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Analyses of *de novo* and common alleles implicate rare missense variants in *SLC6A1* in
schizophrenia

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Abstract

Schizophrenia is a highly polygenic disorder with important contributions from both common and rare risk alleles. We analysed exome-sequencing data for *de novo* variants (DNVs) in a new sample of 613 schizophrenia trios, and combined this with published data for a total of 3,444 trios. In our new data, loss-of-function (LoF) DNVs were significantly enriched among 3,471 LoF intolerant genes, supporting previous findings. In the full dataset, genes associated with neurodevelopmental disorders (n=159) were significantly enriched for LoF DNVs. Within these neurodevelopmental disorder genes, *SLC6A1*, encoding a gamma-aminobutyric acid transporter, was associated with missense-damaging DNVs. In 1,122 trios for which we had genome-wide common variant data, schizophrenia and bipolar disorder polygenic risk were significantly over-transmitted to probands. Probands carrying LoF or deletion DNVs in LoF intolerant or neurodevelopmental disorder genes had significantly less over-transmission of schizophrenia polygenic risk than non-carriers, providing robust support for these DNVs increasing liability to schizophrenia.

Introduction

Genetic liability to schizophrenia involves a combination of rare and common risk alleles distributed across the genome¹. Common schizophrenia risk alleles with odds ratios < 1.3 account for at least a third of genetic liability²⁻⁴, although only a small fraction of this is captured by the 145 genome-wide significant loci that were implicated in the largest published genome-wide association study (GWAS) of the disorder⁵. At the other end of the frequency spectrum, rare copy number variants (CNVs) and rare coding variants, both sometimes occurring as *de novo* variants (DNVs), have been implicated in the disorder⁶⁻⁸. Although CNVs and rare coding variants are enriched in schizophrenia, not all rare variants

observed in individuals with schizophrenia, including those occurring *de novo*, are expected to be aetiologically relevant, as there is a baseline burden of these variants in the general population.

In people with other neurodevelopmental disorders in which CNVs and rare coding variants play a role, particularly autism spectrum disorder (ASD)^{9,10} and developmental delay^{11,12}, the enrichment for rare coding variants is greatest in genes classified as intolerant to loss-of-function (LoF) variants (i.e. variants that introduce premature stop codons or frameshifts in the encoded protein, or are predicted to disrupt mRNA splicing). This indicates that rare coding variants in these genes are more likely to be pathogenic for those disorders than rare coding variants occurring elsewhere in the genome. Moreover, greater enrichment is found for LoF DNVs than for missense DNVs that change an encoded amino acid, indicating the former class of mutation is particularly likely to be pathogenic. Similar observations have been made in schizophrenia, where an excess of LoF DNVs was found to be largely restricted to LoF intolerant genes⁷, although the degree of enrichment is lower than for ASD or developmental disorders.

In studies of ASD and developmental disorders, a significant excess of rare coding variants has been observed for 99 and 93 genes, respectively, with 33 of these genes overlapping between these disorders^{9,11}. Only two genes, *SETD1A*¹³ and *RBM12*¹⁴, are currently associated with rare coding variants in schizophrenia. This is partly because of lower statistical power, as the number of trios that have been exome-sequenced in studies of schizophrenia (n=2,834) is smaller than equivalent studies of developmental disorders (n=7,580)¹¹ and ASD (n = 6,430)⁹, but it also reflects the weaker enrichment in schizophrenia for this type of variant. As a set, genes disrupted by DNVs in neurodevelopmental disorders

are also enriched for DNVs in schizophrenia^{15,16}, and therefore it follows that some of the genes implicated in ASD and developmental disorders by rare coding variants are also involved in the aetiology of schizophrenia. Aiming to contribute to the schizophrenia rare variant discovery effort, we have undertaken exome-sequencing in a new sample of 613 schizophrenia trios, and combined our data with published data from 2,834 trios, which includes 617 trios previously sequenced by our group¹⁵, to provide the largest analysis of coding DNVs in schizophrenia to date. Given the anticipated modest power even of this sample, as we have successfully done before for CNV analysis¹⁷, we exploited the well documented overlap in the genetic aetiologies of schizophrenia, ASD, and developmental disorders, to undertake a hypothesis focused analysis of neurodevelopmental disorder genes in schizophrenia, which highlights *SLC6A1* as a novel risk gene.

The involvement of common variant polygenic risk in schizophrenia is already established^{2,4,18}, but few existing studies have empirically examined the relationships between different classes of rare and common variants. An early case-control exome sequencing study of schizophrenia found evidence for independent additive effects for common alleles, rare CNVs and rare coding variants when cases were compared with controls, but no within-case correlation between the burden of each type¹⁹. More recent evidence indicates a negative correlation within cases for schizophrenia-associated CNV carrier status and common risk variant burden, consistent with the hypothesis that the common and rare alleles co-act^{20,21}. Thus, compared to controls, affected carriers of schizophrenia-associated CNVs have an increased burden of common schizophrenia risk alleles as measured by the polygenic risk score (PRS)²¹, but in a within case analysis, this burden is inversely proportional to the estimated effect size of the implicated CNV²⁰. In ASD and developmental disorders, common variant polygenic risk for those disorders has been shown to be over-transmitted from parents

to probands, but no difference has been reported between those that do or do not carry a disorder-associated DNV^{22,23}. As yet, the relationship between *de novo* mutations and common allele risk has not been studied in schizophrenia. Here, we examine this relationship using the polygenic transmission-disequilibrium test (pTDT)²³. Specifically, we show that people with schizophrenia who are carriers of DNVs in gene sets proposed to be relevant to schizophrenia have a lower common risk allele burden than people with schizophrenia who are not carriers.

Results

De novo mutation rates

After variant quality control, we observed 606 coding *de novo* variants (DNVs) in 613 probands, corresponding to a rate of 0.99 (s.e = 0.041) events per proband, which is not significantly different to the rate observed in a sample of 2,831 previously published schizophrenia trios (previous *de novo* rate = 1.004; rate ratio (95% confidence interval (CI)) = 0.98 (0.9, 1.08); $p = 0.74$; Supplementary Table S1). Of the coding DNVs, 154 were synonymous, 372 were missense, 15 were inframe indels, 2 start-loss, 1 stop-lost, and 62 were LoF (19 stop-gain, 13 splice and 30 frameshift indels). The number of coding DNVs observed per-trio followed the expected Poisson distribution (Supplementary Figure S4).

De novo variant enrichment tests

In the new data set, we observed a significant excess of LoF DNVs among LoF intolerant genes (Fig 1, rate ratio (95% CI) = 2.21 (1.3, 3.75); $p = 2.3 \times 10^{-3}$; Supplementary Table S2). Consistent with previous reports, we found no evidence for DNV enrichment in the following negative control gene set tests: LoF DNVs in LoF tolerant genes (Fig 1), synonymous DNVs in LoF intolerant genes; synonymous DNVs in LoF tolerant genes (Supplementary Table S3).

After combining the new trio data with previously published data from 2,831 trios, LoF DNVs were enriched in LoF intolerant genes with a rate ratio (95% CI) of 1.58 (1.28, 1.96) ($p = 2.5 \times 10^{-5}$) (Fig 1, Supplementary Table S2). Following review, we tested alternative definitions of LoF intolerant genes based on constraint metrics generated from the gnomAD dataset²⁴; the degree of enrichment of LoF DNVs in schizophrenia is similar regardless of the definition of LoF intolerant genes (see Supplementary Material for full results).

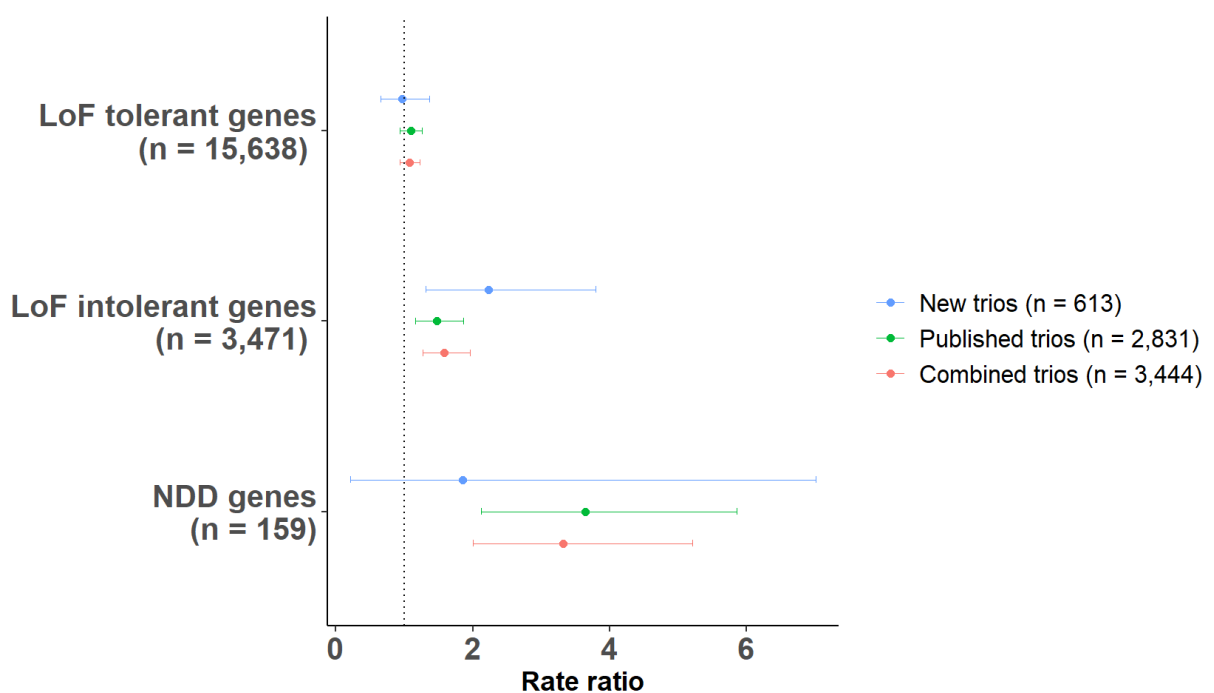


Figure 1. Gene set enrichment for loss-of-function *de novo* variants. Loss-of-function (LoF) DNVs were tested in LoF intolerant genes and neurodevelopmental disorder genes. For LoF intolerant and neurodevelopmental disorder gene sets, rate ratios and 95% confidence intervals are relative to the baseline DNV rate, which is defined as the LoF DNV enrichment observed for all genes outside of the given set. LoF DNV enrichment for LoF tolerant genes are shown as a negative control. A breakdown of the LoF intolerant and neurodevelopmental disorder gene set results is provided in Supplementary Tables S2 and S3. NDD = neurodevelopmental disorder.

In the combined trio data, no individual gene was significantly enriched for LoF DNVs after correction for all genes tested (n=19,109). The most significant novel gene was *CUL1*, which had two LoF DNVs in the new trios and one additional LoF DNV in the published trios (Table 1).

Table 1 about here

We have previously shown that rare CNVs that increase risk of schizophrenia are effectively confined to those that also influence other neurodevelopmental disorders¹⁷. Defining neurodevelopmental disorder genes as those (N=159) that are significantly enriched for rare coding variants in recent large studies of ASD⁹ and developmental disorders¹¹, neurodevelopmental disorder genes were significantly enriched for LoF DNVs in the combined trio data (Fig 1; rate ratio (95% CI) = 3.3 (2.0, 5.17); $p = 8.2 \times 10^{-6}$; Supplementary Table S2), and this enrichment was significantly greater than for LoF intolerant genes (rate ratio (95% CI) = 2.37 (1.41, 3.8); $p = 8.8 \times 10^{-4}$). In the full sample of trios, we observed no enrichment of missense-damaging DNVs for sub-genic regions that have been identified as being depleted for missense variation²⁵ (rate ratio (95% CI) = 1.004 (0.85, 1.18); $p = 0.9$). The rate of missense-damaging DNVs in neurodevelopmental disorder genes was elevated compared with the background rate (rate ratio (95% CI) = 1.53 (0.79, 2.7)), but this is not significant ($p = 0.16$), possibly reflecting the small number of DNVs in neurodevelopmental disorder genes (n=13).

Exploiting the strong enrichment among neurodevelopmental disorder genes for DNVs in schizophrenia, we undertook focused analysis of genes in this set, with the aim of identifying

high probability schizophrenia risk genes. As highlighted in the study of ASD⁹, association to some neurodevelopmental disorder genes is driven by LoF variants alone, a combination of LoF variants and missense variants, and in some cases, primarily by missense variants. Therefore, we considered all those classes of mutation in our analysis. All LoF/missense-damaging DNVs observed in neurodevelopmental disorder genes and, where available, phenotypes observed in these carriers are presented in Supplementary Table S4.

SLC6A1 was significantly associated with missense-damaging DNVs in our new trio data after correcting for three classes of mutation (LoF, missense-damaging and LoF plus missense-damaging) and 159 neurodevelopmental disorder genes (2 damaging-missense DNVs; $p = 7.46 \times 10^{-5}$; $p_{corrected} = 0.036$). This finding was supported in our analysis of all trio data, where we observed one additional missense-damaging DNV (Table 2; 3 missense-damaging DNVs; $p = 5.2 \times 10^{-5}$, $p_{corrected} = 0.025$). It is striking that in the study of ASD⁹, association to *SLC6A1* was also driven by missense variants (n=8) rather than LoF variants (n=1). Following the rationale outlined by the Deciphering Developmental Disorders Study²⁶, we undertook a combined analysis of schizophrenia and ASD DNVs; the evidence for enrichment of missense-damaging DNVs ($MPC \geq 2$) in *SLC6A1* was more than 3 orders of magnitude stronger than for ASD alone, supporting the hypothesis that missense variants in this gene contribute to both disorders (combined $p = 1.6 \times 10^{-14}$; ASD alone $p = 8.0 \times 10^{-11}$).

Table 2 about here

Polygenic transmission disequilibrium tests

Schizophrenia and BD PRS were significantly over-transmitted from parents to probands (Fig 2, Supplementary Material Table S5). These results did not differ when the analysis was

restricted to trios with European ancestry (as defined by principal component analysis; Supplementary Material Table S5).

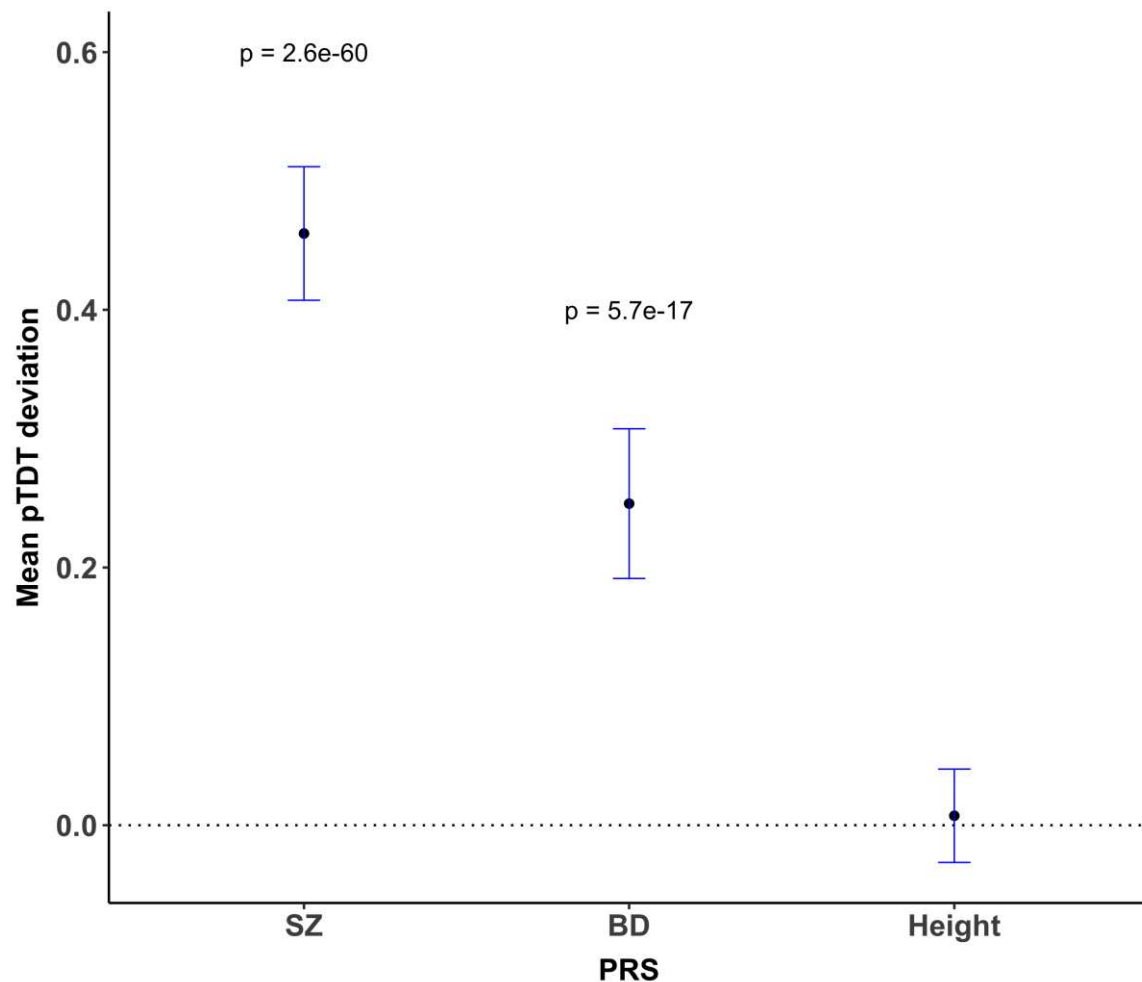


Figure 2. Mean pTDT deviation and 95% confidence intervals for schizophrenia, bipolar disorder (BD), and height polygenic risk scores. Polygenic risk for schizophrenia and bipolar disorder is significantly over-transmitted to schizophrenia probands. PRS = polygenic risk score.

Under a liability threshold model, probands carrying DNVs of large effect size should require less transmission of polygenic risk than probands without such a variant. To test this, the

mean pTDT was compared between carriers of candidate schizophrenia related DNVs and the remainder of the sample. We define candidate schizophrenia related DNVs as LoF DNVs in a LoF intolerant gene or a neurodevelopmental disorder gene. Given CNV deletions disrupting LoF intolerant genes are associated with schizophrenia⁷, we also included *de novo* CNV deletions disrupting one of these genes as candidate schizophrenia related DNVs (CNVs contributing to this analysis are presented in Supplementary Table S6. CNV calling procedure is outlined in the Supplementary Material).

Probands carrying candidate schizophrenia related DNVs had a significantly lower mean pTDT than those who did not carry one of these DNVs (carrier mean pTDT (95% CI) = 0.07 (-0.15, 0.29); non-carrier mean pTDT (95% CI) = 0.48 (0.43, 0.54); $p = 3.5 \times 10^{-4}$; Fig 3). Based on mean pTDT point estimates, the over-transmission of common risk alleles from parents is about 7-fold greater to non-carriers than carriers of candidate schizophrenia related DNVs, although this estimate is imprecise given the width of the confidence intervals (Fig 3). Similar patterns were observed when LoF and deletion DNVs were tested separately (Fig 3). In a negative control test, the mean pTDT did not significantly differ between probands carrying a synonymous DNV in either a LoF intolerant or neurodevelopmental disorder gene and non-carriers (Fig 3).

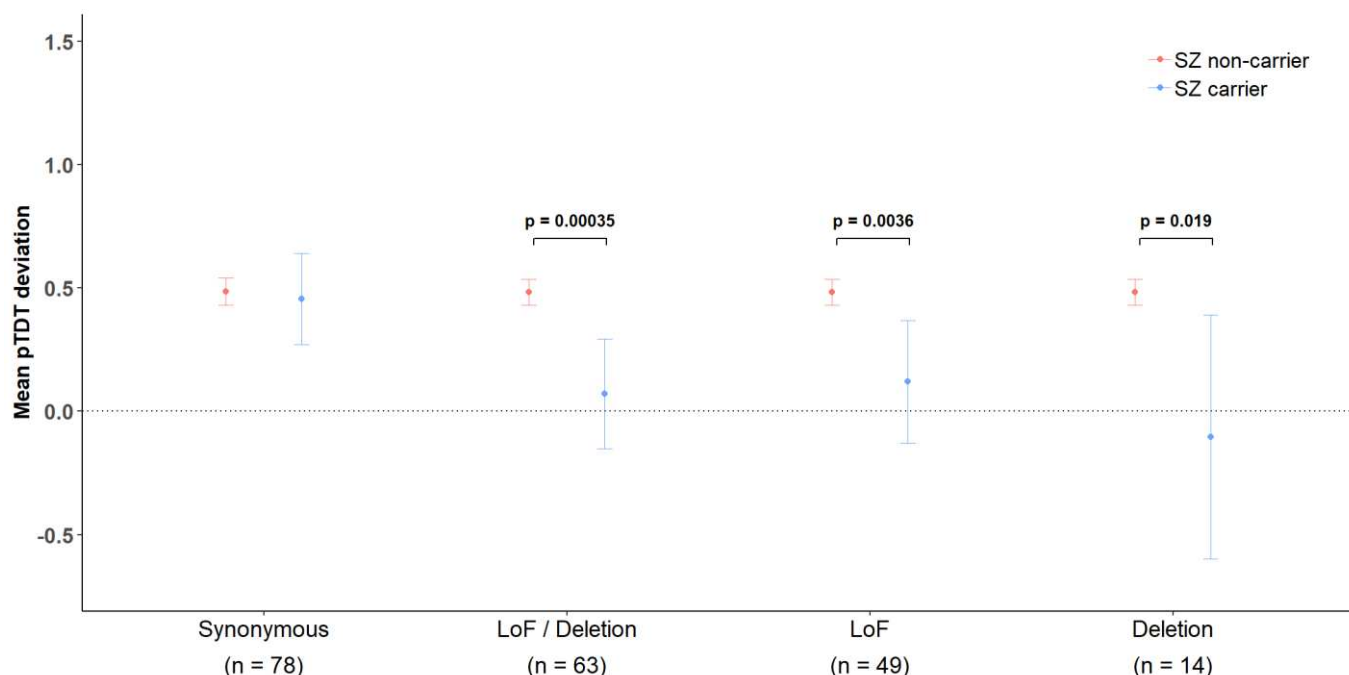


Figure 3. Mean pTDT deviation and 95% confidence intervals for schizophrenia PRS.

Results are shown for probands carrying various classes of *de novo* variant (DNV) in a LoF intolerant gene or a neurodevelopmental disorder gene; our primary analysis defined schizophrenia carriers as probands with a LoF or deletion DNV in a LoF intolerant gene or a neurodevelopmental disorder gene (LoF/deletion label). Results are also shown separately for carriers of LoF and deletion DNVs. LoF = loss-of-function.

The finding that the mean pTDT deviation for schizophrenia PRS was significantly different between probands carrying candidate schizophrenia related DNVs and non-carrying probands was consistent across schizophrenia PRS training *p*-value thresholds (Supplementary Table S7). Although the pTDT method is expected to be robust to population stratification, the efficacy of PRS as a measure of relative liability varies with the extent to which the ancestry of the sample from which risk alleles are derived (the source GWAS) matches the ancestry of those being tested (in our case the trios). Given the source GWAS is primarily of European

ancestry, we tested, and confirmed, that our findings held when we restrict our analysis to trios with European ancestry (Supplementary Figure S5 and S6) despite the smaller sample size (all results for European-only trios are presented in Supplementary Tables S7 and S8).

The mean pTDT in carriers of candidate schizophrenia related DNVs was not significantly greater than the null (Fig 3). Based on the pTDT standard deviation observed for schizophrenia PRS in all trios (0.89), we only had 80% power to detect a significant ($\alpha = 0.05$) mean pTDT of 0.4 in the 63 carriers of candidate schizophrenia related DNVs. Thus, while we can be confident that the over-transmission to candidate DNV carriers is less than to non-carriers, power limitations mean we cannot conclude that candidate DNV carriers have no contribution from common alleles.

During the review process, we performed an exploratory analysis to evaluate whether pTDT was lower in carriers of additional classes of DNV. Despite testing a wide-range of alternative variant filters (e.g. excluding DNVs observed in ExAC/gnomAD), missense annotations (e.g. MPC scores and constrained coding regions), and CNVs intersecting only LoF tolerant genes, no set of DNV carriers had a significantly greater reduction in pTDT than that observed for our primary candidate schizophrenia-related set of DNVs defined above (see Supplementary Material Table S8 for all results).

Discussion

Proband-parent trio studies have identified large numbers of genes associated with DNVs in ASD and developmental disorders^{9,11}. Although similar studies in schizophrenia have revealed general pathophysiological insights into the disorder, such as a role for proteins involved in postsynaptic signaling complexes^{15,27}, schizophrenia gene discovery through

DNV analysis has been hindered by small samples. To add to efforts to overcome this limitation, we performed exome-sequencing of a new sample of 613 schizophrenia trios. We confirmed previous work showing schizophrenia LoF DNVs are significantly enriched among a set of 3,471 genes intolerant to this class of mutation, and identified a stronger enrichment of DNVs in a smaller set of 159 genes that are associated with rare coding variants in neurodevelopmental disorders. GWAS data suggest common risk alleles are under negative selection²⁸ and enriched in highly conserved genes⁵, but are nevertheless maintained by population mechanisms related to background selection and genetic drift^{5,29}. The findings from rare mutations, both CNVs^{7,30} and rare coding variants^{7,31}, also support a role for deleterious point mutations in schizophrenia that are under more intense negative selection than alleles of weak effect. Despite this, the population burden of schizophrenia risk alleles seems to be maintained by mutation-selection balance; for CNVs, strong selection is balanced by their relatively high mutation rates³⁰, while for exonic mutations, in the face of a low per base mutation rate, balance is likely maintained by the large size of the mutational target.

In our analysis of all schizophrenia trios, no novel gene was unequivocally associated with DNVs after correction for all genes tested. Despite conducting the largest analysis of DNVs in schizophrenia to date, it is clear that even larger samples are required to identify specific risk genes with genome-wide levels of significance. However, taking an approach based on the wealth of data showing that rare CNVs that increase risk of schizophrenia are effectively confined to those that also influence other neurodevelopmental disorders¹⁷, and exploiting the observation here for strong enrichment for DNVs in known neurodevelopmental disorder genes, we find evidence for association between *SLC6A1*, which encodes a sodium-dependent γ -aminobutyric acid (GABA) transporter (also known as GAT1), and missense-damaging DNVs. *SLC6A1* is involved in reuptake of the inhibitory neurotransmitter GABA

from the synaptic cleft; our finding therefore adds to the evidence for perturbation of GABAergic neuronal signaling in genetic risk for schizophrenia³². Congruent with our findings, the largest study of rare coding variants in ASD found *SLC6A1* to be the most significant (of only four) genes where association signal was driven by missense-damaging variants (8 missense and 1 LoF DNVs)⁹. In myoclonic atonic epilepsy and developmental disorders, LoF variants account for 54% and 30% of the observed nonsynonymous DNVs, respectively⁹. Given the strong convergent evidence for this gene, and specifically for a role for missense mutations, from other neurodevelopmental disorders, *SLC6A1* is highly likely also to be involved in schizophrenia. This conclusion is further supported by the result of the DNV missense meta-analysis of ASD and schizophrenia, in which the combined evidence for association is more than 3 orders of magnitude stronger than the (already strong) evidence for association to ASD alone, and surpasses genome-wide significance by 8 orders of magnitude. Given the small number of DNVs in *SLC6A1*, it will be important to extend our finding in other samples, and clearly, a larger number of DNVs will be required to establish that risk is conferred largely by missense rather than LoF mutations.

The role of polygenic risk in schizophrenia has been widely studied using large case-control samples. However, to our knowledge, this is the first study to investigate polygenic risk in schizophrenia using the pTDT method. The pTDT method has several advantages over case-control PRS studies as it is not confounded by ancestry or ascertainment bias or the possibility of effects arising from super-healthy controls in discovery GWAS and subsequent PRS test samples²³. Our results provide strong refutation that such effects might explain the PRS effects that have been widely publicised in the literature, including that of overlap in risk between schizophrenia and BD.

More importantly in the present context, our finding that carriers of LoF DNVs in genes defined by LoF intolerance, or in a known neurodevelopmental disorder gene, have significantly lower distortion of transmission of polygenic liability from the mean parental PRS than do non-carriers provides orthogonal evidence that a substantial proportion of this class of *de novo* variant contribute to schizophrenia pathogenesis. This is an important observation given the possibility that previously documented gene set enrichments in cases of these variants could have been driven by errors in the calibration of the expected mutation rate, or technical issues arising from comparing cases and controls (or case and control trios) often derived opportunistically from different studies.

Our limited sample size does not allow accurate estimation of the magnitude of difference in the transmission distortion between probands carrying candidate schizophrenia related DNVs and those that do not, but the point estimate is that the distortion in non-carriers is about 7-fold of that of carriers (and almost 10-fold when restricted to those of European ancestry). This suggests that on average, the candidate DNVs contribute a substantial amount of liability in those who carry them. Indeed in the present study, carriers of candidate schizophrenia related DNVs did not significantly over-inherit a common allele burden from their parents, which is consistent with DNVs in LoF intolerant genes acting as monogenic risk factors in those who carry them. However, it is important to stress that the latter finding is also consistent with limited power (as discussed in the results) rather than no role for common variation in the carriers, and we note the point estimate for the pTDT in candidate DNV carriers is greater than 0. It will be important for future larger studies to determine whether differences in co-action between common and rare risk alleles exist between schizophrenia and neurodevelopmental disorders. Meanwhile, with respect to the genetic architecture of schizophrenia, together with previous findings from CNVs alone^{20,21}, we

interpret our data as being consistent with a polygenic liability threshold model of schizophrenia³³.

In conclusion, we provide further evidence that certain classes of DNV are associated with increased risk for schizophrenia. We highlight strong evidence that mutations in *SLC6A1*, a known ASD, developmental disorders and epilepsy gene, confer high risk of schizophrenia. Through combining exome-sequencing and GWAS data, we show that carriers of candidate schizophrenia related DNVs inherit significantly fewer common risk alleles than non-carrying cases, providing strong orthogonal evidence that these DNVs contribute to schizophrenia liability.

Online Methods

Sample overview

674 schizophrenia proband-parent trios, consisting of 2,000 individuals, were exome-sequenced on Illumina HiSeq 4000 platforms. The proband-parent trios were composed of 653 trios, 9 quads (two affected children) and one family with 3 affected children. None of these samples have been previously exome-sequenced. The families were recruited by six independent groups (Supplementary Table S9), and were ascertained from general psychiatric wards or outpatient clinics. All probands had received a DSM-IV or ICD-10 diagnosis of schizophrenia or schizoaffective disorder. Individuals with a known diagnosis of intellectual disability or other neurodevelopmental disorder were not included. For probands passing quality control (quality control procedure described below), information on family history of schizophrenia/psychosis was available for 552 trios; 66% of probands were recorded as family history negative. Further details on the recruitment and diagnostic

criteria for each cohort are provided in the Sample Description section of the Supplementary Material.

Exome sequence generation

Exome sequence was generated using the Nextera DNA Exome capture kit and HiSeq 3000/4000 PE Cluster Kit and HiSeq 3000/4000 SBS Kit. Raw sequencing reads were processed according to GATK best practice guidelines^{34,35}. Reads were aligned to the human reference genome (GRCh37) using bwa version 0.7.15³⁶. Variants were called using GATK haplotype caller (v3.4) and filtered using the GATK Variant Quality Score Recalibration (VQSR) tool. For all samples passing quality control (criteria outlined below), we generated sequence data for a median of 83% of the exome target at $\geq 10X$ coverage. We discuss sequencing coverage further in the Supplementary Material. For future users of our new dataset, we provide in Supplementary Table S10 the median proportion each gene is covered at $\geq 10X$ coverage.

Sample quality control

Trios (n=27) were excluded for low sequencing coverage if less than 70% of the exome target achieved $\geq 10X$ coverage in the proband or either parent (Supplementary Figure S1). An additional 27 trios were excluded for excess heterozygosity (heterozygote:homozygote ratio > 1.9) or evidence of cross sample contamination (as measured by the FREEMIX sequence only estimate of contamination³⁷) (Supplementary Figure S2). The last two metrics are highly correlated. Identity-by-descent (IBD) analysis (plink v1.9) to ensure expected proband-parent relationships resulted in exclusion of 3 trios. Four additional trios were excluded as outliers for the number of DNVs (Supplementary Figure S3). Following implementation of all the above sample quality control steps, 613 proband-parent trios were retained for DNV analysis.

Variant quality control

In each of our newly sequenced samples, we excluded genotypes if they did not meet the following criteria: depth $\geq 10X$; genotype quality score ≥ 30 ; allele balance ≤ 0.1 and ≥ 0.9 for homozygous genotypes for the reference and alternative allele, respectively; allele balance between 0.2 and 0.8 for heterozygous genotypes. For samples and variants that passed quality control, we observed no difference in the number of heterozygous variants transmitted or non-transmitted from parents to probands (transmission disequilibrium test $p = 0.53$), indicating high data quality.

De novo variant calling

Putative DNVs in the new trios were identified as sites that were heterozygous in the proband and homozygous for the reference allele in both parents. All trio members were required to pass genotype quality control described above. We considered as putative DNVs 1) those where there were no reads for the mutant allele in either parent, and the mutant allele was not called in any other sample of the new trios (parent or proband) and 2) those where the mutant allele met all of the following; an allele count ≤ 3 in all newly sequenced samples, no mutant allele variant reads in either parent, and at least 5 reads of the mutant allele in the proband.

Read alignments for all putative DNVs were manually inspected using IGV

(<http://software.broadinstitute.org/software/igv/>) and variants were reassigned as high or low confidence if there was, respectively, no evidence or evidence for, read misalignment.

We used Sanger sequencing to perform a validation experiment, where DNA was available and primers could be designed, on all high confidence LoF DNVs, as well as additional putative DNVs. In total, primers were successfully designed for 205 putative DNVs. We

observed high validation rates for high confidence DNVs (95.5%) and low rates (3.4%) for low confidence DNVs (Supplementary Table S11). Following these results, in our new trios we included in the downstream analyses all high confidence DNVs (N = 606 coding DNVs, Supplementary Table S12).

Adding published *de novo* data

To increase the power of our analysis, we included previously published DNVs from 2,831 schizophrenia trios. When combined with our new trios, this resulted in a sample size of 3,444 schizophrenia trios. We note that no DNV from our new trios was also observed among the previously published schizophrenia *de novo* data, thus confirming the independence of our new trio dataset. A summary of the published data can be found in Supplementary Table S13.

De novo variant analysis

We tested whether DNVs were enriched in single genes or sets of genes using the statistical framework described in Samocha *et al* 2014³⁸. Here, for a given set of genes we estimated the number of DNVs expected in our new sample using per-gene mutation rates³⁹, adjusted for sequence coverage. When estimating the number of expected DNVs in previously published trios, we did not adjust per-gene mutation rates for coverage as coverage metrics were not available for all samples; the use of unadjusted per-gene mutation would over-estimate the expected number of DNVs in these trios, producing more conservative enrichment results. For our gene-set analysis, we define LoF intolerant genes as genes with a pLi score ≥ 0.9 , using pLi metrics generated from the non-psychiatric component of ExAC³¹ (available from <http://exac.broadinstitute.org/downloads>). For single genes, we tested whether the overall burden of DNVs was significantly greater than that expected using a one-sided Poisson test

(implemented in R). For our primary *de novo* gene set analysis, we controlled for background *de novo* rates by using a two-sample Poisson rate ratio test, which compared the DNV enrichment observed for genes in the set to that in genes outside the set.

DNVs from both the new trios and previously published *de novo* data were annotated using Ensemble Variant Effect Predictor (version 96)⁴⁰. We define LoF variants as stop-gain, splice-acceptor, splice-donor and frameshift mutations. Although we observed a small number of start-loss and stop-loss DNVs, we did not include them in our LoF annotation as mutation rates are not available for these variants. We classify missense-damaging variants as missense variants with an MPC score ≥ 2 , as this metric has proven effective at identifying variants associated with ASD^{9,25}. Missense-damaging mutation rates for individual genes were calculated by summing tri-nucleotide mutation probabilities for all sites with an MPC score ≥ 2 . Following previous work by us and others^{15,16}, if an individual carried multiple *de novo* variants in the same gene, we conservatively considered these to be the result of a single mutation event, and retained for analysis only the variant predicted to be most deleterious.

Polygenic risk scores

Where available (n=1,122 trios), we used SNP genotype data to generate polygenic risk scores. We confirmed that genotype and exome-sequence data belonged to the same individual through IBD analysis (plink v1.9). A summary of the data sets for which we had both exome-sequencing and SNP genotype data can be found in Supplementary Table S14. To derive PRS for schizophrenia, bipolar disorder (BD) and height, we used the largest available GWAS summary statistics that were independent from our trio test data. Given some samples overlapped between our Bulgarian trios and PGC2, we computed schizophrenia PRS in the Bulgarian trios using custom PGC2 GWAS summary statistics that

omitted the Bulgarian samples. We used BD PRS as previous studies have shown that common variant liability to schizophrenia and BD is substantially shared⁴¹. Height PRS was used as a negative control. A summary of the training data used to generate PRS can be found in Supplementary Table S14.

For quality control purposes, SNP genotype data were first harmonised to the Haplotype Reference Consortium panel using the Genotype Harmonizer package⁴² and then subjected to standard quality control, which included exclusion of samples with a call rate < 95%, SNPs with a MAF < 0.1, SNPs with > 1% missingness, or SNPs with a Hardy-Weinberg equilibrium exact test p value < 1×10^{-6} . PRS were generated using PRSice 2 software⁴³, where SNPs were clumped based on a window of 250 kb and a maximum r^2 of 0.2. We generated PRS across a range of training data P-value thresholds ($P < 0.5, 0.1, 0.05, 0.001$).

pTDT deviation

To test for a significant over-transmission of polygenic risk, we used the polygenic transmission disequilibrium test (pTDT) as described in Weiner *et al* (2017)²³. Here, pTDT deviation scores were generated for each trio by subtracting the mean-parental PRS from the child PRS (Equation 1). pTDT deviation scores were standardised by dividing them by the cohort-specific mean-parental PRS standard deviation.

$$pTDT\ deviation = \frac{PRS_{proband} - PRS_{parental\ mean}}{SD(PRS_{parental\ mean})}$$

Equation 1.

We tested whether the mean pTDT deviation was significantly greater than 0, representing an over-transmission of polygenic risk, by using a one-sided one-sample *t* test. A one-sided two-sample *t* test was used to compare mean pTDT deviation scores across groups of trios.

The primary pTDT results were produced using PRS generated with a *P*-threshold of 0.05, as this threshold explained the most case-control variance in the 2014 schizophrenia PGC analysis⁴. However, we also present in the Supplementary Material Table S5 pTDT results obtained for PRS generated across different *P*-value thresholds.

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MCOD, MJO, JTRW, PH and ER conceived and designed the research. ER analysed the data. JH, JM and NC performed and managed the sequencing experiments. NR, TL, NK, VG, MP, JGP, CA, GI, MG, GK, JTRW, MCOD and MJO led the acquisition of the clinical samples. ER, MCOD, MJO wrote the manuscript, which was read, edited and approved by all authors.

Competing interests

The authors declare no competing interests.

Data availability

Where permitted by ethical approval, Exome sequence generated from this project will be deposited into the European Genome-Phenome Archive (<https://www.ebi.ac.uk/ega/home>).

De novo mutations discovered from the new trios are published in Supplementary Table S12.

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Figure Legends

Figure 1. Gene set enrichment for loss-of-function *de novo* variants. Loss-of-function (LoF) DNVs were tested in LoF intolerant genes and neurodevelopmental disorder genes. For LoF intolerant and neurodevelopmental disorder gene sets, rate ratios and 95% confidence intervals are relative to the baseline DNV rate, which is defined as the LoF DNV enrichment observed for all genes outside of the given set. LoF DNV enrichment for LoF tolerant genes are shown as a negative control. A breakdown of the LoF intolerant and neurodevelopmental disorder gene set results is provided in Supplementary Tables S2 and S3. NDD = neurodevelopmental disorder.

Figure 2. Mean pTDT deviation and 95% confidence intervals for schizophrenia, bipolar disorder (BD), and height polygenic risk scores. Polygenic risk for schizophrenia and bipolar disorder is significantly over-transmitted to schizophrenia probands. PRS = polygenic risk score.

Figure 3. Mean pTDT deviation and 95% confidence intervals for schizophrenia PRS.

Results are shown for probands carrying various classes of *de novo* variant (DNV) in a LoF intolerant gene or a neurodevelopmental disorder gene; our primary analysis defined schizophrenia carriers as probands with a LoF or deletion DNV in a LoF intolerant gene or a neurodevelopmental disorder gene (LoF/deletion label). Results are also shown separately for carriers of LoF and deletion DNVs. LoF = loss-of-function.

Tables

Gene	New trios (n=613)		Published trios (n=2,831)		All trios (n=3,444)	
	LoF DNVs	P	LoF DNVs	P	LoF DNVs	P
<i>SETD1A</i>	0	1	3	1.90E-06	3	3.00E-06
<i>CUL1</i>	2	3.60E-05	1	0.04	3	2.00E-05
<i>TAF13</i>	0	1	2	2.40E-05	2	3.30E-05
<i>GALNT9</i>	0	1	2	2.90E-05	2	4.20E-05
<i>HENMT1</i>	0	1	2	5.50E-05	2	7.90E-05
<i>PAF1</i>	1	0.0028	1	0.013	2	0.00013
<i>SV2B</i>	0	1	2	0.00016	2	0.00023
<i>NRXN3</i>	0	1	2	0.00022	2	0.0003
<i>HIVEP3</i>	0	1	2	0.00026	2	0.00035
<i>RB1CC1</i>	0	1	2	0.00046	2	0.00065
<i>SMARCC2</i>	0	1	2	0.0005	2	0.00068
<i>MKI67</i>	0	1	2	0.00085	2	0.0012
<i>CHD8</i>	0	1	2	0.0009	2	0.0013
<i>TENM1</i>	1	0.0077	1	0.046	2	0.0014
<i>TRIO</i>	0	1	2	0.0012	2	0.0016
<i>SCN2A</i>	1	0.012	1	0.057	2	0.0024
<i>DNAH9</i>	0	1	2	0.0018	2	0.0026
<i>KMT2C</i>	0	1	2	0.0086	2	0.012
<i>KIAA1109</i>	0	1	2	0.01	2	0.015
<i>TTN</i>	1	0.16	2	0.22	3	0.092

Table 1. Genes disrupted by 2 or more LoF *de novo* variants. The most significant gene, *SETD1A*, has been previously identified as a schizophrenia risk gene¹³.

Gene	Observed DNVs			<i>P</i> (uncorrected)		
	Miss _{dam}	LoF	Miss _{dam} + LoF	Miss _{dam}	LoF	Miss _{dam} + LoF
<i>SLC6A1</i>	3	0	3	5.20E-05*	1	7.90E-05*
<i>SCN2A</i>	1	2	3	0.15	0.0024	0.0019
<i>SMARCC2</i>	0	2	2	1	0.00068	0.0019
<i>PUF60</i>	1	1	2	0.056	0.022	0.003
<i>MED13L</i>	1	1	2	0.048	0.064	0.0062
<i>DEAF1</i>	0	1	1	1	0.0082	0.011
<i>TRIO</i>	0	2	2	1	0.0016	0.014
<i>CHD8</i>	0	2	2	1	0.0013	0.023
<i>CHD4</i>	1	1	2	0.2	0.04	0.03
<i>KMT2C</i>	0	2	2	1	0.012	0.04
<i>PTEN</i>	1	0	1	0.029	1	0.044
<i>GNAO1</i>	1	0	1	0.042	1	0.052
<i>TEK</i>	0	1	1	1	0.025	0.057
<i>AUTS2</i>	0	1	1	1	0.03	0.057
<i>CSNK2A1</i>	1	0	1	0.052	1	0.064
<i>POGZ</i>	0	1	1	1	0.048	0.066
<i>NACC1</i>	1	0	1	0.08	1	0.085
<i>KDM5B</i>	0	1	1	1	0.084	0.092
<i>TLK2</i>	1	0	1	0.075	1	0.1
<i>KDM6B</i>	0	1	1	1	0.025	0.13
<i>GRIN2B</i>	1	0	1	0.15	1	0.17
<i>SYNGAP1</i>	0	1	1	1	0.026	0.21

Table 2. Neurodevelopmental disorder genes with at least 1 LoF or missense-damaging *de novo* variant observed in schizophrenia. Enrichment *P* values are derived from the analysis of all schizophrenia trios (n = 3,444). Miss_{dam} = missense-damaging (MPC score ≥ 2). * indicate *p* values which survive correction for 159 neurodevelopmental disorder genes and three mutation classes (LoF, missense-damaging and LoF plus missense-damaging).