risk genes (FDR < 10%)	66	1.28	0.97-1.69	0.0095	3.96	1.65-9.94	0.019	2.21	1.75-2.79	0.00033	9.50×10^{-6}	0.0017
Darnell *et al.* targets of FMRP	790	1.24	1.13-1.36	8.5×10^{-6}	1.31	0.83-2.09	0.17	1.32	1.2-1.47	0.0032	9.30×10^{-7}	0.00038
Cotney *et al.* CHD8-targeted promoters (hNSC and human brain tissue)	2920	1.09	1.02-1.16	0.0008	1.77	1.36-2.31	0.00025	1.11	1.05-1.18	0.027	1.10×10^{-6}	0.00038
G2CDB: mouse cortex post-synaptic density consensus	1527	1.20	1.11-1.3	2.5×10^{-6}	1.57	1.06-2.33	0.028	1.04	0.96-1.11	0.32	3.90×10^{-6}	0.00097
Weynvanhentenryck *et al.* CLIP targets of RBFOX	967	1.21	1.11-1.33	4.8×10^{-5}	1.84	1.21-2.8	0.0085	1.07	0.98-1.17	0.2	1.30×10^{-5}	0.002
NMDAR network (defined in Purcell *et al.)*	61	1.66	1.09-2.54	0.0061	5.60	2.06-16.09	0.017	2.46	1.78-3.4	0.0028	3.70×10^{-5}	0.0044
GOBP: chromatin modification (GO:0016568)	519	1.29	1.13-1.49	0.00018	2.26	1.32-3.94	0.0099	1.12	0.99-1.28	0.18	4.20×10^{-5}	0.0046

Table 1: Gene sets enriched for rare coding variants conferring risk for schizophrenia at FDR < 1%. The effect sizes and corresponding *P*-values from enrichment tests of each variant type (case-control SNVs, DNM, and case-control CNVs) are shown for each gene set, along with the Fisher's combined P-value (P_{meta}) and the FDR-corrected Q-value (Q_{meta}). We only show the most significant gene set if there are multiple ones from the same data set or biological process (see Supplementary Table 1 for all 1,766 gene sets). N_{genes}: number of genes in the gene set; Est: effect size estimate and its lower and upper bound assuming a 95% CI; DNM: *de novo* mutation.

627 ## Supplementary Table captions
628
629 **Supplementary Table 1:** Full results from enrichment analyses of 1,766 gene
630 sets. The *P*-values from enrichment tests of each variant type (case-control SNVs,
631 DNM, and case-control CNVs) are shown for each gene set, along with the
632 Fisher's combined P-value (P_{meta}) and the FDR-corrected Q-value (Q_{meta}). N_{genes}:
633 number of genes in the gene set; SNV: single nucleotide variants from whole-
634 exome data; DNM: *de novo* mutations.
635
636 **Supplementary Table 2:** Gene sets enriched for rare coding variants conferring
637 risk for schizophrenia at FDR < 5%. The effect sizes and corresponding *P*-values
638 from enrichment tests of each variant type (case-control SNVs, DNM, and case-
639 control CNVs) are shown for each gene set, along with the Fisher's combined P-
640 value (P_{meta}) and the FDR-corrected Q-value (Q_{meta}). N_{genes}: number of genes in
641 the gene set; Est: effect size estimate and its lower and upper bound assuming a
642 95% CI; SNV: single nucleotide variants from whole-exome data; DNM: *de novo*
643 mutations.
644
645 **Supplementary Table 3:** Results from enrichment analyses of FDR < 5% gene
646 sets, conditional on brain-expressed and ExAC LoF intolerant genes. We restrict
647 enrichment analyses to genes that reside in two different background gene sets,
648 one defined on brain-enriched expression in GTeX, and the second on genic
649 constraint (ExAC LoF intolerant genes), and determined if gene sets with FDR <
650 5% in the meta-analysis still had significance above the specific background. The
651 *P*-values from enrichment tests of each variant type (case-control SNVs, DNM,
652 and case-control CNVs) are shown for each gene set, along with the Fisher's
653 combined P-value (P_{meta}). SNV: single nucleotide variants from whole-exome
654 data; DNM: *de novo* mutations
655
656 **Supplementary Table 4:** Results from enrichment analyses of rare loss-of-
657 function variants in LoF intolerant genes and developmental disorder genes
658 comparing schizophrenia cases stratified by information on cognitive function
659 and matched controls. Each comparison is defined in the Table, and the *P*-values
660 shown were calculated using the variant threshold method comparing LoF
661 burden between the corresponding case and baseline samples. N_{case}: number of
662 case samples; $N_{comparison}$: number of comparison samples; Estimates: effect size
663 estimate and its lower and upper bound assuming a 95% CI.

664 ## Online Methods

665 ## Sample collections
666
667 The ascertainment, data production, and quality control of the
668 schizophrenia case-control whole-exome sequencing data set had been
669 described in detail in an earlier publication[18]. Briefly, the data set was composed
670 of schizophrenia cases recruited as part of eight collections in the UK10K
671 sequencing project, and matched population controls from non-psychiatric arms
672 of the UK10K project, healthy blood donors from the INTERVAL project, and five

673 Finnish population studies. The UK10K data set was combined and analyzed
674 with published data from a Swedish schizophrenia case-control study[35]. The data
675 production, quality control, and analysis of the case-control CNV data set was
676 described in an earlier publication[29]. The schizophrenia cases were recruited as
677 part of the CLOZUK and CardiffCOGS studies, which consisted of both
678 schizophrenia individuals taking the antipsychotic clozapine and a general
679 sample of cases from the UK. Matched controls were selected from four publicly
680 available non-psychiatric data sets. All samples were genotyped using Illumina
681 arrays, and processed and called under the same protocol. Sanger-validated *de*
682 *novo* mutations identified through whole exome-sequencing in seven published
683 studies of schizophrenia parent-proband trios were aggregated and re-annotated
684 for enrichment analyses[13,44–49]. A full description of each trio study, including
685 sequencing and capture technology and sample recruitment was previously
686 described[18].

687 **Sample and variant quality control**

688

689 We jointly called each case data set with its nationality-matched controls,
690 and excluded samples based on contamination, coverage, non-European
691 ancestry, and excess relatedness[18]. A number of empirically derived filters were
692 applied at the variant and genotype level, including filters on GATK VQSR,
693 genotype quality, read depth, allele balance, missingness, and Hardy-Weinberg
694 disequilibrium[18]. After variant filtering, the per-sample transition-to-
695 transversion ratio was ~3.2 across the entire data set, as expected for
696 populations of European ancestry[50]. For the case-control CNV analysis, we
697 similarly excluded samples based on excess relatedness, and only CNVs
698 supported by more than 10 probes and greater than 10 kilobases in size were
699 retained to ensure high quality calls. All *de novo* mutations in our study had been
700 validated using Sanger sequencing.

701

702 We used the Ensembl Variant Effect Predictor (VEP) version 75 to
703 annotate all variants (SNVs and CNVs) according to Gencode v.19 coding
704 transcripts. We defined frameshift, stop gained, splice acceptor, and donor
705 variants as loss-of-function (LoF), and missense or initiator codon variants with
706 the recommended CADD Phred score cut-off of greater than 15 as damaging
707 missense[51]. A gene was annotated as disrupted by a deletion if part of its coding
708 sequence overlapped the copy number event. We more conservatively defined
709 genes as duplicated only if the entire canonical transcript of the gene overlapped
710 with the duplication event.

711

712 Statistical tests of the case-control exome data used case-control
713 permutations within each population (UK, Finnish, Swedish) to generate
714 empirical *P*-values to test hypotheses. No genome-wide inflation was observed in
715 burden tests of individual genes[18]. In the curated set of *de novo* mutations, we
716 observed the expected exome-wide number of synonymous mutations given
717 gene mutation rates from previously validated models[24], suggesting variant
718 calling was generally unbiased across Gencode v.19 coding genes. Lastly, the
719 case-control CNV data set had been previously analyzed for burden of CNVs
720 affecting individual genes, and enrichment analyses in targeted gene sets[7,29].

721 ## Rare variant gene set enrichment analyses

722 **Case-control enrichment burden tests** For the case-control SNV data set, we
723 performed permutation-based gene set enrichment tests using an extension of
724 the variant threshold method[30]. This method assumed that variants with a MAF
725 below an unknown threshold T were more likely to be damaging than variants
726 with a MAF above T, and this threshold was allowed to differ for every gene or
727 pathway tested. To consider different possible values for threshold T, a gene or
728 gene set test statistic $t(T)$ was calculated for every allowable T, and the
729 maximum test-statistic, or $t_{\max}$, was selected. The statistical significance of $t_{\max}$
730 was evaluated by permuting phenotypic labels, and calculating $t_{\max}$ from the
731 permuted data such that different values of T could be selected following each
732 permutation. In Price *et al.*, $t(T)$ was defined as the z-score calculated from
733 regressing the phenotype on the sum of the allele counts of variants in a gene
734 with MAF $< T$. We extended this method to test for enrichment in gene sets by
735 regressing schizophrenia status on the total number of damaging alleles in the
736 gene set of interest with MAF $< T$ ($X_{in,T}$) while correcting for the total number of
737 damaging alleles genome-wide with MAF $< T$ ($X_{all,T}$). $X_{all,T}$ controlled for
738 exome-wide differences between schizophrenia cases and controls, ensuring any
739 significant gene set result was significant beyond baseline differences. $t(T)$ was
740 defined as the t-statistic testing if the regression coefficient of $X_{in,T}$ deviated
741 from 0. We then calculated $t(T)$ for all observed thresholds below a minor allele
742 frequency of 0.1%, and selected the maximum value for the $t_{\max}$ based on the
743 observed data. To calculate a null distribution for $t_{\max}$, we performed two
744 million case-control permutations within each population (UK, Finnish, and
745 Swedish) to control for batch and ancestry, and calculated $t_{\max}$ for each
746 permuted sample while allowing T to vary. The P-value for each gene set was
747 calculated as the fraction of the two million permuted samples that had a greater
748 $t_{\max}$ than what was observed in the unpermuted data. The odds ratio and 95%
749 confidence interval of each gene set was calculated using a logistic regression
750 model, regressing schizophrenia status on X_{in} while controlling for total number
751 of variants genome-wide (X_{all}) and population (UK, Finnish, and Swedish).
752 Unlike gene set P-values which were calculated using permutation across
753 multiple frequency thresholds, the odds ratios an d 95% CI were calculated using
754 only variants observed once in our data set (allele count of 1) to ensure they
755 were comparable between tested gene sets.

756 **CNV logistic regression** We adapted a logistic regression framework described in
757 Raychaudhuri *et al.* and implemented in PLINK to compare the case-control
758 differences in the rate of CNVs overlapping a specific gene set while correcting
759 for differences in CNV size and total genes disrupted[7,19,31]. We first restricted our
760 analyses to coding deletions and duplications, and tested for enrichment using
761 the following model:

762 $$\log\left(\frac{P_{i,\text{case}}}{1-P_{i,\text{case}}}\right) = \beta_0 + \beta_1 s_i + \beta_2 g_{\text{all}} + \beta_3 g_{\text{in}} + \epsilon,$$

763 where for individual i, p_i is the probability they have schizophrenia i, s_i is the
764 total length of CNVs, g_{all} is the total number of genes overlapping CNVs, and g_{in} is
765 the number of genes within the gene set of interest overlapping CNVs. It has been
766 shown that β_1 and β_2 sufficiently controlled for the genome-wide differences in

767 the rate and size of CNVs between cases and control, while β_3 captured the true
768 gene set enrichment above this background rate[7,19,31]. For each gene set, we
769 reported the one-sided *P*-value, odds ratio, and 95% confidence interval of β_3.

770 **Weighted permutation-based sampling of *de novo* mutations** For each variant
771 class of interest, we first determined the total number of *de novo* mutations
772 observed in the 1,077 schizophrenia trios. We then generated 2 million random
773 samples with the same number of *de novo* mutations, weighting the probability
774 of observing a mutation in a gene by its estimated mutation rate. The baseline
775 gene-specific mutation rates were obtained using the method described in
776 Samocha *et al.* and adapted to produce LoF and damaging missense rates for
777 each Gencode v.19 gene. These mutation rates adjusted for both sequence
778 context and gene length, and were successfully applied in the primary analyses
779 of large-scale exome sequencing of autism and severe developmental disorders
780 with replicable results[23,32,40]. For each gene set, one-sided enrichment *P*-values
781 were calculated as the fraction of two million random samples that had a greater
782 or equal number of *de novo* mutations in the gene set of interest than what is
783 observed in the 1,077 trios. The effect size of the enrichment was calculated as
784 the ratio between the number of observed mutations in the gene set of interest
785 and the average number of mutations in the gene set across the two million
786 random samples. We adapted a method in Fromer *et al*. to calculate 95% credible
787 intervals for the enrichment statistic[13]. We first generated a list of one thousand
788 evenly spaced values between 0 and ten times the point estimate of the
789 enrichment. For each value, the mutation rates of genes in the gene set of
790 interest were multiplied by that amount, and 50,000 random samples of *de novo*
791 mutations were generated using these weighted rates. The probability of
792 observing the number of mutations in the gene set of interest given each effect
793 size multiplier was calculated as the fraction of samples in which the number of
794 mutations in the gene set is the same as the observed number in the 1,077 trios.
795 We normalized the probabilities across the 1,000 values to generate a posterior
796 distribution of the effect size, and calculated the 95% credible interval using this
797 empirical distribution.
798
799 **Combined joint analysis** Gene set *P*-values calculated using the case-control SNV,
800 case-control CNV, and *de novo* data were meta-analyzed using Fisher's combined
801 probability method with *df* = 6 to provide a single test statistic for each gene set.
802 We corrected for the number of gene sets tested in the discovery analysis (n =
803 1,776) by controlling the false discovery rate (FDR) using the Benjamini-
804 Hochberg approach, and reported only results with a *q*-value of less than 5%.
805

806 ## Description of gene sets

807
808 The full list of tested gene sets is found in Supplementary Table 1, and a
809 detailed description is provided in the Supplementary Note. Briefly, we tested all
810 gene sets with more than 100 genes from five public pathway databases. We
811 additionally tested additional gene sets selected based on biological hypotheses
812 about schizophrenia risk, and genome-wide screens investigating rare variants
813 in intellectual disability, autism spectrum disorders, and other
814 neurodevelopmental disorders. All gene identifiers were mapped to the

815 GENCODE v.19 release, and all non-coding genes were excluded. A total of 1,766
816 gene sets were included in our analysis.

817 Selection of allele frequency thresholds and consequence severity

818

819 For the case-control whole-exome data, we applied an extension of the
820 variant threshold model (described above). With this method, we tested
821 damaging variants at a number of frequency thresholds without specifying an *a*
822 *priori* MAF cut-off. All thresholds below a MAF of 0.1% observed in our data
823 were tested, and we assessed statistical significance by permutation testing. For
824 all the whole-exome data (case-control and trio data), we restricted our analyses
825 to loss-of-function variants. These variants have a clear and severe predicted
826 functional consequence in that they putatively cause a single-copy loss of a gene.
827 Furthermore, this class of variants had been demonstrated to have the strongest
828 genome-wide enrichment between cases and controls across
829 neurodevelopmental and psychiatric disorders[18,32,40]. When selecting MAF cut-
830 offs for case-control CNVs, we found that while the bulk of the test statistics were
831 not inflated, the tail of gene set *P*-values were dramatically inflated even when
832 testing for enrichment in the random gene sets (Supplementary Figure 1). This
833 inflation in the tail of the Q-Q plot was driven in part by very large (overlapping
834 more than 10 genes), more common (MAF between 0.1% and 1%) CNVs
835 observed mainly in cases or controls. Some of these, such as the known
836 syndromic CNVs, likely harbored true risk genes. However, because these CNVs
837 were highly recurrent in cases and depleted in controls, and disrupted a large
838 number of genes, any gene set that included even a single gene within these
839 CNVs would appear to be significant, even after controlling for total CNV length
840 and genes overlapped. To ensure our model was well calibrated and its *P*-values
841 followed a null distribution for random gene sets, we explored different
842 frequency and size thresholds, and conservatively restricted our analysis to copy
843 number events overlapping less than seven genes (excluding the largest 10% of
844 CNVs) with MAF < 0.1% (Supplementary Figure 1). Our main conclusions
845 remained unchanged even if we selected a more stringent (excluding the largest
846 15% of CNVs) or less stringent (excluding the largest 5% of CNVs) size threshold.
847

848 Robustness of enrichment analyses

849

850 We uniformly sampled genes from the genome (as defined by Gencode
851 v.19) to generate random gene sets with the same size distribution as the 1,776
852 gene sets in our discovery analysis. For each random set, we calculated gene set
853 *P*-values for the case-control SNV data, case-control CNV data, and *de novo* data
854 using the appropriate method and frequency cut-offs across all variant classes. A
855 Q-Q plot was generated using *P*-values from enrichment tests of each data set
856 and variant type. Reassuringly, we observed null distributions in all such Q-Q
857 plots (Supplementary Figure 3).
858

859 Comparison of *de novo* enrichment with broader neurodevelopmental
860 disorders

861

We aggregated and re-annotated *de novo* mutations from four studies: 1,113 severe DD probands[40], 4,038 ASD probands[23,32], and 2,134 control probands[28,32]. We used the Poisson exact test to calculate differences in *de novo* rates in constrained genes between schizophrenia, ASD, and DD and controls. Counts in each functional class (synonymous, missense, damaging missense, and LoF) were tested separately, and the one-sided *P*-value, rate ratio, and 95% CI of each comparison were reported and plotted in Figure 2, Supplementary Figure 4 and 5.

Conditional analyses

In each of the three methods we used for gene set enrichment, we restricted all variants analyzed to those that reside in the background gene list, and tested for an excess of rare variants in genes shared between the gene set of interest (K) and the background list (B). Brain-enriched genes from GTEx, and the ExAC LoF intolerant genes (pLI > 0.9) were used as backgrounds (see above). For the case-control SNV data, we modified the variant threshold method to regress schizophrenia status on the total number of damaging alleles in genes present in both the gene set of interest and the background gene set ($K \cap B$), while correcting for the total number of damaging alleles in the set of all background genes (B). The logistic regression model for the case-control CNV data was modified to:

$$\log\left(\frac{P_{i,\text{case}}}{1-P_{i,\text{case}}}\right) = \beta_0 + \beta_1 s_i + \beta_2 g_B + \beta_3 g_{K \cap B} + \epsilon,$$

where g_B is the total number of background genes overlapping a CNV, and $g_{K \cap B}$ is the number of genes in the intersection of the gene set of interest and the background list overlapping a CNV. Finally, we determined the total number of *de novo* mutations within the background gene list observed in the 1,077 schizophrenia trios, and generated 2 million random samples with the same number of *de novo* mutations. For each gene set, one-sided enrichment *P*-values were calculated as the fraction of two million random samples that had a greater or equal number of *de novo* mutations in genes in $K \cap B$ than what is observed in the 1,077 trios. Gene set *P*-values were combined using Fisher's method. We restricted our conditional enrichment analysis to gene sets with *q*-value < 5% in the discovery analysis, and adjusted for multiple testing using Bonferroni correction (P = 0.00071, or 0.05/67 tests; see Supplementary Table 3).

Rare variants and cognition in schizophrenia

Within the UK10K study, 97 individuals from the MUIR collection were given discharge diagnoses of mild learning disability and schizophrenia (ICD-8 and -9). The recruitment guidelines of the MUIR collection were described in detail in a previous publication[52]. In brief, evidence of remedial education was a prerequisite to inclusion, and individuals with pre-morbid IQs below 50 or above 70, severe learning disabilities, or were unable to give consent were excluded. The Schizophrenia and Affective Disorders Schedule-Lifetime version (SADS-L) in people with mild learning disability, PANSS, RDC, and DSM-III-R, and St. Louis Criterion were applied to all individuals to ensure that any diagnosis of

908 schizophrenia was robust. Using the clinical information provided alongside the
909 Swedish and Finnish case-control data sets, we identified additional 182
910 schizophrenia individuals who were similarly diagnosed with intellectual
911 disability, for a total of 279 individuals.

912 Cognitive testing and educational attainment data available for a subset of
913 samples were used identify schizophrenia individuals without cognitive
914 impairment. For 502 individuals from the Cardiff collection in the UK10K study,
915 we acquired their pre-morbid IQ as extrapolated from National Adult Reading
916 Test (NART), and identified 412 individuals for analysis after excluding all
917 individuals with predicted pre-morbid IQ of less than 85 (or below one standard
918 deviation of the population distribution for IQ). We additionally acquired
919 information on educational attainment in 54 schizophrenia individuals in the
920 UK10K London collection, and retained 27 individuals without intellectual
921 disability and who completed at least 12 years of schooling. Lastly, the California
922 Verbal Learning Test was conducted on 124 Finnish schizophrenia individuals
923 sequenced as part of UK10K, and a composite score was generated from
924 measures of verbal and visual working memory, verbal abilities,
925 visuoconstructive abilities, and processing speed. All individuals with intellectual
926 disability had been excluded from cognitive testing. Within this set of samples,
927 we additionally excluded any individuals who ranked in the lowest decile in
928 CVLT composite score, and retained 92 individuals for analysis. According to
929 these criteria, we identified 531 of 697 schizophrenia individuals from the UK
930 and Finnish data sets with cognitive data as not having intellectual disability. We
931 additionally acquired data on educational attainment for the Swedish
932 schizophrenia cases and controls from the Swedish National Registry. After
933 excluding individuals with intellectual disability, we identified 1,527
934 schizophrenia individuals who did not complete secondary school (less than 12
935 years of schooling), and 634 schizophrenia individuals who completed at least
936 compulsory and upper secondary schooling (at least 12 years of schooling). The
937 last group with the greatest educational attainment and without intellectual
938 disability was defined as cases without cognitive impairment. In the Swedish
939 sample, 49.4% of control samples had lower educational attainment than the
940 634 individuals with schizophrenia defined as having no cognitive impairment,
941 suggesting that our definition was sufficiently strict. In total, combining the UK,
942 Finnish, and Swedish data, we identified 1,165 schizophrenia individuals without
943 cognitive impairment.

944 Using the variant threshold method, we tested for differences in rare LoF
945 burden between the three case groups (intellectual disability, did not complete
946 secondary school, no cognitive impairment) against controls. We restricted these
947 analyses to three gene sets (LoF intolerant genes, genes in which LoF variants
948 are diagnostic for severe developmental disorders, and LoF intolerant genes
949 after excluding severe developmental disorders genes), and adjusted for multiple
950 testing using Bonferroni correction (P = 0.0038, or 0.05/13 tests).
951 Supplementary Table 4 enumerated all the statistical tests performed. To
952 estimate the per-exome excess of rare singleton (defined as having an allele
953 count of one in our data set) LoF variants in cases compared to controls, we
954 regressed X_{in} (the number of LoF variants in the gene set of interest) on case
955 status (0 or 1) while controlling for X_{all} (the total number of LoF variants

genome-wide) and population (UK, Finnish, and Swedish). The effect size and 95% CI of the regression coefficient of case status predictor were reported.

Data Availability

Sequence data and processed VCFs for the UK10K project were deposited into the European Genome-phenome Archive (EGA) under study accession code EGAO00000000079. The processed VCFs from the Swedish case-control study were deposited in dbGAP under accession code (phs000473.v1.p1). Rare variant counts, and gene-level association results from combining the whole-exome sequencing data sets were described in a previous publication[18] and were made available on the PGC results and download page (https://www.med.unc.edu/pgc/results-and-downloads).

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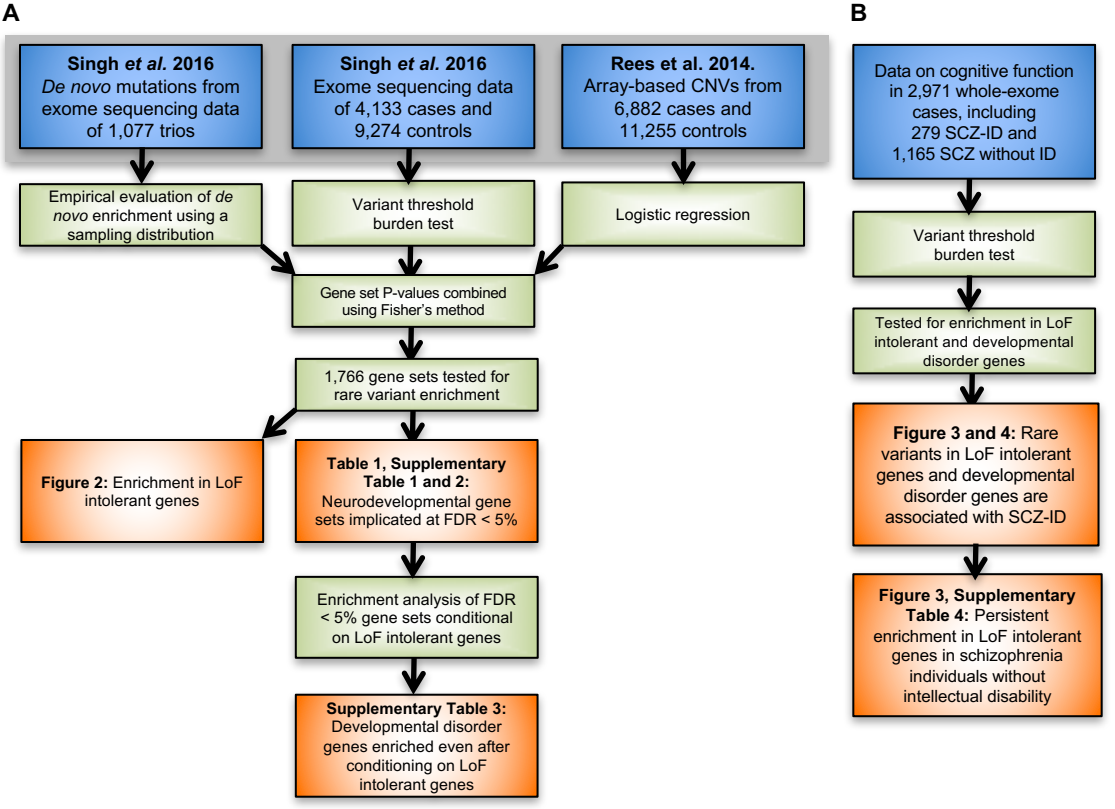
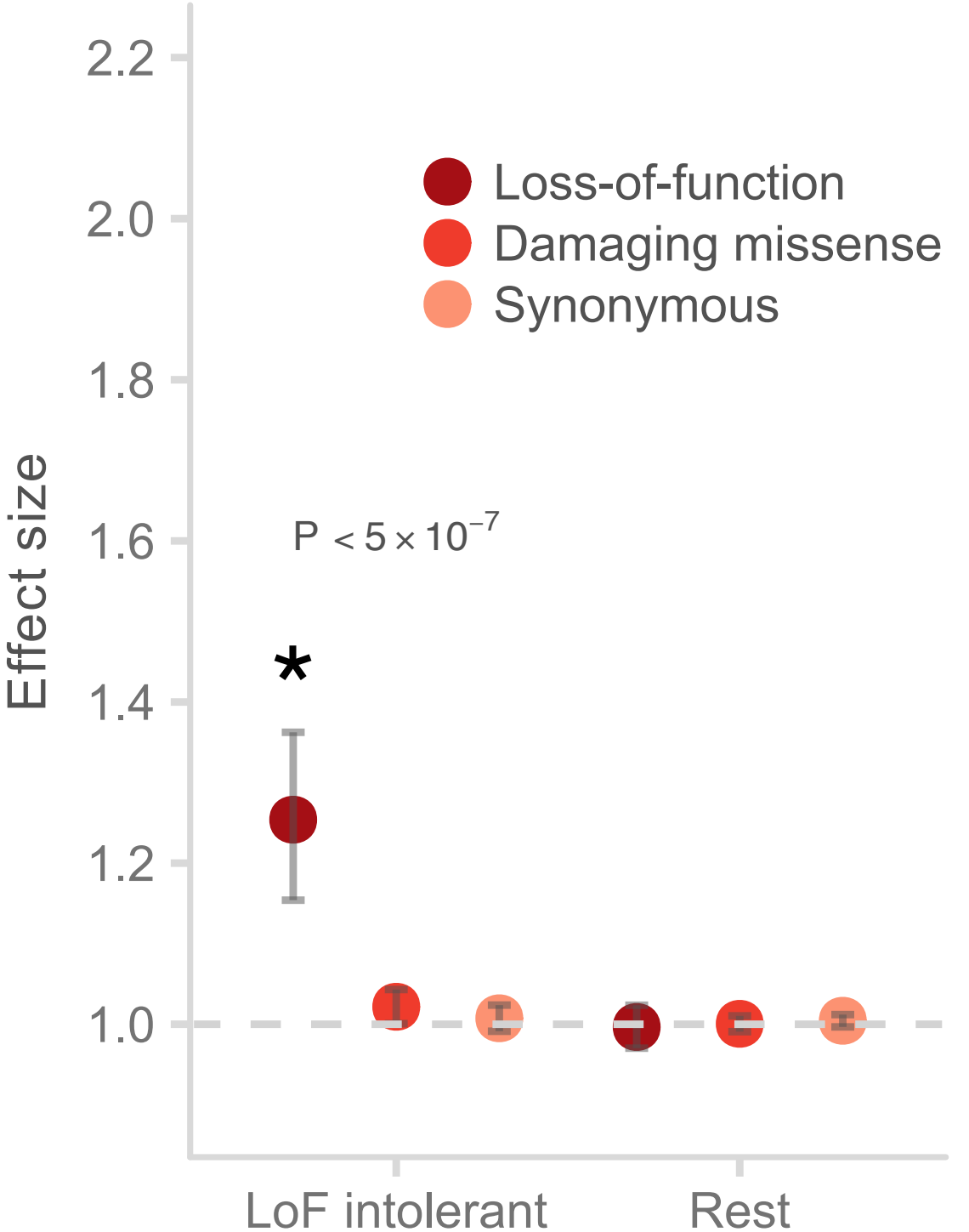
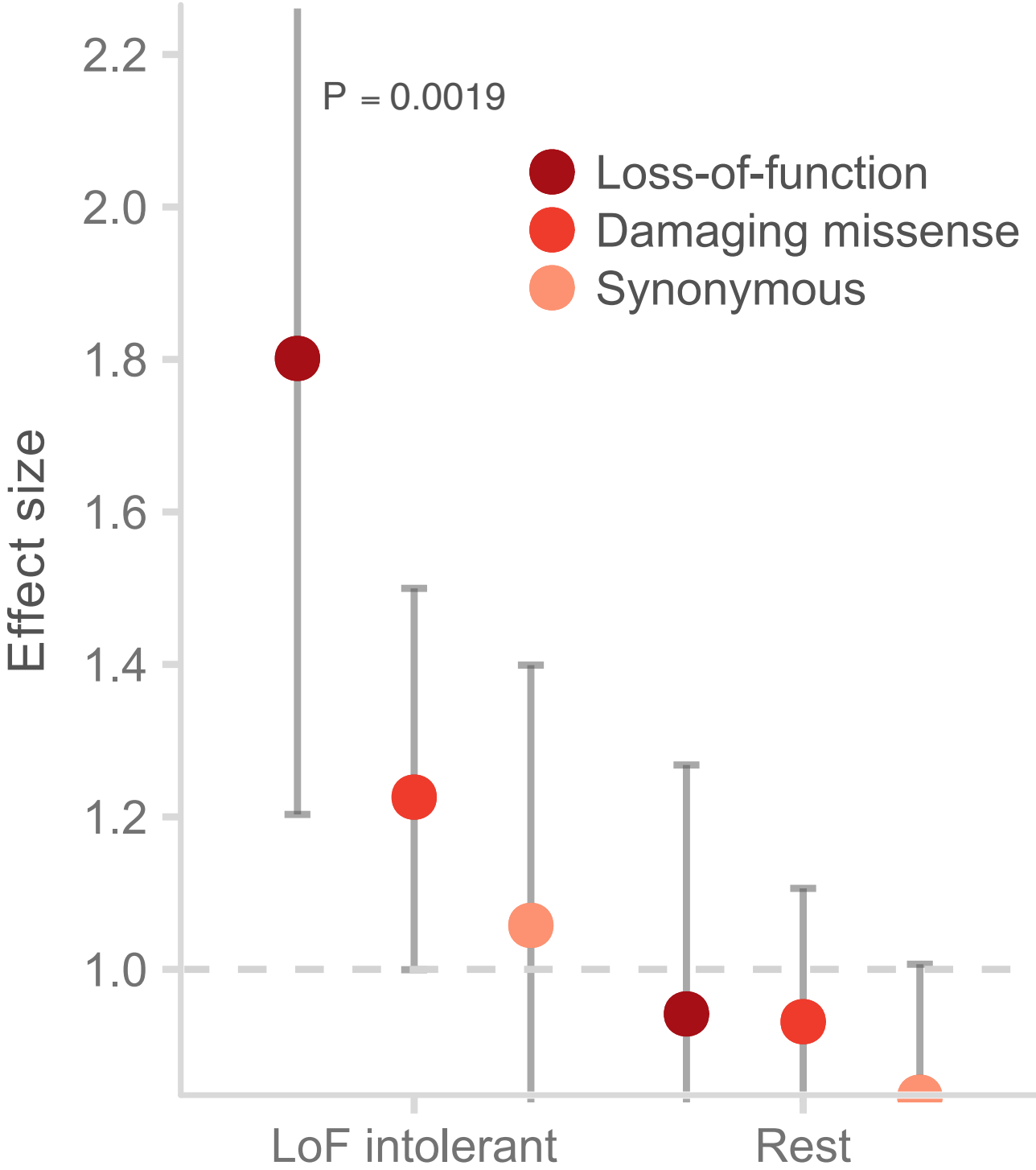
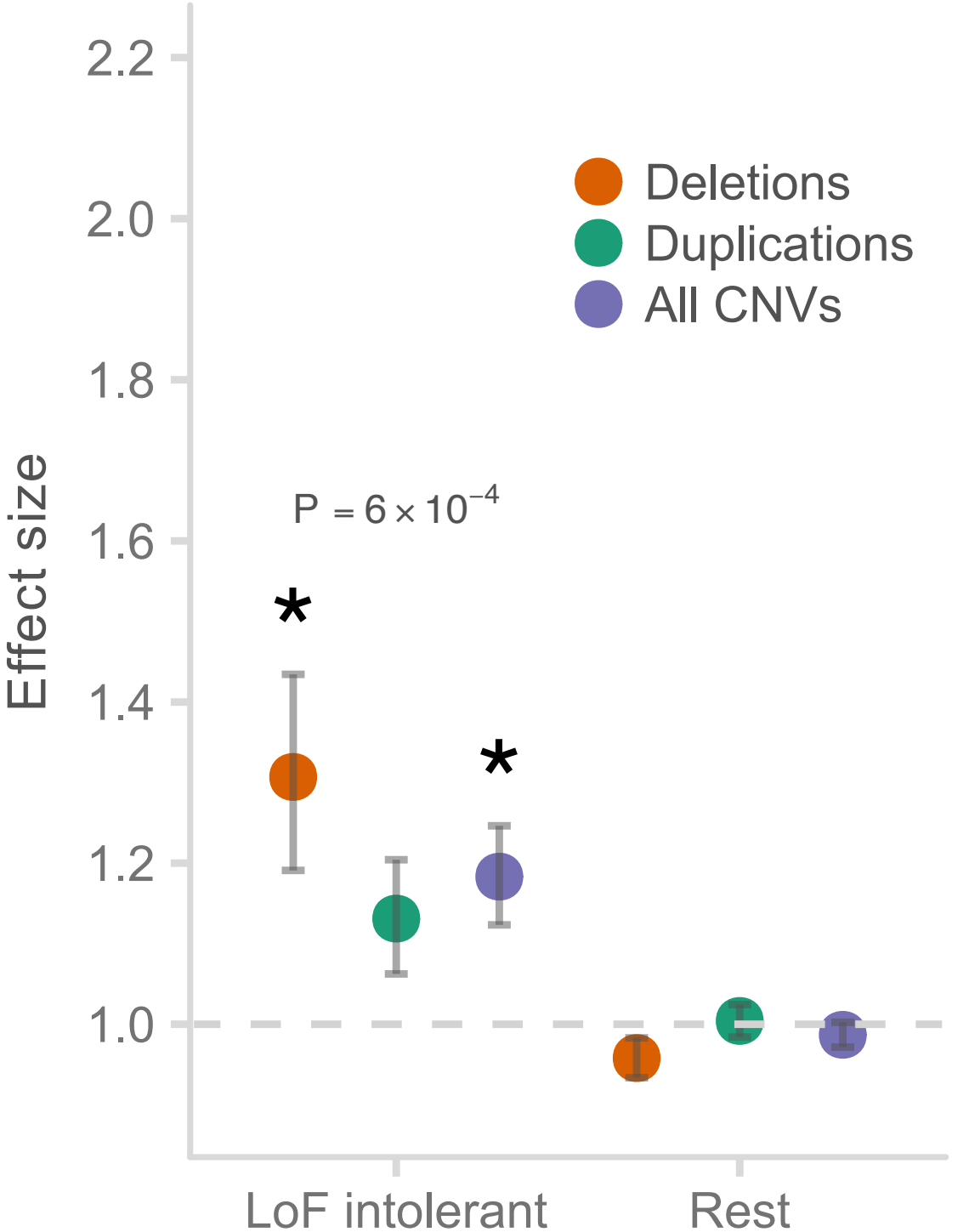
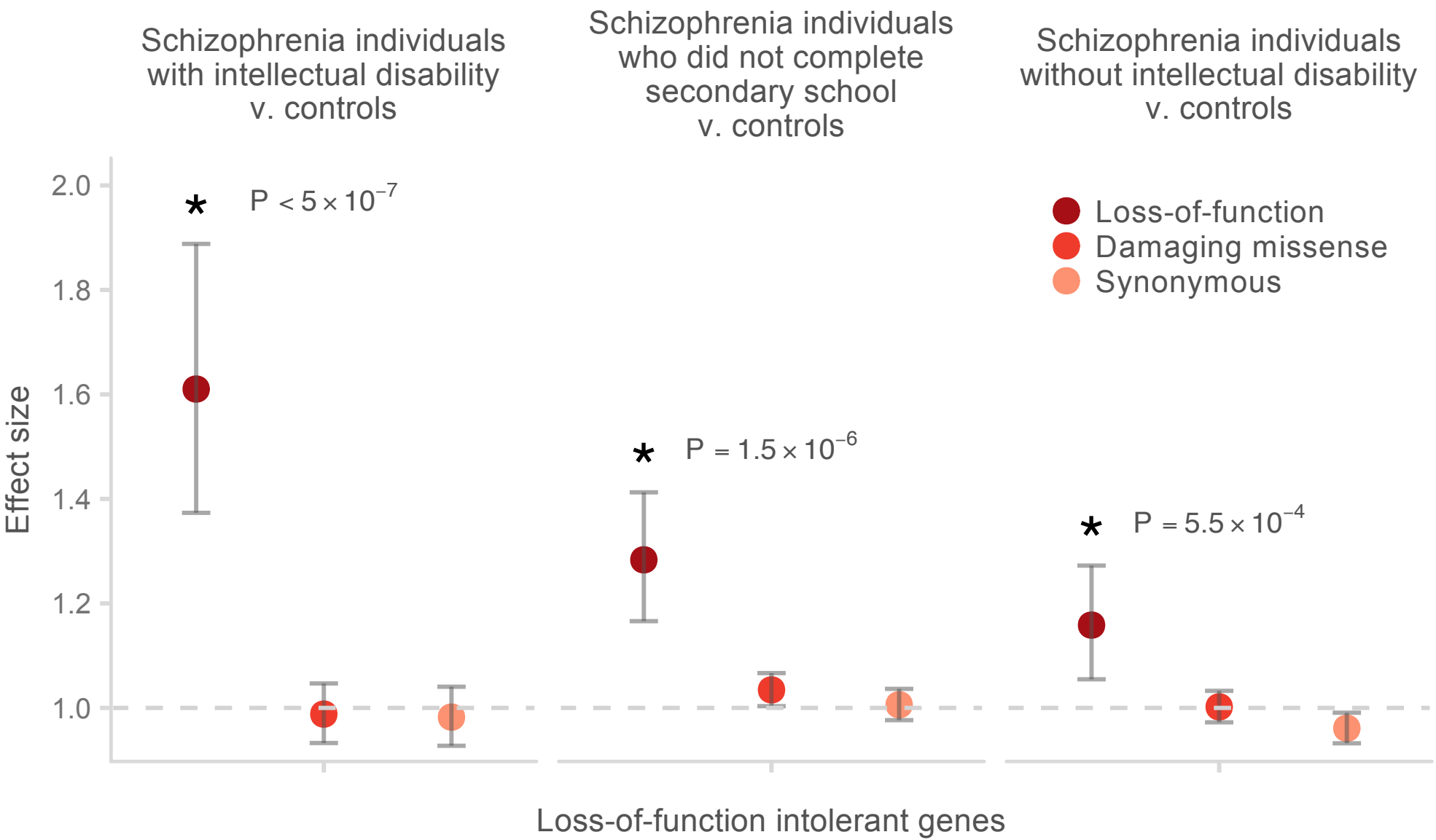
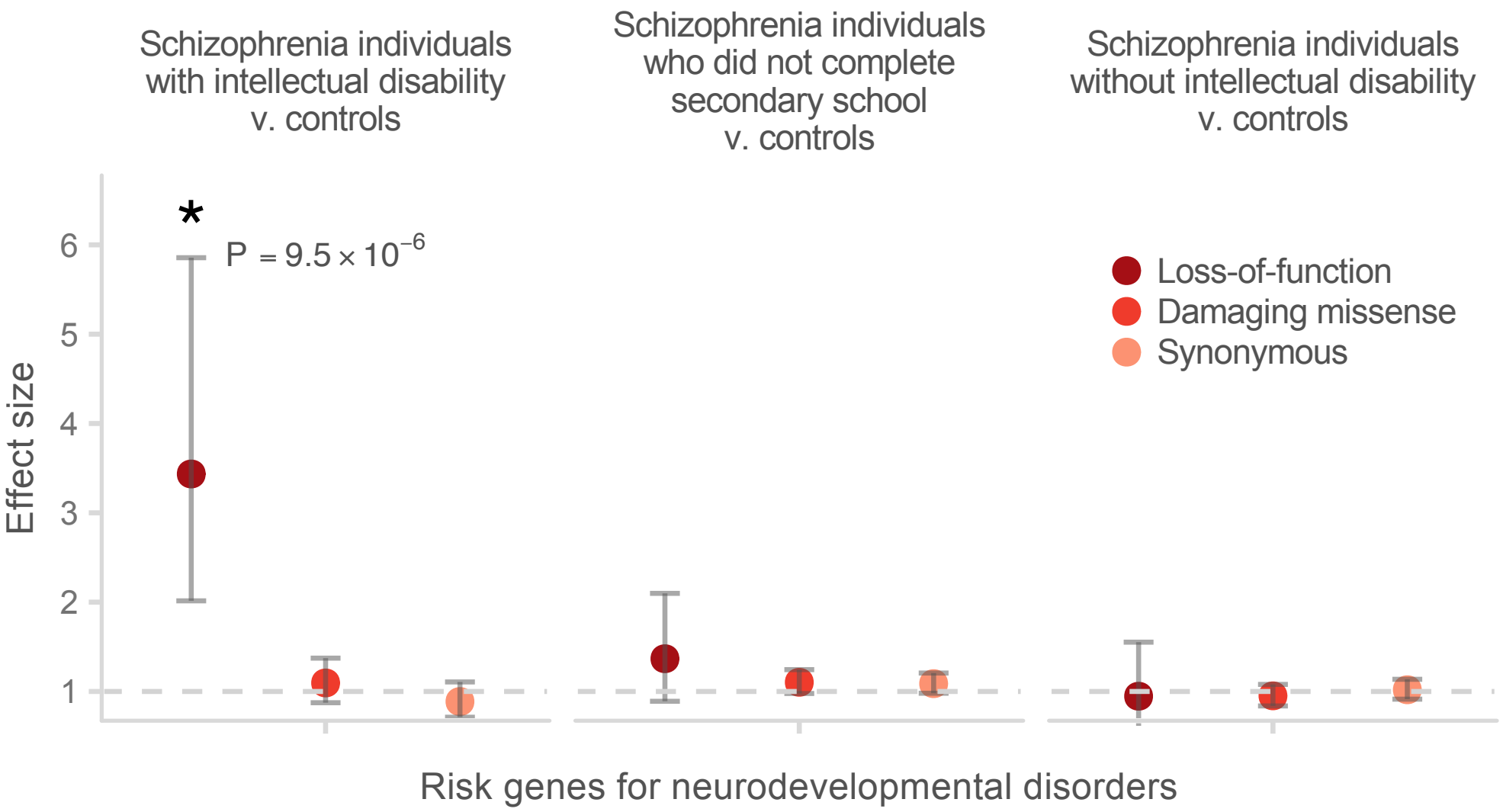

A
Singh et al. 2016
De novo mutations from exome sequencing data of 1,077 trios
Singh et al. 2016
Exome sequencing data of 4,133 cases and 9,274 controls
Rees et al. 2014.
Array-based CNVs from 6,882 cases and 11,255 controls
Empirical evaluation of de novo enrichment using a sampling distribution
Variant threshold burden test
Logistic regression
Gene set P-values combined using Fisher's method
1,766 gene sets tested for rare variant enrichment
Figure 2: Enrichment in LoF intolerant genes
Table 1, Supplementary Table 1 and 2: Neurodevelopmental gene sets implicated at FDR < 5%
Enrichment analysis of FDR < 5% gene sets conditional on LoF intolerant genes
Supplementary Table 3: Developmental disorder genes enriched even after conditioning on LoF intolerant genes
B
Data on cognitive function in 2,971 whole-exome cases, including 279 SCZ-ID and 1,165 SCZ without ID
Variant threshold burden test
Tested for enrichment in LoF intolerant and developmental disorder genes
Figure 3 and 4: Rare variants in LoF intolerant genes and developmental disorder genes are associated with SCZ-ID
Figure 3, Supplementary Table 4: Persistent enrichment in LoF intolerant genes in schizophrenia individuals without intellectual disability


Effect size
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Loss-of-function
Damaging missense
Synonymous
P < 5 × 10^−7
*
LoF intolerant
Rest


P = 0.0019
Loss-of-function
Damaging missense
Synonymous
Effect size
2.2
2.0
1.8
1.6
1.4
1.2
1.0
LoF intolerant
Rest


Effect size
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Deletions
Duplications
All CNVs
P = 6 × 10^−4
*
*
LoF intolerant
Rest


Schizophrenia individuals with intellectual disability v. controls
Schizophrenia individuals who did not complete secondary school v. controls
Schizophrenia individuals without intellectual disability v. controls
Loss-of-function
Damaging missense
Synonymous
P < 5 × 10^−7
P = 1.5 × 10^−6
P = 5.5 × 10^−4
Effect size
2.0
1.8
1.6
1.4
1.2
1.0
Loss-of-function intolerant genes


Schizophrenia individuals with intellectual disability v. controls
Schizophrenia individuals who did not complete secondary school v. controls
Schizophrenia individuals without intellectual disability v. controls
*
P = 9.5 × 10^−6
Loss-of-function
Damaging missense
Synonymous
Effect size
6
5
4
3
2
1
Risk genes for neurodevelopmental disorders