

A contribution of novel CNVs to schizophrenia from a genome-wide study of 41,321 subjects

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Abstract

Genomic copy number variants (CNVs) have been strongly implicated in the etiology of schizophrenia (SCZ). However, apart from a small number of risk variants, elucidation of the CNV contribution to risk has been difficult due to the rarity of risk alleles, all occurring in less than 1% of cases. We sought to address this obstacle through a collaborative effort in which we applied a centralized analysis pipeline to a SCZ cohort of 21,094 cases and 20,227 controls. We observed a global enrichment of CNV burden in cases ($OR=1.11$, $P=5.7e^{-15}$), which persisted after excluding loci implicated in previous studies ($OR=1.07$, $P=1.7e^{-6}$). CNV burden is also enriched for genes associated with synaptic function ($OR = 1.68$, $P = 2.8e^{-11}$) and neurobehavioral phenotypes in mouse ($OR = 1.18$, $P = 7.3e^{-5}$). We identified genome-wide significant support for eight loci, including 1q21.1, 2p16.3 (NRXN1), 3q29, 7q11.2, 15q13.3, distal 16p11.2, proximal 16p11.2 and 22q11.2. We find support at a suggestive level for nine additional candidate susceptibility and protective loci, which consist predominantly of CNVs mediated by non-allelic homologous recombination (NAHR).

Introduction

Studies of genomic copy number variation (CNV) have established a role for rare genetic variants in the etiology of SCZ¹. There are three lines of evidence that CNVs contribute to risk for SCZ: genome-wide enrichment of rare deletions and duplications in SCZ cases relative to controls^{2,3}, a higher rate of *de novo* CNVs in cases relative to controls⁴⁻⁶, and association evidence implicating a small number of specific loci (**Extended data table 1**). All CNVs that have been implicated in SCZ are rare in the population, but confer significant risk (odds ratios 2-60).

To date, CNVs associated with SCZ have largely emerged from mergers of summary data for specific candidate loci⁷⁻⁹; yet even the largest genome-wide scans (sample sizes typically <10,000) remain under-powered to robustly confirm genetic association for the majority of pathogenic CNVs reported so far, particularly for those with low frequencies (<0.5% in cases) or intermediate effect sizes (odds ratios 2-10). It is important to address the low power of systematic CNV studies with larger samples given that this type of mutation has already proven useful for highlighting some aspects of SCZ related biology^{6,10-13}.

The limited statistical power provided by small samples is a significant obstacle in studies of rare and common genetic variation. In response, global collaborations have been formed in order to attain large sample sizes, as exemplified by the study of the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC) in which 108 independent schizophrenia associated loci were identified¹⁴. Recognizing the need for similarly large samples in studies of CNVs for psychiatric disorders, we formed the PGC CNV Analysis Group. Our goal was to enable large-scale analyses of CNVs in psychiatry using centralized and uniform methodologies for CNV calling, quality control, and statistical analysis. Here, we report the largest genome-wide analysis of CNVs for any psychiatric disorder to date, using datasets assembled by the Schizophrenia Working Group of the PGC.

Data processing and meta-analytic methods

Raw intensity data were obtained from 57,577 subjects from 43 separate datasets (**Extended data table 2**). After CNV calling and quality control (QC), 41,321 subjects were retained for analysis. In large datasets derived from multiple studies, variability in CNV detection between studies and array platforms presents a significant challenge. To minimize the technical variability across different studies, we developed a centralized pipeline for systematic calling of CNVs for Affymetrix and Illumina platforms. (**Methods** and **Extended data figure 1**). The pipeline included multiple CNV callers run in parallel. Data from Illumina platforms were processed using PennCNV¹⁵ and iPattern¹⁶. Data from Affymetrix platforms were analyzed using PennCNV and Birdsuite¹⁷. Two additional methods, iPattern and C-score¹⁸, were applied to data from the Affymetrix 6.0 platform. The CNV calls from each program were converted to a standardized format and a consensus call set was constructed by merging CNV outputs at the sample level. Only CNV segments that were detected by all algorithms were retained. We performed rigorous QC at the platform level to exclude samples with poor probe intensity and/or an excessive CNV load (number and length). Larger CNVs that appeared to be fragmented were merged and retained. CNVs spanning centromeres or those with >50% overlap with segmental duplications or regions prone to VDJ recombination (e.g.,

immunoglobulin or T cell receptor loci) were excluded. A final set of rare, high quality CNVs was defined as those >20kb in length, at least 10 probes, and <1% MAF.

Genetic associations were investigated by case-control tests of CNV burden at four levels: (1) genome-wide (2) pathways, (3) genes, and (4) probes. Analyses controlled for SNP-derived principal components, sex, genotyping platform, and individual-level probe intensity. Multiple-testing thresholds for genome-wide significance were estimated from family-wise error rates drawn from permutation

Genome wide analysis of CNV burden reveals an enrichment of ultra-rare variants

An elevated burden of rare CNVs has been well established among SCZ cases². We applied our meta-analytic framework to measure the consistency of overall CNV burden across the genotyping platforms, and whether a measurable amount of CNV burden persists outside of previously implicated CNV regions. Consistent with previous estimates, the overall CNV burden is significantly greater among SCZ cases when measured as total Kb covered (OR=1.12, $p = 5.7e^{-15}$), genes affected (OR=1.21, $p = 6.6e^{-21}$), or CNV number (OR=1.03, $p = 1e^{-3}$). Focusing on genes affected by CNV, our strongest signal of enrichment, the effect size is consistent across all genotyping platforms (**Figure 1A**). When we split by CNV type, the effect size for copy number losses (OR=1.40, $p = 4e^{-16}$) is greater than for gains (OR=1.12, $p = 2e^{-7}$) (**Extended data figures 2-3**). Partitioning by CNV frequency (based on 50% reciprocal overlap with the full call set, **Methods**), CNV burden is enriched among cases across a range of frequencies, up to counts of 80 (MAF = 0.2%) in the combined sample (**Figure 1B**).

A primary question in this study is the contribution of novel loci to the excess CNV burden in cases. After removing nine previously implicated CNV loci (where reported p -values exceed our designated multiple testing threshold, **Extended data table 1**), excess CNV burden in SCZ remains significantly enriched (genes affected OR=1.11, $p = 1.3e^{-7}$, **Figure 1B**). CNV burden also remained significantly enriched after removal of all reported loci from **Extended data table 1**, but the effect-size was greatly reduced (OR = 1.08) compared to the enrichment overall (OR = 1.21). When we partition CNV burden by frequency, we find that much of the

previously unexplained signal is restricted to comparatively rare events (i.e., MAF < 0.1%, **Figure 1B**).

Gene-set (pathway) burden

We assessed whether CNV burden was concentrated within defined sets of genes involved in neurodevelopment or neurological function. A total of 36 gene-sets were evaluated (for a description see **Extended data table 3**), consisting of gene-sets representing neuronal function, synaptic components and neurological and neurodevelopmental phenotypes in human (19 sets), gene-sets based on brain expression patterns (7 sets), and human orthologs of mouse genes whose disruption causes phenotypic abnormalities, including neurobehavioral and nervous system abnormality (10 sets). Some gene-sets can be considered “negative controls”, including genes not expressed in brain (1 set) or associated with abnormal phenotypes in mouse organ systems unrelated to brain (7 sets). We mapped CNVs to genes if they overlapped by at least one exonic bp.

Gene-set burden was tested using logistic regression deviance test⁶. In addition to using the same covariates included in genome-wide burden analysis, we controlled for the total number of genes per subject spanned by rare CNVs to account for signal that merely reflects the global enrichment of CNV burden in cases¹⁹. Multiple-testing correction (Benjamini-Hochberg False Discovery Rate, BH-FDR) was performed separately for each gene-set group and CNV type (gains, losses). After multiple test correction (Benjamini-Hochberg FDR ≤ 10%) 15 gene-sets were enriched for rare loss burden in cases and 4 for rare gains in cases, all of which are brain-related gene sets (**Figure 2**).

Of the 15 sets significant for losses, the majority consist of synaptic or other neuronal components (9 sets) from gene-set group (a); in particular, “GO synaptic” (GO:0045202) and “ARC complex” rank first based on statistical significance and effect-size respectively (“GO synaptic” deviance test p-value = 2.8e-11, “ARC complex” regression odds-ratio > 1.8, **Figure 2a**). Losses in cases were also significantly enriched for genes involved in nervous system or behavioral phenotypes in mouse but not for gene-sets related to other organ system phenotypes (**Figure 2c**). To account for dependency between synaptic and neuronal gene-sets,

we re-tested loss burden following a step-down logistic regression approach, ranking gene-sets based on significance or effect size (**Extended data table 4**). Only GO synaptic and ARC complex were significant in at least one of the two step-down analyses, suggesting that burden enrichment in the other neuronal categories is mostly accounted by the overlap with synaptic genes. Following the same approach, the mouse neurological/neurobehavioral phenotype set remained nominally significant, pointing to the existence of additional signal not captured by the synaptic set. Pathway enrichment was less pronounced for duplications, consistent with the smaller burden effects for this class of CNV. Duplication burden was significantly enriched for NMDA receptor complex, highly brain-expressed genes, medium/low brain-expressed genes and prenatally expressed brain genes (**Figure 2b**).

Given that synaptic gene sets were robustly enriched for deletions in cases, and with an appreciable contribution from loci that have not been strongly associated with SCZ previously, pathway-level interactions of these sets were further investigated. A protein-interaction network was seeded using the synaptic and ARC complex genes that were intersected by rare deletions in this study (**Figure 3**). A graph of the network highlights multiple subnetworks of synaptic proteins including pre-synaptic adhesion molecules (NRXN1, NRXN3), post-synaptic scaffolding proteins (DLG1, DLG2, DLGAP1, SHANK1, SHANK2), glutamatergic ionotropic receptors (GRID1, GRID2, GRIN1, GRIA4), and complexes such as Dystrophin and its synaptic interacting proteins (DMD, DTNB, SNTB1, UTRN). A subsequent test of the Dystrophin glycoprotein complex (DGC) revealed that deletion burden of the synaptic DGC proteins (intersection of “GO DGC” GO:0016010 and “GO synapse” GO:0045202) was enriched in cases (Deviance test $P = 0.05$), but deletion burden of the full DGC was not significant ($P = 0.69$).

Gene CNV burden

To define specific loci that confer risk for SCZ, we tested CNV burden at the level of individual genes, using logistic regression deviance test and the same covariates included in genome-wide burden analysis. To correctly account for large CNVs that affect multiple genes, we aggregated adjacent genes into single loci if their copy number was highly correlated across subjects. CNVs were mapped to genes if they overlapped one or more exons. The criterion for genome-wide

significance used the Family-Wise Error Rate (FWER) < 0.05 . The criterion for suggestive evidence used a Benjamini-Hochberg False Discovery Rate (BH-FDR) < 0.05 .

Of nineteen independent CNV loci with gene-based BH-FDR < 0.05 , two were excluded based on CNV calling accuracy or evidence of a batch effect (**Supplementary Information**). The seventeen loci that remained after these additional QC steps are listed in **Table 1**. P-values for this summary table were obtained by re-running our statistical model across the entire region (**Supplementary Results**). These seventeen loci represent a set of novel ($n=6$), previously reported ($n=4$), and previously implicated ($n=7$) loci. Manhattan plots of the gene association for losses and gains are provided in **Figure 4**. A permutation-based false discovery rate yielded similar estimates to BH-FDR.

Eight loci attain genome-wide significance, including copy number losses at 1q21.1, 2p16.3 (NRXN1), 3q29, 15q13.3, 16p11.2 (distal) and 22q11.2 along with gains at 7q11.23 and 16p11.2 (proximal). An additional nine loci meet criterion for suggestive association. Based on our estimation of False Discovery Rates (BH and permutations), we expect to observe less than two associations meeting suggestive criteria by chance.

Probe level CNV burden

With our current sample size and uniform CNV calling, many individual CNV loci can be tested with adequate power at the probe level, potentially facilitating discovery at a finer grain than locus-wide tests. Tests for association were performed at each CNV breakpoint using the residuals of case-control status after controlling for analysis covariates, with significance determined through permutation. Results for losses and gains are shown in **Extended data figure 4**. Four independent CNV loci surpass genome-wide significance, all of which were also identified in the gene-based test, including the 15q13.2-13.3 and 22q11.21 deletions, 16p11.2 duplication, and 1q21.1 deletion and duplication. While these loci represent less than half of the previously implicated SCZ loci, we do find support for all loci where the association originally reported meets the criteria for genome-wide correction in this study. We examined association among all previously reported loci showing association to SCZ, including 12 CNV

losses and 20 CNV gains (**Extended data table 5**), and 14 of the 33 loci were associated with SCZ at $p < .05$.

When a probe-level test is applied, associations at some loci become better delineated. For instance, The *NRXN1* gene at 2p16.3 is a CNV hotspot, and exonic deletions of this gene are significantly enriched in SCZ^{9,20}. In this large sample, we observe a high density of “non-recurrent” deletion breakpoints in cases and controls. The probe-level Manhattan plot reveals a saw tooth pattern of association, where peaks correspond to transcriptional start sites and exons of *NRXN1* (**Figure 5**). This example highlights how, with high diversity of alleles at a single locus, the association peak may become more refined, and in some cases converge toward individual functional elements. Similarly, a high density of duplication breakpoints at previously reported SCZ risk loci on 16p13.2 (<http://bit.ly/1NPgluq>) and 8q11.23 (<http://bit.ly/1PwYtTt>) exhibit patterns of association that better delineate genes in these regions.

[the above URLs link to a PGC CNV browser display of the respective genomic regions. The browser can also be accessed directly at the following URL
http://pgc.tcag.ca/gb2/gbrowse/pgc_hg18/]

Novel risk loci are predominantly NAHR-mediated CNVs

Many CNV loci that have been strongly implicated in human disease are hotspots for non-allelic homologous recombination (NAHR), a process which in most cases is mediated by flanking segmental duplications²¹. Consistent with the importance of NAHR in generating CNV risk alleles for schizophrenia, most of the loci in **Table 1** are flanked by segmental duplications. After excluding loci that have been implicated in previous studies, we investigated whether NAHR mutational mechanisms were also enriched among novel associated CNVs. We defined a CNV as “NAHR” when both the start and end breakpoint is located within a segmental duplication. Across all loci with FDR < 0.05 in the gene-base burden test, NAHR-mediated CNVs were significantly enriched, 6.03-fold ($P=0.008$; **Extended data figure 5**), when compared to a null distribution determined by randomizing the genomic positions of associated genes

(Supplemental Material). These results suggest that novel SCZ CNVs tend to occur in regions prone to high rates of recurrent mutation.

Discussion

The present study of the PGC SCZ CNV dataset includes the majority of all microarray data that has been generated in genetic studies of SCZ to date. In this, the best body of evidence to date with which to evaluate CNV associations, we find definitive evidence for eight loci and we find significant evidence for a contribution from novel CNVs conferring both risk and protection. The complete results, including CNV calls and statistical evidence at the gene or probe level, can be viewed using the PGC CNV browser (URLs). Our data suggest that the novel risk loci that can be detected with current genotyping platforms lie at the ultra-rare end of the frequency spectrum and still larger samples will be needed to identify them at convincing levels of statistical evidence.

Collectively, the eight SCZ risk loci that surpass genome-wide significance are carried by a small fraction (1.4%) of SCZ cases in the PGC sample. We estimate 0.85% of the variance in SCZ liability is explained by carrying a CNV risk allele within these loci (**Supplementary Results**). As a comparison, 3.4% of the variance in SCZ liability is explained by the 108 genome-wide significant loci identified in the companion PGC GWAS analysis. Combined, the CNV and SNP loci that have been identified to date explain a small proportion (<5%) of heritability.

The large dataset here provides an opportunity to evaluate the strength of evidence for a variety of loci where an association with schizophrenia has been reported previously. Of 33 published findings from the recent literature, we find evidence for 14 loci ($P < 0.05$, **Extended data table 5**); thus, nearly half of the existing candidate loci are supported by our data. However we also find a lack of evidence for many. A lack of strong evidence in this dataset (which includes samples that overlap with many of the previous studies) may in some cases simply reflect that statistical power is limited for very rare variants, even in large samples. However, it is likely that some of these original findings represent spurious associations. Indeed, the loci that are not supported by our data consist largely of loci for which the original statistical evidence was modest (**Extended data table 5**). Thus, our results help to refine the list

of promising candidate CNVs. Continued efforts to evaluate the growing number of candidate variants has considerable value for directing future research efforts focused on specific loci.

Novel candidate loci meeting suggestive criteria in this study highlight strong candidate loci that have not been previously implicated in SCZ. Two such associations are located on the X chromosome in a region of Xq28 that is highly prone to recurrent rearrangements²²⁻²⁴

(Extended data figure 6). Gains at the distal Xq28 locus are enriched in cases in this study; similar duplications have been reported in association with intellectual disability, while reciprocal deletions of this region are associated with embryonic lethality in males²⁵.

Duplications at the proximal Xq28 locus, including a single gene *MAGEA11*, are enriched in controls in this study, and to our knowledge have not been documented in other disorders.

We observed multiple “protective” CNVs that showed a suggestive enrichment in controls, including duplications of 22q11.2, *MAGEA11*, and *ZMYM5* along with deletions and duplications of *ZNF92*. No protective effects were significant after genome-wide correction. Moreover, a rare CNV that confers reduced risk for SCZ may not confer a general protection from neurodevelopmental disorders. For example, microduplications of 22q11.2 appear to confer protection from SCZ²⁶; however, such duplications have been shown to increase risk for developmental delay and a variety of congenital anomalies in pediatric clinical populations²⁷. It is probable that some of the undiscovered rare alleles in SCZ are variants that confer protection but larger sample sizes are needed to determine this unequivocally. If true, our estimates of the excess CNV burden in cases may not fully account for the variation SCZ liability that is explained by rare CNVs.

Our results provide strong evidence that deletions in SCZ are enriched within a highly connected network of synaptic proteins, consistent with previous studies^{2,6,10,28}. The large CNV dataset here allows a more detailed view of the synaptic network and highlights subsets of genes account for the excess deletion burden in SCZ, including synaptic cell adhesion and scaffolding proteins, glutamatergic ionotropic receptors and protein complexes such as the ARC complex and DGC. Modest CNV evidence implicating Dystrophin (DMD) and its binding partners is intriguing given that the involvement of certain components of the DGC have been

postulated^{29, 30} and disputed³¹ previously. Larger studies of CNV are needed to define a role for this and other synaptic sub-networks in SCZ.

This study represents a milestone. Large-scale collaborations in psychiatric genetics have greatly advanced discovery through genome-wide association studies. Here we have extended this framework to rare CNVs. Our knowledge of the contribution from lower frequency variants gives us confidence that the application of this framework to large newly acquired datasets has the potential to further the discovery of loci and identification of the relevant genes and functional elements. The PGC CNV resource is now publicly available through a custom browser at http://pgc.tcag.ca/gb2/gbrowse/pgc_hg18/.

Author Contributions

Management of the study, core analyses and content of the manuscript was the responsibility of the CNV Analysis Group chaired by J.S. and jointly supervised by S.W.S. and B.M.N. together with the Schizophrenia Working Group chaired by M.C.O'D. Core analyses were carried out by D.P.H., D.M., and C.R.M. Data Processing pipeline was implemented by C.R.M., B.T., W.W., D.G., M.G., A.S. and W.B. The A custom PGC CNV browser was developed by C.R.M., D.P.H., and B.T. Additional analyses and interpretations were contributed by W.W., D.A. and P.A.H. The individual studies or consortia contributing to the CNV meta-analysis were led by R.A., O.A.A., D.H.R.B., A.D.B., E. Bramon, J.D.B., A.C., D.A.C., S.C., A.D., E. Domenici, H.E., T.E., P.V.G., M.G., H.G., C.M.H., N.I., A.V.J., E.G.J., K.S.K., G.K., J. Knight, T. Lencz, D.F.L., Q.S.L., J. Liu, A.K.M., S.A.M., A. McQuillin, J.L.M., P.B.M., B.J.M., M.M.N., M.C.O'D., R.A.O., M.J.O., A. Palotie, C.N.P., T.L.P., M.R., B.P.R., D.R., P.C.S., P. Sklar, D.St.C., P.F.S., D.R.W., J.R.W., J.T.R.W. and T.W. The remaining authors contributed to the recruitment, genotyping, or data processing for the contributing components of the meta-analysis. J.S., B.M.N., C.R.M., D.P.H., and D.M. drafted the manuscript, which was shaped by the management group. All other authors saw, had the opportunity to comment on, and approved the final draft.

Competing Financial Interest

Several of the authors are employees of the following pharmaceutical companies: F.Hoffman-La Roche (E.D., L.E.), Eli Lilly (D.A.C., Y.M., L.N.) and Janssen (A.S., Q.S.L). None of these companies influenced the design of the study, the interpretation of the data, the amount of data reported, or financially profit by publication of the results, which are pre-competitive. The other authors declare no competing interests.

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URLs

PGC CNV browser, http://pgc.tcag.ca/gb2/gbrowse/pgc_hg18.

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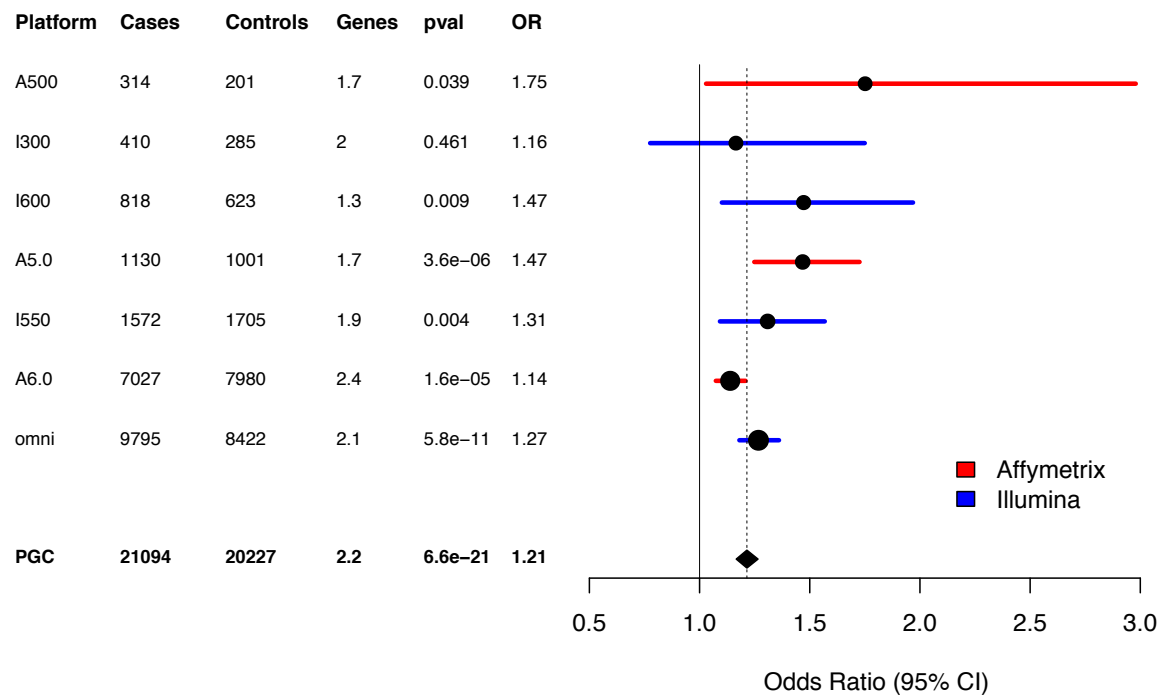
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CHR	BP1	BP2	Locus (GENE)	Putative CNV Mechanism	CNV test	Direction	FWER	BH-FDR	Cases	Controls	Regional <i>p</i> -value	Odds Ratio [95% C
22	17,400,000	19,750,000	22q11.21	NAHR	loss	risk	yes	3.54E-15	64	1	5.70E-18	67.7 [9.3-492.8]
16	29,560,000	30,110,000	16p11.2 (proximal)	NAHR	gain	risk	yes	5.82E-10	70	7	2.52E-12	9.4 [4.2-20.9]
2	50,000,992	51,113,178	2p16.3 (NRXN1)	NHEJ	loss	risk	yes	3.52E-07	35	3	4.92E-09	14.4 [4.2-46.9]
15	28,920,000	30,270,000	15q13.3	NAHR	loss	risk	yes	2.22E-05	28	2	2.13E-07	15.6 [3.7-66.5]
1	144,646,000	146,176,000	1q21.1	NAHR	loss+gain	risk	yes	0.00011	60	14	1.50E-06	3.8 [2.1-6.9]
3	197,230,000	198,840,000	3q29	NAHR	loss	risk	yes	0.00024	16	0	1.86E-06	NA [0-Inf]
16	28,730,000	28,960,000	16p11.2 (distal)	NAHR	loss	risk	yes	0.0029	11	1	5.52E-05	20.6 [2.6-162.2]
7	72,380,000	73,780,000	7q11.23	NAHR	gain	risk	yes	0.0048	16	1	1.68E-04	16.1 [3.1-125.7]
X	153,800,000	154,225,000	Xq28 (distal)	NAHR	gain	risk	no	0.049	18	2	3.61E-04	8.9 [2.0-39.9]
22	17,400,000	19,750,000	22q11.21	NAHR	gain	protective	no	0.024	3	16	4.54E-04	0.15 [0.04-0.52]
7	64,476,203	64,503,433	7q11.21 (ZNF92)	NAHR	loss+gain	protective	no	0.033	131	180	6.71E-04	0.66 [0.52-0.84]
13	19,309,593	19,335,773	13q12.11 (ZMYM5)	NHAR	gain	protective	no	0.024	15	38	7.91E-04	0.36 [0.19-0.67]
X	148,575,477	148,580,720	Xq28 (MAGEA11)	NAHR	gain	protective	no	0.044	12	36	1.06E-03	0.35 [0.18-0.68]
15	20,350,000	20,640,000	15q11.2	NAHR	loss	risk	no	0.044	98	50	1.34E-03	1.8 [1.2-2.6]
9	831,690	959,090	9p24.3 (DMRT1)	NHEJ	loss+gain	risk	no	0.049	13	1	1.35E-03	12.4 [1.6-98.1]
8	100,094,670	100,958,984	8q22.2 (VPS13B)	NHEJ	loss	risk	no	0.048	7	1	1.74E-03	14.5 [1.7-122.2]
7	158,145,959	158,664,998	7p36.3 (VIPR2/WDR60)	NAHR	loss+gain	risk	no	0.046	20	6	5.79E-03	3.5 [1.3-9.0]

Table 1: Significant CNV loci from gene-based association test

All seventeen loci listed contain at least one gene with Benjamini-Hochberg false discovery rate (BH-FDR) < 0.05 in the gene-based test, with eight loci containing at least one gene surpassing the family-wise error rate (FWER) < 0.05. Genomic positions listed are using hg18 coordinates. For putative CNV mechanisms, non-allelic homologous recombination (NAHR) and non-homologous end joining (NHEJ) are listed as the likely genomic feature driving CNV formation at each locus. Regional *p*-values and odds ratios listed are from a regional test at each locus, where we combine CNV overlapping the implicated region and run the same test as used for each gene (logistic regression with covariates and deviance test *p*-value).

A



B

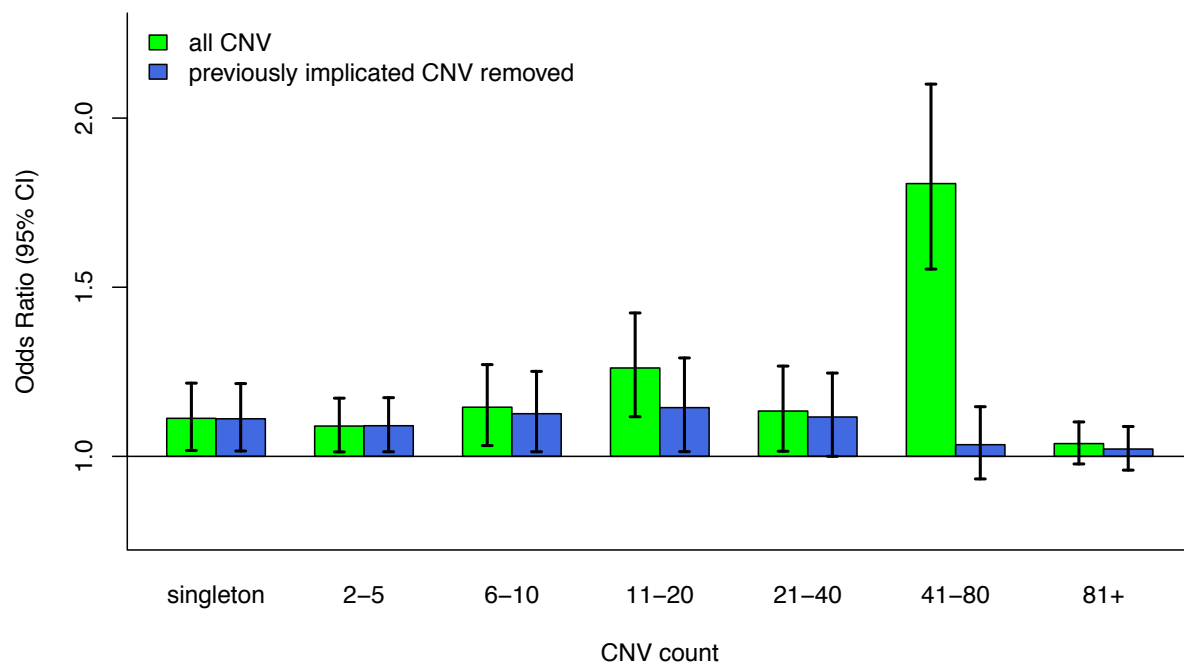


Figure 1. CNV Burden

(A) Forest plot of CNV burden (measured here as genes affected by CNV), partitioned by genotyping platform, with the full PGC sample at the bottom. CNV burden is calculated by combining CNV gains and losses. Case and control counts are listed, and “genes” is the rate of genes affected by CNV in controls. Burden tests use a logistic regression model predicting SCZ case/control status by CNV burden along with covariates (see methods). The odds ratio is the exponential of the logistic regression coefficient, and odds ratios above one predict increased SCZ risk. **(B)** CNV burden partitioned by CNV frequency. For reference, a CNV with MAF 0.1% in the PGC sample would have ~41 CNVs. Using the same model as above, each CNV was placed into a single CNV frequency category based on a 50% reciprocal overlap with other CNVs. CNV burden with inclusion of all CNVs are shown in green, whereas CNV burden excluding previously implicated CNV loci are shown in blue.

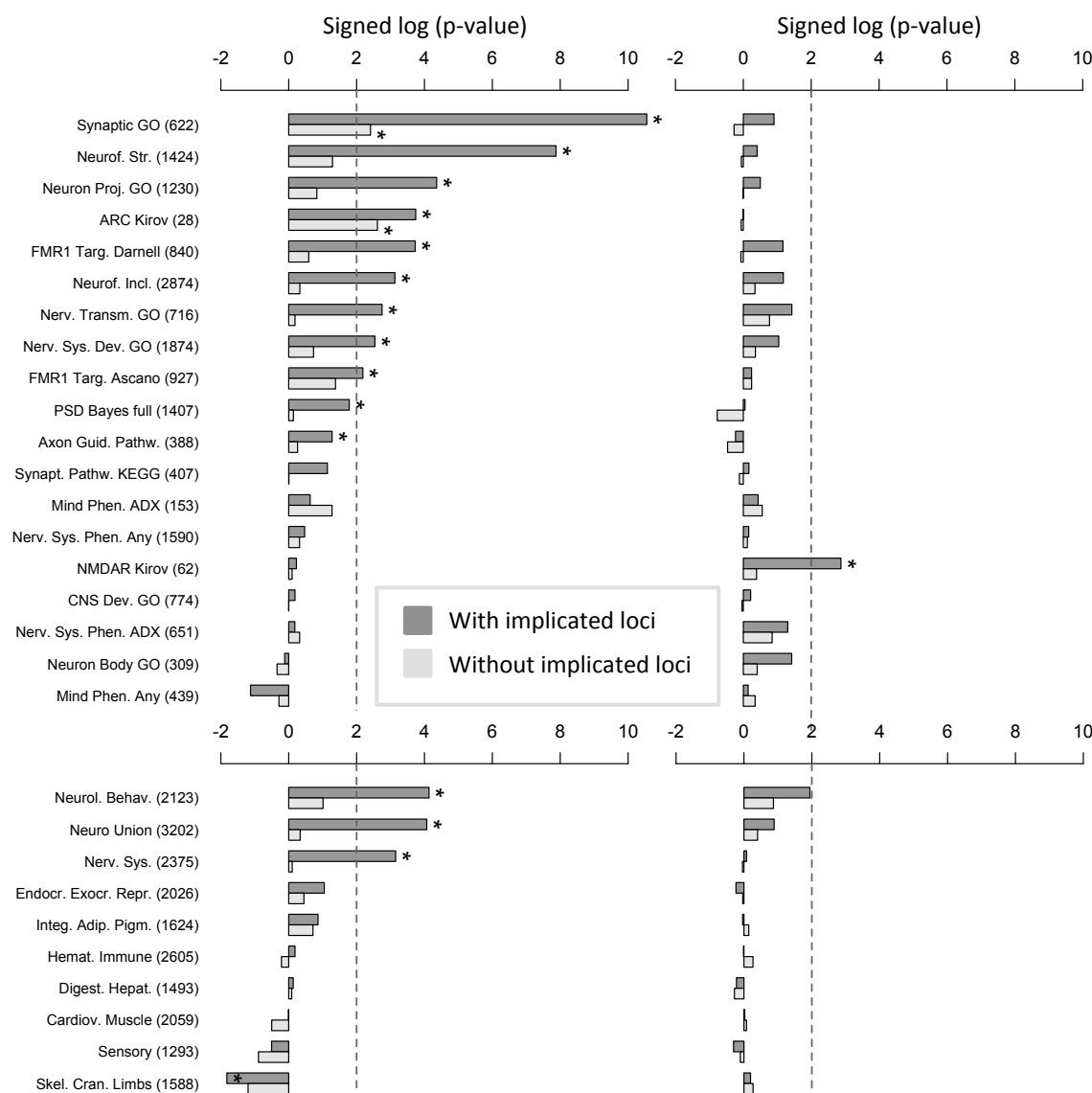


Figure 2: Gene-set Burden

Gene-set burden test results for rare losses (a, c) and gains (b, d); frames a-b display gene-sets for neuronal function, synaptic components, neurological and neurodevelopmental phenotypes in human; frames c-d display gene-sets for human homologs of mouse genes implicated in abnormal phenotypes (organized by organ systems); both are sorted by $-\log_{10}$ of the logistic regression deviance test p-value multiplied by the beta coefficient sign, obtained for rare losses when including known loci. Gene-sets passing the 10% BH-FDR threshold are marked with “*”.

Gene-sets representing brain expression patterns were omitted from the figure because only a few were significant (losses: 1, gains: 3).

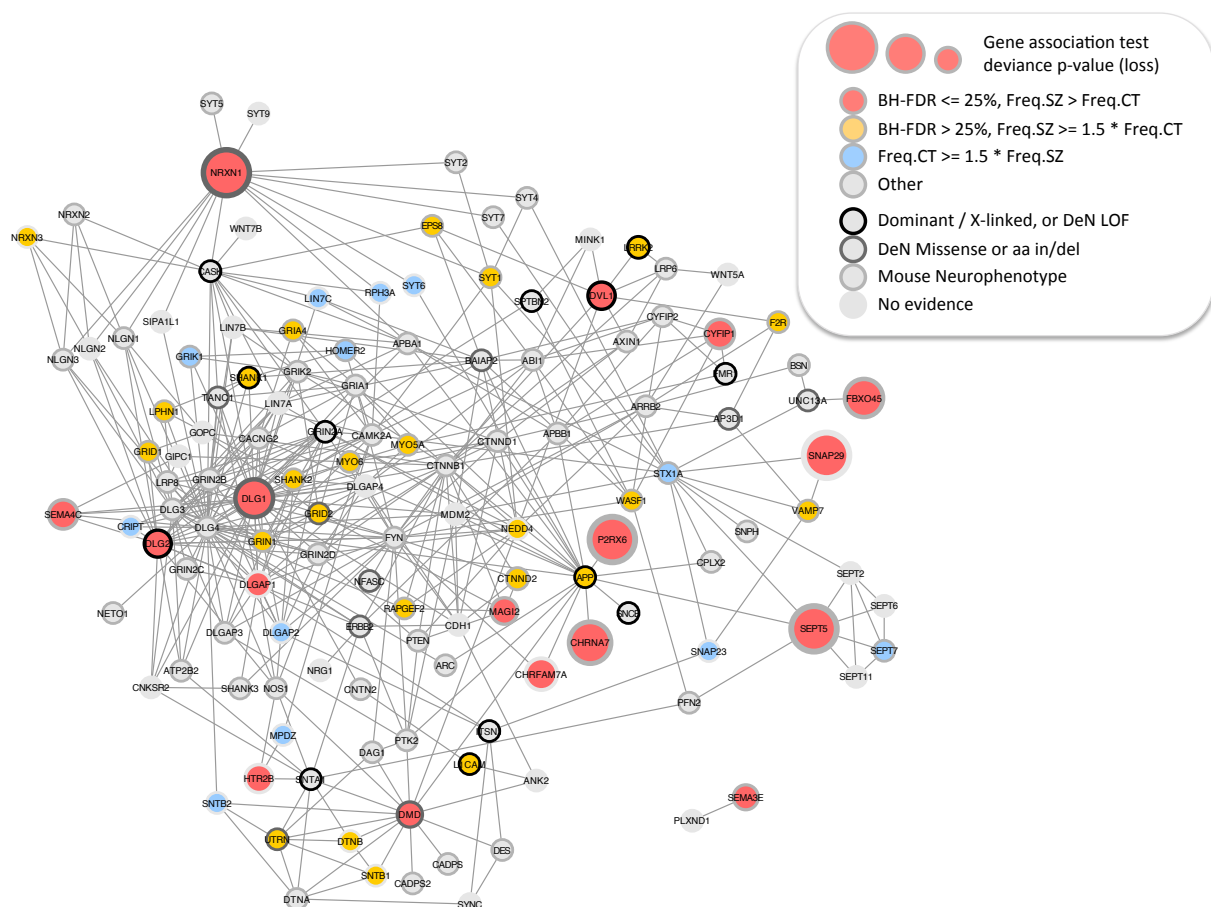


Figure 3: Protein Interaction Network for Synaptic Genes

Synaptic and ARC-complex genes intersected by a rare loss in at least 4 case or control subjects and with genic burden Benjamini-Hochberg FDR $\leq 25\%$ (red discs) were used to query GeneMANIA³² and retrieve additional protein interaction neighbors, resulting in a network of 136 synaptic genes. Genes are depicted as disks; disk centers are colored based on rare loss frequency (Freq.SZ and Freq.CT) being prevalent in cases or controls; disk borders are colored to mark (i) gene implication in human dominant or X-linked neurological or neurodevelopmental phenotype, (ii) *de novo* mutation (DeN) reported by Fromer et al.²⁸, split between LOF (frameshift, stop-gain, core splice site) and missense or amino acid insertion / deletion, (iii) implication in mouse neurobehavioral abnormality. Pre-synaptic adhesion molecules (NRXN1, NRXN3), post-synaptic scaffolds (DLG1, DLG2, DLGAP1, SHANK1, SHANK2)

and glutamatergic ionotropic receptors (GRID1, GRID2, GRIN1, GRIA4) constitute a highly connected subnetwork with more losses in cases than controls.

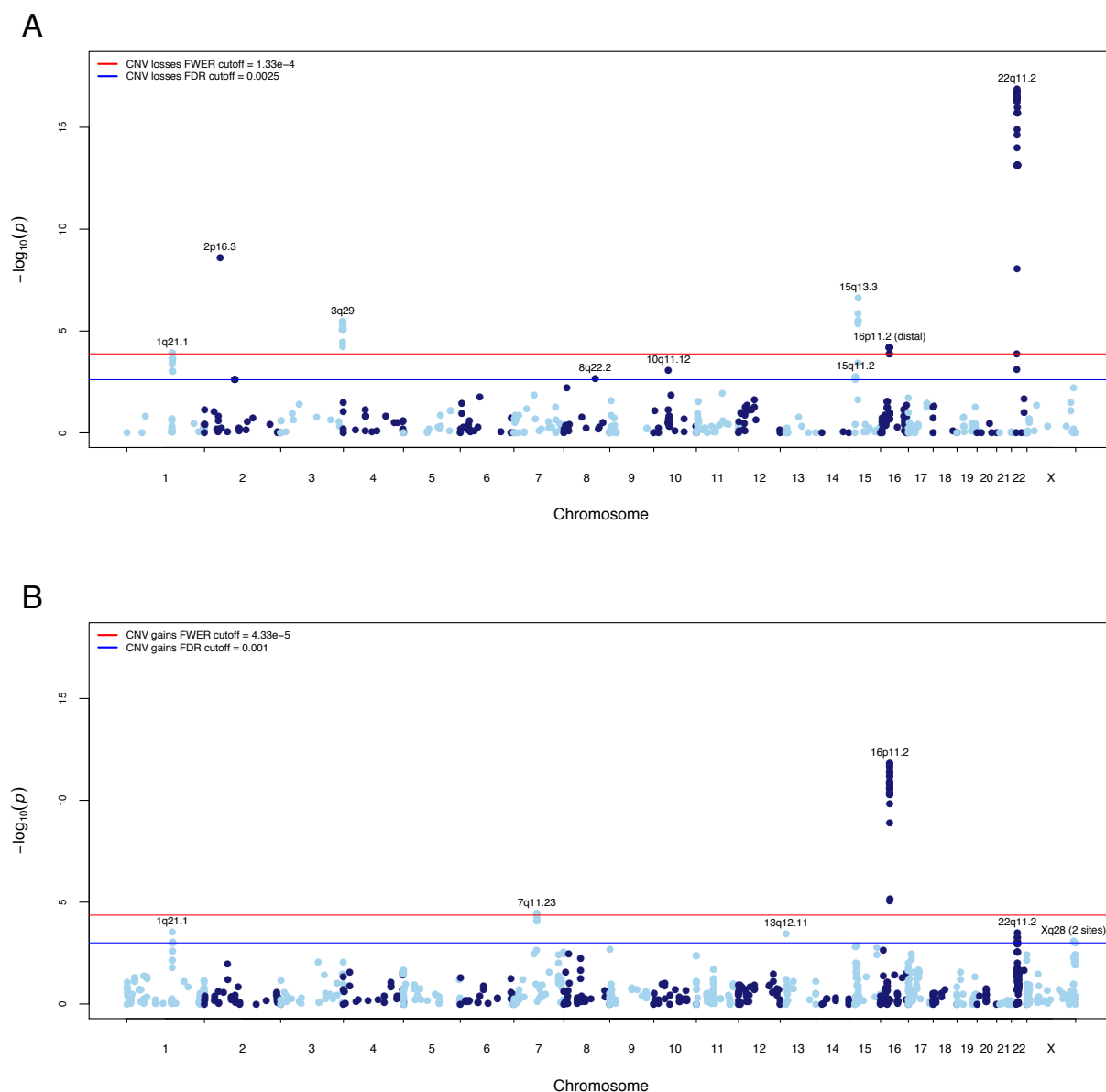


Figure 4: Gene Based Manhattan Plot.

Manhattan plot displaying the $-\log_{10}$ deviance p -value for **(A)** CNV losses and **(B)** CNV gains the gene-based test. P -value cutoffs corresponding to FWER < 0.05 and BH-FDR < 0.05 are highlighted in red and blue, respectively. Loci significant after multiple test correction are labeled.

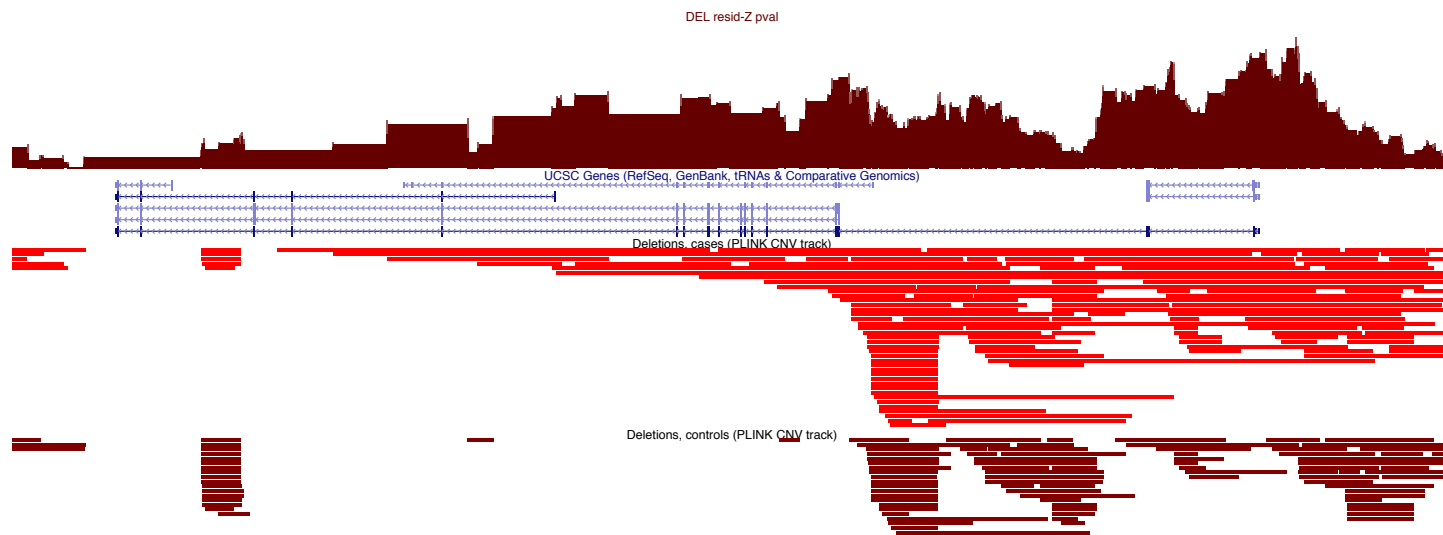


Figure 5: Manhattan plot of probe-level associations across the Neurexin-1 locus

Empirical p -values at each deletion breakpoint reveal a saw-tooth pattern of association. Predominant peaks correspond to exons and transcriptional start sites of NRXN1 isoforms.

Locus	CNV type	Gene or region name	Initial SCZ association reference (see legend)	Tested in Rees et al. 2014	Reported <i>p</i> -value	SCZ CNV carrier %	Control CNV carrier %	Reported Odds Ratio
22q11.2	deletion	multigenic	1	yes	4.40E-40	0.29	0	Inf
16p11.2	duplication	proximal duplication	2	yes	2.90E-24	0.35	0.03	11.52
1q21.1	deletion	multigenic	3,4	yes	4.10E-13	0.17	0.021	8.35
2p16.3	deletion	NRXN1 exons	5,6	yes	1.30E-11	0.18	0.02	9.01
15q11.2	deletion	multigenic	3	yes	2.50E-10	0.59	0.28	2.15
3q29	deletion	multigenic	7,11	yes	1.50E-09	0.082	0.0014	57.65
15q13.2-13.3	deletion	multigenic	3,4	yes	5.60E-06	0.14	0.019	7.52
15q11.2-13.1	duplication	AS/PWS	8	yes	5.60E-06	0.083	0.0063	13.2
8q11.23	duplication	RB1CC1	9	no	1.29E-05	0.106	0.014	8.58
16p13.11	duplication	multigenic	8	yes	5.70E-05	0.31	0.13	2.3
7q11.23	duplication	Williams-Beuren	10	yes	6.90E-05	0.066	0.0058	11.35
1q21.1	duplication	multigenic	11	yes	9.90E-05	0.13	0.037	3.45
16p13.2	duplication	C16orf72/USP7	11	no	1.00E-04	0.254	0.0197	12.9
1p36.33	duplication	multigenic	12	no	5.00E-04	0.065	0.0075	8.66
22q11.2	duplication	multigenic	13	no	8.60E-04	0.014	0.085	0.17
17p12	deletion	HNPP	14	yes	1.20E-03	0.094	0.026	3.62
9q34.3	duplication	intergenic	15	no	1.40E-03	1.47	0.43	3.38
16p12.1	deletion	multigenic	12	no	1.60E-03	0.15	0.057	2.72
15q21.3	duplication	CGNL1	12	no	1.90E-03	0.32	0.19	1.71
11q25	deletion	GLB1L3/GLB1L2	11	no	3.00E-03	0.38	0.123	3
2q37.3	duplication	AQP12A/KIF1A	12	no	3.00E-03	0.34	0.24	1.43
17q12	deletion	RCAD	16	yes	0.0072	0.036	0.0054	6.64
9p24.2	deletion	GLIS3	12	no	8.40E-03	0.033	0	Inf

9p24.2	deletion	SLC1A1	12	no	9.80E-03	0.047	0.0075	6.19
16p11.2	deletion	distal deletion	17	yes	0.017	0.063	0.018	3.39
7q36.3	duplication	WDR60/VIPR2	11,18	yes	0.27	0.11	0.069	1.54

Extended Data Table 1: Previously reported CNV association

We assembled a list of 26 reported CNV associations to SCZ, where an odds ratio and p -value were available. At each CNV locus, we list the odds ratios and p -values from the largest sample collection available in the literature. Results from all CNV loci meta-analyzed in Rees et al. (2014), when available, were used. Throughout this article we refer to this entire list as “previously reported” loci. Reported p -values for nine loci shown in bold surpass the multiple testing threshold drawn from the current dataset using a Cochran-Mantel Haenszel test stratified by genotyping platform. Throughout this article we refer to these nine loci as “previously implicated”.

Extended Data Tables 2-4 are separate .xlsx sheets available upon request.

ED Table 2 datasets.xlsx – datasets and sample sizes used in the current study

ED Table 3 NeuroGeneSsets.xlsx – Gene sets investigated in the current study

ED Table 4 stepdown_withLegend.xlsx – Unique contribution of significant gene sets in step-down regression models

Locus	CNV type	Gene or region	Reference (see ED table 1)	Z-score p-value	CMH p-value	CNV carriers N = 41321	SCZ Case count N = 21094	Control count N = 20227	CMH test Odds Ratio
22q11.21	Deletion	Multigenic	1	6.20E-13	4.40E-13	58	58	0	NA
16p11.2	Duplication	Proximal duplication	2	2.60E-10	9.40E-12	67	63	4	13.8
15q13.2-13.3	Deletion	Multigenic	3,4	6.10E-06	2.50E-06	33	30	3	10.55
3q29	Deletion	Multigenic	7	6.20E-05	1.60E-04	16	16	0	NA
2p16.3	Deletion	NRXN1	5,6	9.40E-05	3.00E-04	27	23	4	5.87
16p11.2	Deletion	Distal deletion	17	1.00E-04	5.20E-03	12	11	1	12.68
22q11.21	Duplication	Multigenic	12	1.60E-04	6.30E-04	27	4	23	0.18
1q21.1	Deletion	Multigenic	3,4	2.90E-04	2.70E-05	39	33	6	5.42
16p13.2	Duplication	C16orf72/USP7	11	3.80E-04	1.10E-04	27	24	3	9.02
7q11.23	Duplication	Williams-Beuren	10	4.90E-04	1.70E-03	13	13	0	NA
15q11.2-13.1	Duplication	AS/PWS	8	6.60E-04	4.40E-04	15	15	0	NA
8q11.23	Duplication	FAM150/RB1CC1	9	9.20E-04	5.10E-04	14	14	0	NA
15q11.2	Deletion	Multigenic	3	1.70E-03	1.30E-03	142	95	47	1.8
1q21.1	Duplication	Multigenic	11	2.00E-03	0.02	21	19	2	6.28
16q22.1	Duplication	WWP2	19	0.003	0.08	5	5	0	NA
7q36.3	Duplication	WDR60/VIPR2	11,18	4.10E-03	2.40E-03	14	13	1	12.12
17q12	Duplication	RCAD duplication	19	0.009	0.02	20	16	4	3.81
9q33.1	Deletion	NA	19	0.02	0.09	11	9	2	4.02
22q11.23	Duplication	Multigenic	19	0.02	0.03	20	15	5	3.28
5q21.2	Deletion	NA	19	0.03	0.07	32	22	10	2.16
8p22	Duplication	SGCZ	19	0.03	0.08	5	5	0	NA
9p24.2	Deletion	SLC1A1	12	0.03	0.02	8	8	0	NA
16p12.1	Deletion	Multigenic	12	0.03	0.006	33	26	7	3.22
15q21.3	Duplication	CGNL1	12	0.04	1.30E-03	103	69	34	1.99
17q12	Deletion	RCAD	16	0.04	0.13	4	4	0	NA
16p13.11	Del/Dup	Multigenic	8	0.08	0.03	139	84	55	1.49
7q11.21	Duplication	NA	19	0.09	0.35	64	26	38	0.76
12q23.1	Duplication	ANKS1B/UHRF1BP1L	19	0.1	0.73	28	16	12	1.23
1p36.33	Duplication	Multigenic	12	0.11	0.06	15	12	3	3.98
5q33.1	Deletion	NA	12	0.11	0.1	11	9	2	4.19
9q21.33	Duplication	AGTPBP1	11	0.2	0.21	22	15	7	1.94
9q34.3	Duplication	C9orf62	15	0.23	0.03	409	190	219	0.8
6q24.2	Duplication	PHACTR2	12	0.26	0.15	10	8	2	4.03
3q26.1	Deletion	NA	11	0.27	0.43	5	4	1	3.5
4q35.2	Deletion	TRIML1/TRIML2	12	0.35	0.21	26	17	9	1.82
18q21.31	Duplication	NEDD4L	11	0.39	0.7	2	2	0	NA
11q25	Deletion	GLB1L3/GLB1L2	11	0.42	0.22	58	34	24	1.44
9p24.2	Deletion	GLIS3	12	0.43	0.99	10	5	5	0.99
18q23	Duplication	GALR1	12	0.57	0.81	7	4	3	1.22

4q35.2	Duplication	FAM149A/CYP4V2	12	0.69	0.77	12	5	7	0.71
2q37.2	Duplication	AQP12A/KIF1A	12	0.72	0.14	125	72	53	1.34
17p12	Deletion	HNPP	14	0.82	0.89	22	12	10	1.06
4q25	Duplication	ELOVL6	12	0.9	0.99	13	7	6	1
10q11.21	Duplication	Likely common CNV	19	NA	NA	NA	NA	NA	NA

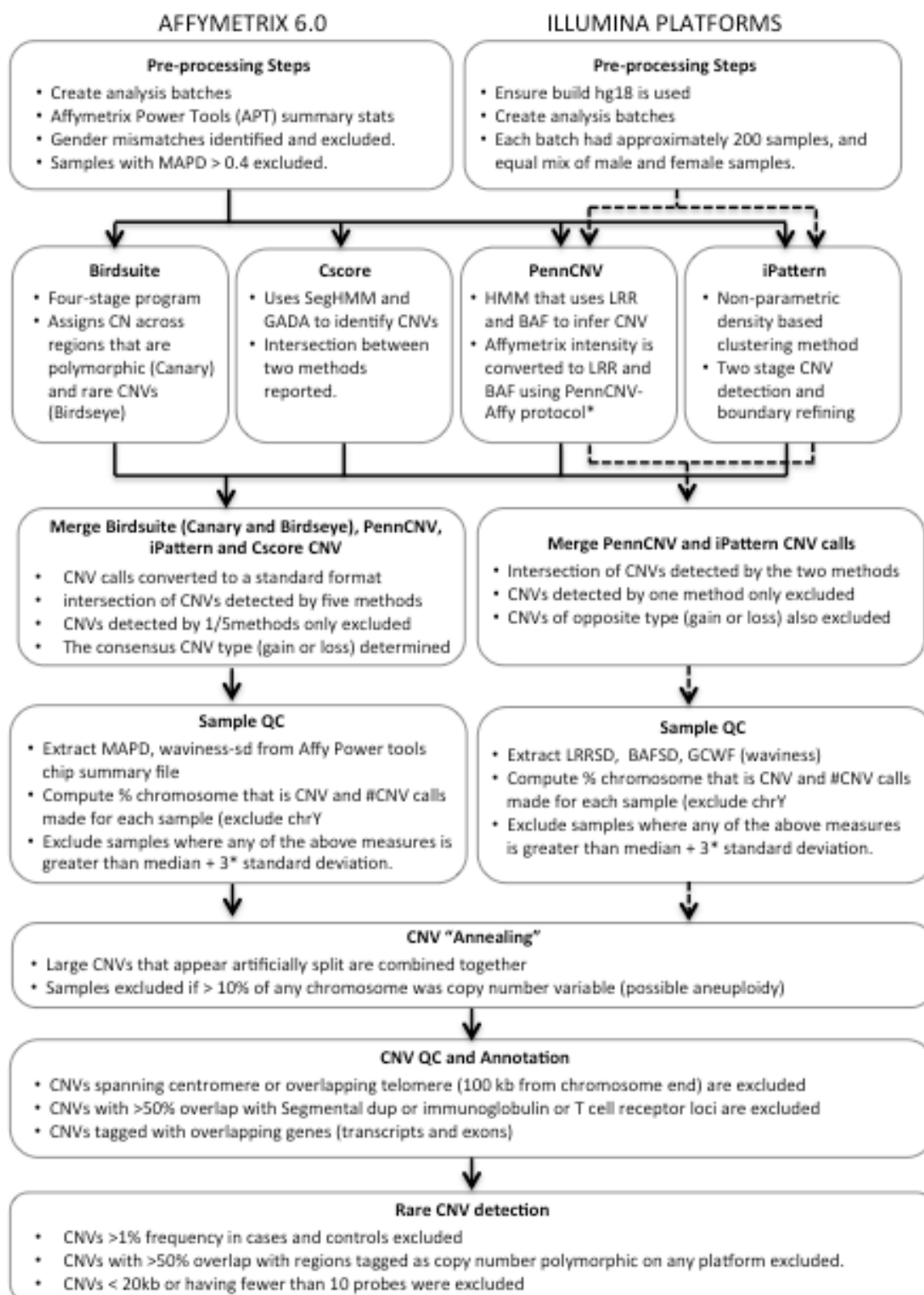
Extended data Table 5: CNV probe-level results – Previously reported CNVs

Probe-level association results for all previously reported CNVs from genome-wide scans of SCZ. We report association results from the SCZ residual phenotype and from a CMH test stratified by genotyping platform. CNV loci in bold make up previously implicated loci, in which the most recent published *p*-value surpassed genome-wide correction.

Extended data table references

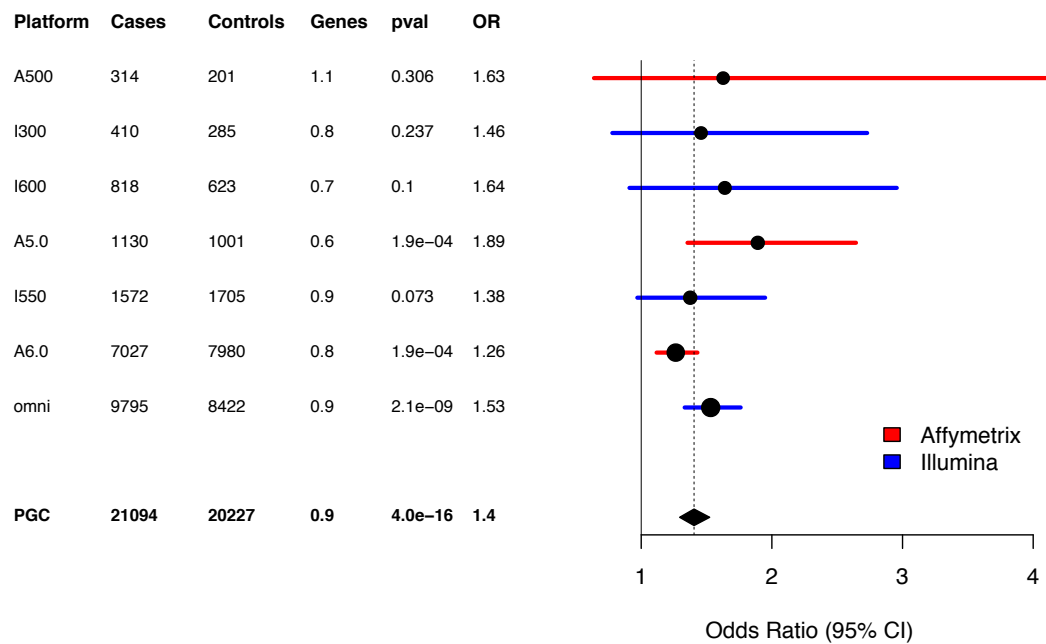
1. Shprintzen, R.J., Goldberg, R., Golding-Kushner, K.J. & Marion, R.W. Late-onset psychosis in the velo-cardio-facial syndrome. *Am J Med Genet* **42**, 141-2 (1992).
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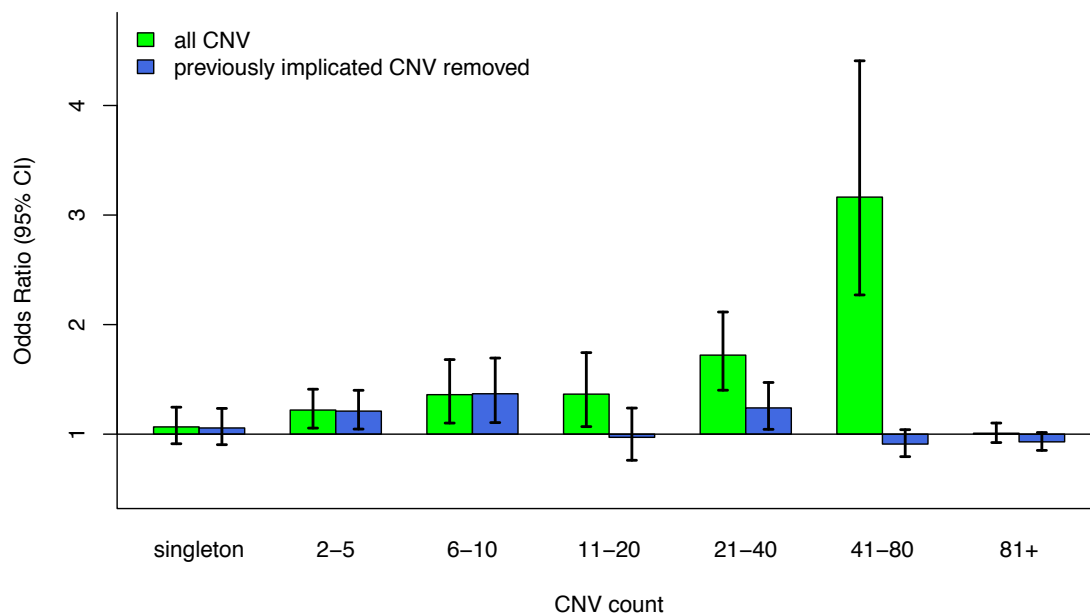


Extended Data Figure 1: CNV pipeline workflow

A

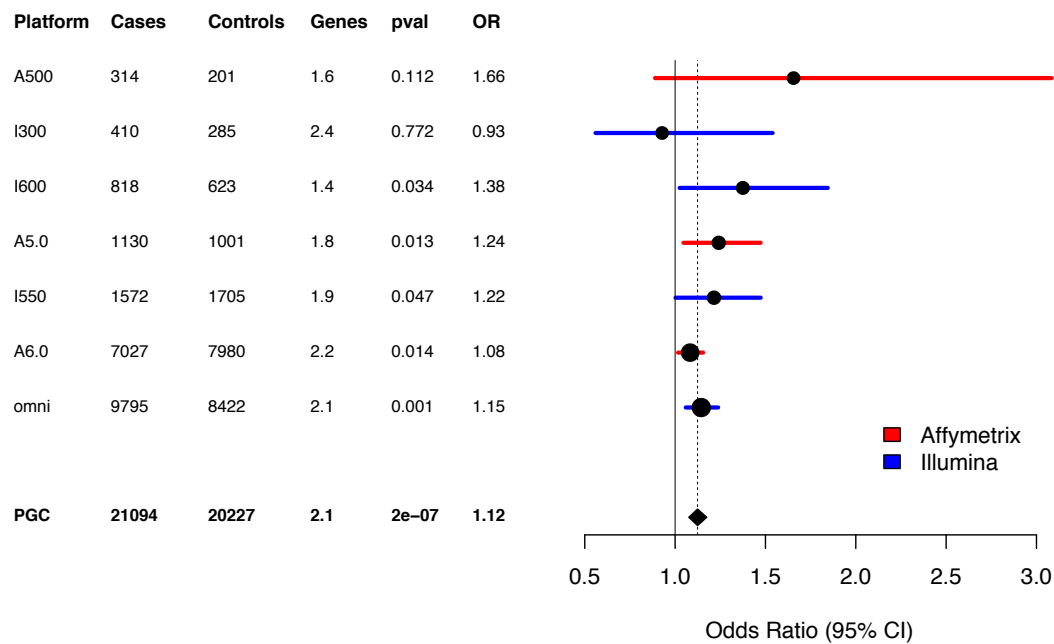


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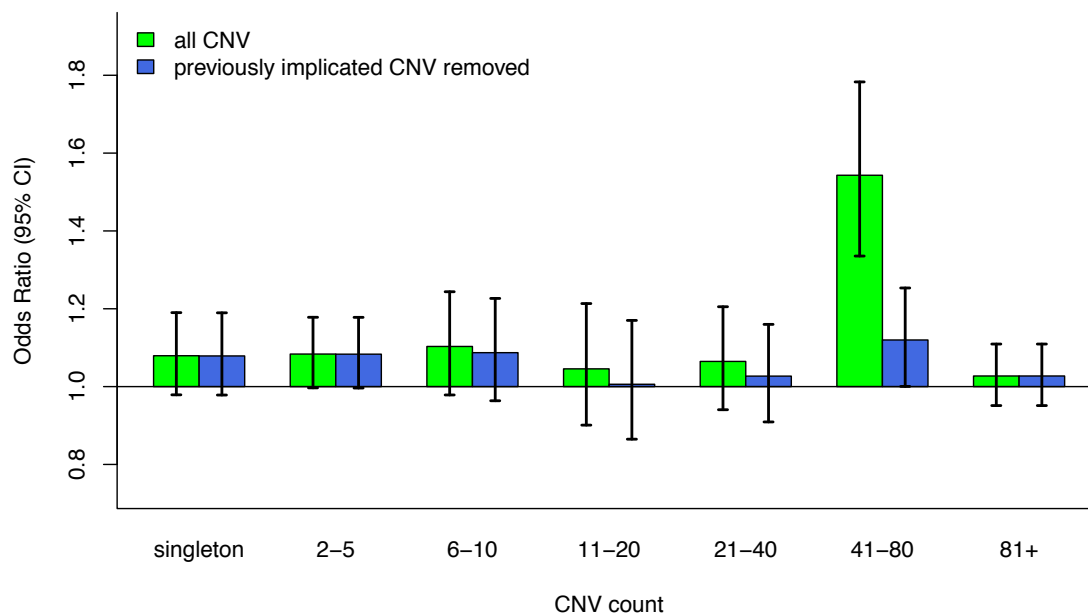


Extended Data Figure 2: CNV burden for losses: Fig 3A: Forest plot of CNV burden (genes affected) partitioned by genotyping platform. Fig 3B: CNV burden partitioned by CNV frequency.

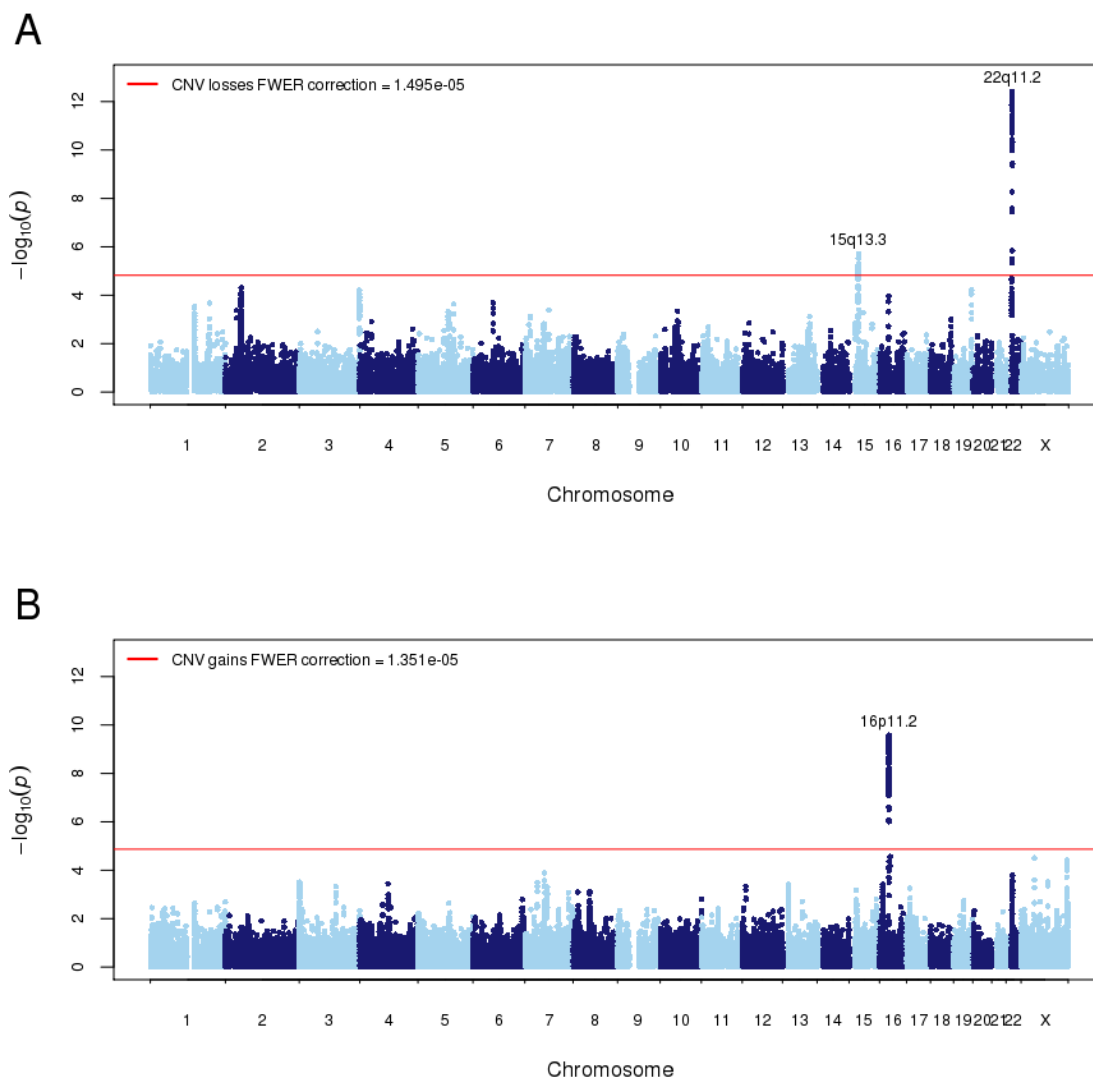
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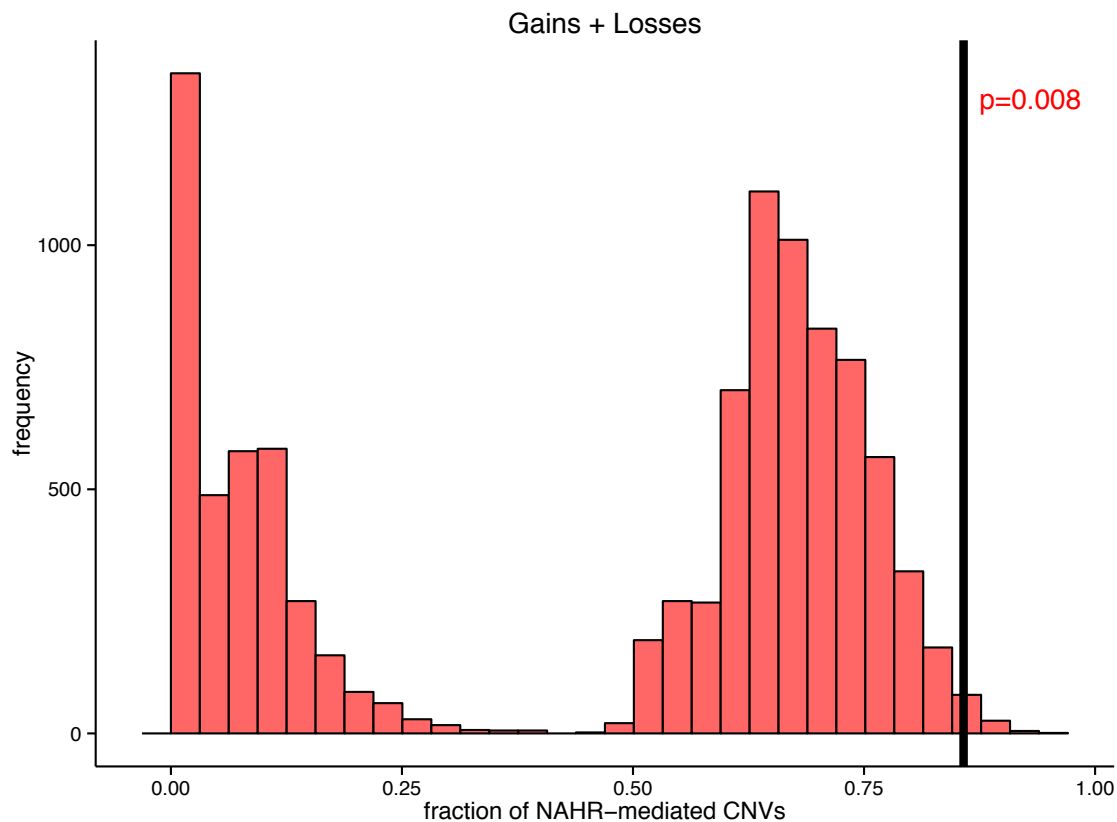
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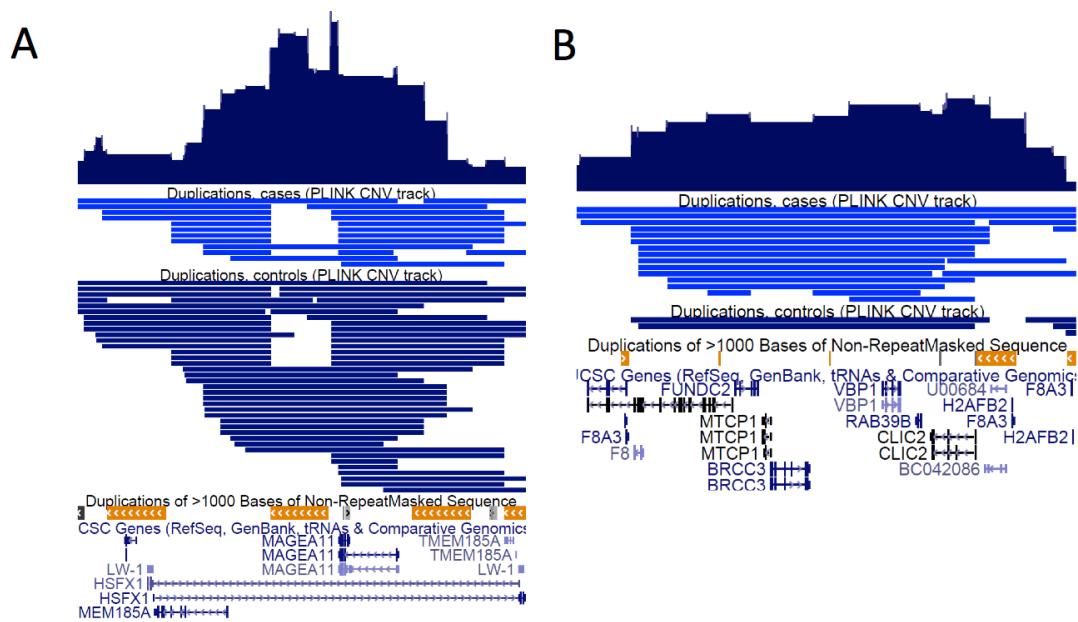
Extended Data Figure 3: CNV burden for gains: Fig 4A: Forest plot of CNV burden (genes affected) partitioned by genotyping platform. Fig 4B: CNV burden partitioned by CNV frequency.



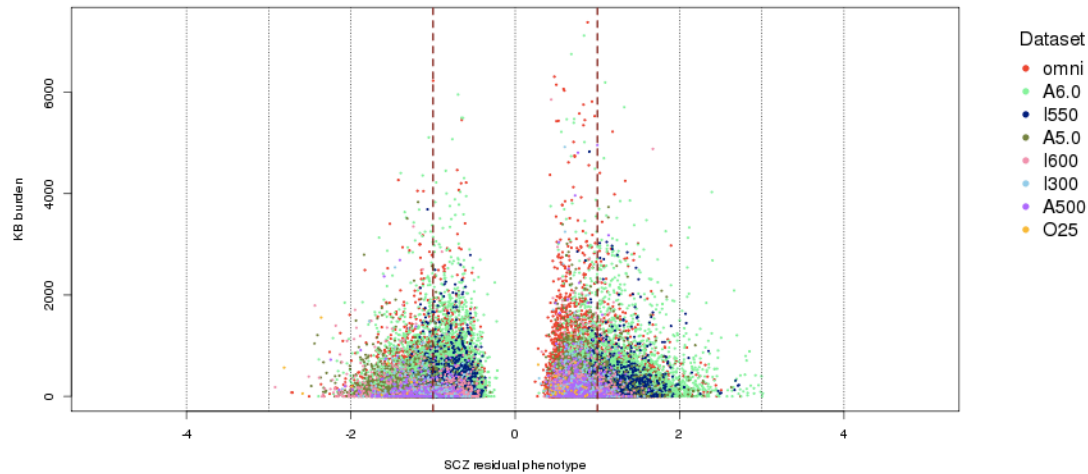
Extended Data Figure 4: CNV probe level Manhattan plot: Manhattan plot of probe-level association results from the SCZ residual phenotype. Fig 5A: CNV losses Fig 5B: CNV gains. Genome-wide correction was determined using the family-wise error rate (FWER) drawn from permutation.



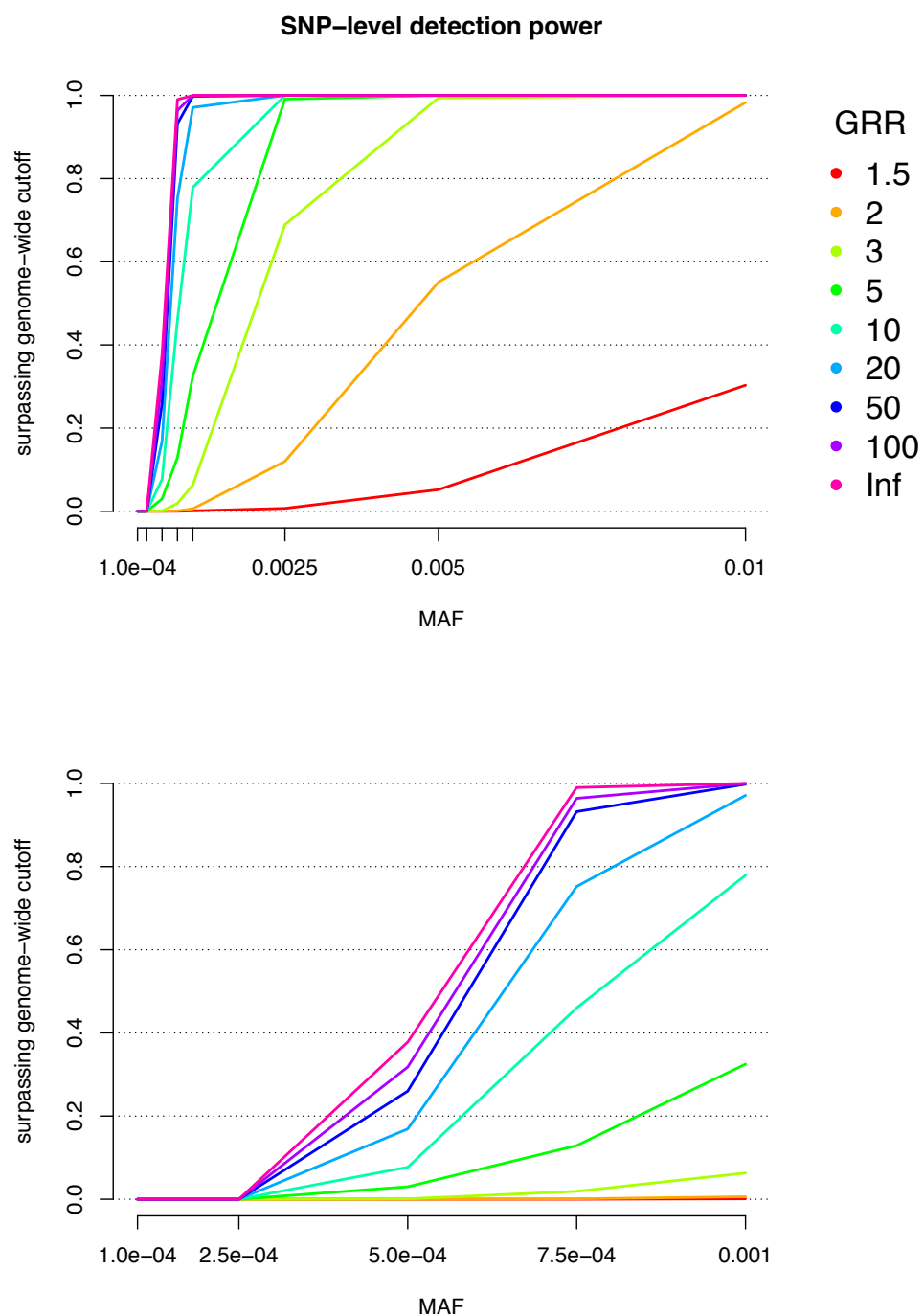
Extended Data Figure 5: Permutation of NAHR-mediated CNVs: Permutation results from drawing frequency-match CNV loci and testing for fraction of NAHR-mediated CNVs. To test for the enrichment of NAHR-mediated loci in our suggestive results from the gene-based test, each permutation selected an equivalent number of independent CNV loci and tested the fraction of NAHR-mediated CNVs.



Extended Data Figure 6: Xq28 CNV hotspot: Fig 6A: Protective CNV gain association peak around the MAGEA11 and TMEM185A gene, both within an intron of the HSFX1 gene. Fig 6B: Risk CNV gain association peak at the distal end of Xq28 overlapping ten genes.



Extended Data Figure 7: SCZ phenotype residual distribution: X-axis: Distribution of phenotype residual values after regressing case/control status on selected covariates. Plotted against overall CNV Kb burden (Y-axis) to visually inspect if individuals with large residuals have an excess of CNV burden, which can lead to higher false positive associations. SCZ cases have positive residual values and controls negative residual values.



Extended Data Figure 8: Detection power for CNV losses: Power is the proportion of simulated causal CNV loci detected (e.g. surpassing genome-wide FWER correction) using probe-level association. Each graph plots power across various MAF (x-axis) and

genotype relative risk (GRR: colored lines). Simulations use the sample size and FWER cutoff from the current study.

Methods

Overview

We assembled a CNV analysis group with members from Broad Institute, Children's Hospital of Philadelphia, University of Chicago, University of California San Diego, University of Michigan, University of North Carolina, Colorado University Boulder, and University of Toronto/SickKids Hospital. Our aim was to leverage the extensive expertise of the group to develop a fully automated centralized pipeline for consistent and systematic calling of CNVs for both Affymetrix and Illumina platforms. An overview of the analysis pipeline is shown in **Extended Data Figure 1**. After an initial data formatting step we constructed batches of samples for processing using four different methods, PennCNV, iPattern, C-score (GADA and HMMSeg) and Birdsuite for Affymetrix 6.0. For Affymetrix 5.0 data we used Birdsuite and PennCNV, for Affymetrix 500 we used PennCNV and C-score, and for all Illumina arrays we used PennCNV and iPattern. We then constructed a consensus CNV call dataset by merging data at the sample level and further filtered calls to make a final dataset **Extended data table 2**. Prior to any filtering, we processed raw genotype calls for a total of **57,577** individuals, including **28,684** SCZ cases and **28,893** controls.

Study Sample

A complete list of datasets that were included in the current study can be found in **Extended Data Table 2**. A more detailed description of the original studies can be found in a previous publication¹

Copy Number Variant Analysis Pipeline Architecture and Sample Processing

All aspects of the CNV analysis pipeline were built on the Genetic Cluster Computer (GCC) in the Netherlands. PGC members sent external drives of raw data to the Netherlands for upload to the server as well as the corresponding sample metadata files.

Input Acceptance and Preprocessing: For Affymetrix we used the *.CEL files (all converted to the same format) as input, whereas for Illumina we required Genome or Beadstudio exported *.txt files with the following values: Sample ID, SNP Name, Chr, Position, Allele1 – Forward, Allele2 – Forward, X, Y, B Allele Freq and Log R Ratio. Samples were then partitioned into ‘batches’ to be run through each pipeline. For Affymetrix samples we created analysis batches based on the plate ID (if available) or genotyping date. Each batch had approximately 200 samples with an equal mix of male and female samples. Affymetrix Power Tools (APT - apt-copynumber-workflow) was then used to calculate summary statistics about chips analyzed. Gender mismatches identified and excluded as were experiments with MAPD > 0.4. For Illumina data, we first determined the genome build and converted to hg18 if necessary and created analysis batches based on the plate ID or genotyping date. Each batch had approximately 200 samples, and equal mix of male and female samples.

Composite Pipeline: The composite pipeline comprises CNV callers PennCNV², iPattern³, Birdsuite⁴ and C-Score⁵ organized into component pipelines. We used all four callers for Affymetrix 6.0 data, PennCNV and C-Score for Affymetrix 500, Probe annotation files were preprocessed for each platform. Once the array design files and probe annotation files were pre-processed, each individual pipeline component pipeline was run in two steps: 1) processing the intensity data by the core pipeline process to produce CNV calls, 2) parsing the specific output format of the core pipeline and converting the calls to a standard form designed to capture confidence scores, copy number states and other information computed by each pipeline

Merging of CNV data and Quality control filtering

Merging of CNV data: After standardization of outputs from each algorithm, CNV calls from each algorithm were merged at the sample level to increase specificity³. For CNVs generated from Affymetrix 6.0 array, we took the intersection of the four outputs

(Birdsuite, iPattern, C-Score, PennCNV) at the sample level to create a consensus CNV. For the Affymetrix 500, Affymetrix 5.0, and Illumina platforms, CNV merging was performed by taking the intersection of the calls made by the two algorithms (PennCNV and C-Score for Affymetrix 500, Birdsuite and PennCNV for Affymetrix 5.0, and iPattern and PennCNV for Illumina) at the sample level. CNV calls that were made by only one of the algorithm were excluded. Calls discordant for type of CNV (gain or loss) were also excluded.

Quality control filtering: Following merging we applied filtering criteria for removal of arrays with excessive probe variance or GC bias and removal of samples with mismatches in gender or ethnicity or chromosomal aneuploidies. For Affymetrix data, we extracted the MAPD and waviness-sd from the APT summary file. We also calculated the proportion of each chromosome (excluding chrY) tagged as copy number variable and computed the number of CNV calls made for each sample. We then retained experiments if each of these measures was within 3 SD of the median. For Illumina data, we extracted LRRSD, BAFSD, GCWF (waviness) from PennCNV log files. As with the Affymetrix data, we calculated the proportion of each chromosome (excluding chrY) tagged as copy number variable and computed the number of CNV calls made for each sample. We retained samples if each of the above measures was within 3 SD of the median. For both Illumina and Affymetrix datasets, large CNVs that appeared artificially split were combined together if one of the methods detected a CNV spanning the gap. However, samples where > 10% of the chromosome was copy number variable were excluded as possible aneuploidies. Further, we excluded CNVs that: 1) spanned the centromere or overlapped the telomere (100 kb from the ends of the chromosome); 2) had > 50% of its length overlapping a segmental duplication; 3) had >50% overlap with immunoglobulin or T cell receptor. The final filtered CNV dataset was annotated with Refseq genes (transcriptions and exons). After this stage of quality control (QC), we had a total of **52,511** individuals, with **27,034** SCZ cases and **25,448** controls.

Filtering for rare CNVs: To make our final dataset of rare CNVs for all subsequent analysis we universally filtered out variants that present at $\geq 1\%$ (50% reciprocal overlap) frequency in cases and controls combined. CNVs that overlapped $> 50\%$ with regions tagged as copy number polymorphic on any other platform were also excluded. CNVs $< 20\text{kb}$ or having fewer than 10 probes were also excluded.

Post-CNV Calling QC

Overview: A number of steps were undertaken after CNV calling and initial filtering QC to minimize the impact of technical artifacts and potential confounds. In summary, we removed individuals not present in the PGC2 GWAS analysis ¹, removed datasets with non-matching case or control samples that could not be reconciled using consensus platform probes, and removed any additional outliers with respect to overall CNV burden, CNV calling metrics, or SCZ phenotype residuals. All steps are described in more detail below.

Merging with GWAS cohort: By matching the unique sample identifiers, we retained only individuals that also passed QC filtering from the companion PGC GWAS study in Schizophrenia ¹. This step filtered out samples with low-quality SNP genotyping, related individuals, and repeated samples across cohorts. An additional benefit of the PGC analytical framework is the ability to account for population stratification across cohorts using principal components derived from probe level analysis. After the post-CNV calling quality control steps described below, we re-calculated principal components using the Eigenstrat software package ⁶. Sample information and subsequent CNV and GWAS filtered sample sets are presented in **Extended data table 2**. In the process of matching to the GWAS-specific cohort, all individuals of non-European ancestry were removed from analysis ($\sim 5.8\%$ of the post-QC sample comprising three separate datasets). We also removed 42 samples that had discordant phenotype designations between the GWAS analysis and CNV genotype submission.

Individual dataset removal: Some datasets submitted to the PGC consisted of only case or control samples, affected trios, or recruited external samples as controls. This asymmetry in case-control ascertainment and genotyping can present serious biases for CNV analysis, as the sensitivity to detect CNV will vary considerably across genotyping platforms, as well as within dataset and genotyping batch. Unlike imputation protocols commonly used for SNP genotyping, there is no equivalent process to infer unmeasured probe intensity from nearby markers. We took a number of steps to identify and remove datasets that showed strong signs of case-control ascertainment or genotyping asymmetry:

- 1) Identify genotyping platforms where case-control ratio was not between 40-60%
- 2) Where possible, merge similar genotyping platforms using consensus probes prior to CNV-calling pipeline in order to improve case-control ratio.
- 3) Examine overall CNV burden and association peaks for spurious results
- 4) Remove datasets that remain problematic due to unusual CNV burden or multiple spurious CNV associations.

The genotyping platforms identified and processed are listed in **Extended data table 2**. We were able to combine the Illumina OmniExpress and Illumina OmniExpress plus Exome Chip platforms with success by removing probe content specific to the Exome chip platform. We removed the *caws* Affymetrix 500 datasets due to a number of strong CNV association peaks not seen in any other dataset. We also remove the *fii6* dataset due to a 2-fold CNV burden in cases relative to controls. In order to improve case-control balance, we had to remove the affected proband trio datasets (*boco*, *lacw*, and *lemu*) in the Illumina 610 platform, and the control-only *uclo* dataset in the Affymetrix 500 platform.

Individual sample removal: We re-analyzed CNV burden estimates in the reduced sample to flag any lingering outliers missed in the initial QC. We identified outliers for

CNV count and Kb burden in the autosome (> 30 CNVs or 8 Mb, respectively) and in the X chromosome (> 10 CNVs or 5 Mb, respectively), removing an additional 15 individuals.

Genome-wide CNV intensity and quality measurements produced by CNV calling algorithms (i.e. “CNV metrics”) were examined for additional outliers and potential relationships with case-control status. Each CNV metric was re-examined across studies to assess if any additional outliers were present. Only three outliers were removed as their mean B allele (or minor allele) frequency deviated significantly from 0.5. Many CNV metrics are auto-correlated, as they measure similar patterns of variation in the probe intensity. Thus, we focused on the main intensity metrics - median absolute pairwise difference (MAPD) for projects genotyped on the Affymetrix 6.0 platform, and Log R Ratio standard deviation (LRRSD) in all other genotyping platforms. Among Affymetrix 6.0 datasets, MAPD did not differ between cases and controls ($t=1.14$, $p = 0.25$). However, among non-Affymetrix 6.0 datasets, LRRSD showed significant differences between cases and controls ($t=-35.3$, $p < 2e^{-16}$), with controls having a higher standardized mean LRRSD (0.227) than cases (-0.199). To control for any spurious associations driven by CNV calling quality, we included LRRSD (MAPD for Affymetrix 6.0 platforms) as a covariate in downstream analysis. CNV metrics were normalized with their genotyping platform prior to inclusion in the combined dataset.

Regression of potential confounds on case-control ascertainment

The PGC cohorts are a combination of many datasets drawn from the US and Europe, and it is important to ensure that any bias in sample ascertainment does not drive spurious association to SCZ. In order to ensure the robustness of the analysis, we controlled for a number of covariates that could potential confound results. Burden and gene-set analyses included covariates in a logistic regression framework. Due to the number of tests run at probe level association, we employed a step-wise logistic regression approach to allow for the inclusion of covariates in our case-control association, which we term the *SCZ residual* phenotype.

Covariates include sex, genotyping platform, CNV metrics, and ancestry principal components derived from SNP genotypes on the same samples in a previous study¹. We were unable to control for dataset or genotyping batch, as a subset of the contributing datasets are fully confounded with case/control status. CNV metric is normalized within genotyping platform prior to inclusion in the logistic model. Only principal components that showed a significant association to small CNV burden were used (small CNV being defined as autosomal CNV burden with CNV < 100 kb in size). Among the top 20 principal components, only the 1st, 2nd, 3rd, 4th, and 8th principal component showed association with small CNV burden (with $p < 0.01$ used as the significance cutoff). To calculate the SCZ residual phenotype, we first fit a logistic regression model of covariates to affection status, and then extracted the Pearson residual values for use in a quantitative association design for downstream analyses. Residual phenotype values in cases are all above zero, and controls below zero, and are graphed against overall kb burden in **Extended data figure 7**. We removed three individuals with an SCZ residual phenotype greater than three (or negative three in controls). After the post-processing round of QC, we retained a dataset with a total of **41,321** individuals comprising **21,094** SCZ cases and **20,227** controls.

Identifying previously implicated CNV loci in the literature

To delineate CNV burden effects coming from CNV loci that have previously been reported as putative SCZ risk factors from CNV in remainder of the genome, we flagged CNV loci with $p < 0.01$ that have either been reviewed^{7,8} or otherwise reported⁸⁻¹⁰ as potential SCZ risk factors in the literature. Previously reported loci meeting inclusion are listed in **Extended data table 1**. While a number of CNV loci have been reported in multiple studies, we sought the most recent reports that incorporated the largest sample sizes. To identify putatively associated CNV loci with SCZ from the full list, we applied the genome-wide p -value cutoff of $8e^{-5}$, derived from the Cochran-Mantel-Haenszel (CMH) test in the current probe-level analysis as the p -value cutoff for inclusion

as SCZ implicated CNV loci. While the CMH test is not the primary probe-level test in the current PGC analysis, it corresponds more closely to the tests used in published reports. In all, nine independent CNV loci from published reports surpass genome-wide correction. All published CNV loci, even those excluded as an SCZ implicated regions, are examined in the probe-level association analysis.

CNV burden analysis

We analyzed the overall CNV burden in a variety of ways to discern which general properties of CNV are contributing to SCZ risk. Overall individual CNV burden was measured in 3 distinct ways – 1) Kb burden of CNVs, 2) Number of genes affected by CNVs, and 3) Number of CNVs. In particular, we only counted gene as affected when the CNV overlapped a coding exon. We also partitioned our analyses by CNV type, size, and frequency. CNV type is defined as copy number losses (or deletions), copy number gains (or duplications), and both copy number losses and gains. To assign a specific allele frequency to a CNV, we used the `--cnv-freq-method2` command in PLINK, whereby the frequency is determined as the total number of CNV overlapping the target CNV segment by at least 50%. This method differs from other methods that assign CNV frequencies by genomic region, whereby a single CNV spanning multiple regions may be included in multiple frequency categories.

For **Figure 1**, and **Extended data figures 2 and 3**, we partitioned CNV burden by genotyping platform, and the abbreviations for each platform are expanded below:

A500: Affymetrix 500

I300: Illumina 300K

I600: Illumina 610K and Illumina 660W

A5.0: Affymetrix 5.0

A6.0: Affymetrix 6.0

omni: OmniExpress and OmniExpress plus Exome

Due to the small size of the Omni 2.5 array (28 cases and 10 controls), they were excluded from presentation in the figure, but are included in all burden analyses with the total PGC sample. Burden tests use a logistic regression framework with the inclusion of covariates detailed above. Using a logistic regression framework, we predicted SCZ status using CNV burden as an independent predictor variable, thus allowing us to get an accurate estimate of the unique contribution of CNV burden in a multiple regression framework. To gain insight into the proportion of CNV burden risk coming from loci outside of the previously implicated SCZ regions, we ran all burden analyses after removing CNV that overlapped previously implicated CNV boundaries by more than 10%.

CNV probe level association

Genome-wide interrogation of CNV signals was tested at each respective CNV. Probe level tests were examined at the start, end, and single base position after the end of the called CNV. Three categories of CNV were tested: CNV deletions, CNV duplications, and deletions and duplications together. All analyses were run using PLINK software ¹¹.

We ran probe level association using the SCZ residual phenotype as a quantitative variable, with significance determined through permutation of phenotype residual labels. An additional z-scoring correction, explained below, is used to control for any extreme values in the SCZ residual phenotype and efficiently estimate two-sided empirical *p*-values for highly significant loci. To ensure against the potential loss of power from the inclusion of covariates, we also ran a single degree of freedom Cochran-Mantel-Haenzel (CMH) test stratified by genotyping platform, with a 2 (CNV carrier status) x 2 (phenotype status) x N (genotyping platform) contingency matrix. While the CMH test does not account for more subtle biases that could drive false positive signals, it is robust to signals driven by a single platform and allows for each CNV carrier to be

treated equally. Loci that surpassed genome-wide correction in either test were followed up for further evaluation.

Z-score recalibration of empirical testing: Probe level association p -values from the SCZ residual phenotype were initially obtained by performing one million permutations at each CNV position, whereby each permutation shuffles the SCZ residual phenotype among all samples, and retains the SCZ residual mean for CNV carriers and non-carriers. For extremely rare CNV, however, CNV carriers at the extreme ends of the SCZ residual phenotype can produce highly significant p -values. While we understand that such rare events are unable to surpass strict genome-wide correction, we wanted to retain all tests to help delineate the potential fine-scale architecture within a single region of association. To properly account for the increased variance when only a few individuals are tested, we applied an empirical Z-score correction to the CNV carrier mean. In order to get an empirical estimate of the variance for each test, we calculated the standard deviation of residual phenotype mean differences in CNV carriers and non-carriers from 5,000 permutations. Z-scores are calculated as the observed case-control mean difference divided by the empirical standard deviation, with corresponding p -values calculated from the standard normal distribution. Concordance of the initial empirical and z-score p -values are close to unity for association tests with six or more CNV, whereas Z-score p -values are more conservative among tests with less than six CNV. Furthermore, the Z-score method naturally provides an efficient manner to estimate highly significant empirical p -values that would involve hundreds of millions of permutations to achieve.

Genome-wide correction for multiple tests

Beyond identifying significant CNV at the probe level, we also estimated the genome-wide testing space for rare CNV analysis. With the large PGC cohort being called through a consistent pipeline, we saw an opportunity to characterize the null expectation of segregating and recurrent *de novo* rare CNV in populations of European ancestry.

Accepted thresholds for significance among published risk CNV have been limited in scope, as accurate population estimates of rare CNV frequency and distribution across the genome require large representative samples.

Genome-wide significance thresholds were calculated using the 5% family-wise error rate from 5,000 permutations in both the SCZ residual phenotype and CMH test. Specifically, we selected the 95th percentile of the minimum p -values obtained across permutations. Below are the genome-wide correction p -value thresholds determined in this manner:

SCZ residual phenotype FWER correction:

CNV losses and gains: $6.73e^{-6}$

CNV losses: $1.5e^{-5}$

CNV gains: $1.35e^{-5}$

CMH test FWER correction:

CNV losses and gains: $3.65e^{-5}$

CNV losses: $8.25e^{-5}$

CNV gains: $7.8e^{-5}$

This method differs slightly from those used in **Levinson et al.**⁹ to estimate the multiple test correction for rare CNV, however their genome-wide correction of $p = 1e^{-5}$ corresponds quite closely to the estimates observed using the SCZ residual phenotype. The observed family-wise correction serves as good approximation of the independent rare CNV signals found among European ancestry populations for array-based CNV capture, but as sample sizes increase, so too will the effective number of tests, necessitating further evaluation of the multiple testing burden.

Gene-set burden enrichment analysis: gene-sets

Gene-sets with an a priori expectation of association to neuropsychiatric disorders were compiled based on gene annotations (Gene Ontology and curated pathway databases, downloaded June 2013) and published article materials (for details, see **Extended Data Table 3**). Gene-sets based on brain expression were compiled by processing the BrainSpan RNA-seq gene expression data-set (<http://www.brainspan.org/static/download.html>, downloaded Sept 2012). Four roughly equally sized gene-sets (about 4600 genes each) were derived to represent four expression tiers (very high, medium-to-high, medium-to-low, very low or absent); genes were selected if they passed a fixed expression threshold in at least 5/508 experimental data points (corresponding to different regions of donor brains, different donor ages corresponding to different developmental brain stages, and different donor sexes). Gene-sets based on mouse phenotypes were assembled by downloading MPO (Mammalian Phenotype Ontology) annotations from MGI (www.informatics.jax.org, downloaded August 2013), up-propagating annotations following ontology relations, and mapping to human orthologs using NCBI Homologene (www.ncbi.nlm.nih.gov/homologene); finally, top-level organ systems with fewer genes were aggregated while striving to preserve biological homogeneity, so to have roughly equal-sized sets (2,600-1,300 genes). For all gene-sets, gene identifiers in the primary source were mapped to Entrez-gene identifiers using the R/Bioconductor package *org.Hs.eg.db*.

Gene-set burden enrichment analysis: pre-processing

Subjects were restricted to the ones with at least one rare CNV. For copy number gains and losses, we separately calculated the following subject-level totals: variant number, variant length and number of genes impacted; these covariates are then used to model global burden and correct gene-set burden to ensure it is specific (i.e. not a mere reflection of genome-wide burden with some stochastic deviation due to sampling). The subject-level total number of genes impacted was also calculated for each gene-set, again separately for gains and losses. Subjects were flagged if they carried at least one

CNV matching a locus previously implicated in schizophrenia (see section “Identifying previously implicated CNV loci in the literature”); this was then used to analyzed gene-set burden for all subjects, or excluding subjects with an already implicated CNV.

Gene-set burden enrichment analysis: statistical test

For each gene-set, we fit the following logistic regression model (as implemented by the R function *glm* of the *stats* package), where subjects are statistical sampling units:

$$y \sim \text{covariates} + \text{global} + \text{gene-set}$$

Where:

- *y* is the dicotomic outcome variable (schizophrenia = 1, control = 0)
- *covariates* is the set of variables used as covariates also in the genome-wide burden and probe association analysis (sex, genotyping platform, CNV metric, and CNV associated principal components)
- *global* is the measure of global burden; for the results in the main text, we used the total gene number (abbreviated as *U* from universe gene-set count); we also calculated results for total length (abbreviated as *TL*) and variant number plus variant mean length (abbreviated as *CNML*)
- *gene-set* is the gene-set gene count

The gene-set burden enrichment was assessed by performing a chi-square deviance test (as implemented by the R function *anova.glm* of the *stats* package) comparing these two regression models:

$$y \sim \text{covariates} + \text{global}$$

$$y \sim \text{covariates} + \text{global} + \text{gene-set}$$

We reported the following statistics:

- coefficient beta estimate (abbreviated as *Coeff*)
- t-student distribution-based coefficient significance p-value (as implemented by the R function *summary.glm* of the *stats* package, abbreviated as *Pvalue_glm*)
- deviance test p-value (abbreviated as *Pvalue_dev*)
- gene-set size (i.e. number of genes is the gene-set, regardless of CNV data)

- BH-FDR (Benjamini-Hochberg False Discovery rate)
- percentage of schizophrenia and control subjects with at least 1 gene, 2 genes, etc... impacted by a CNV of the desired type (loss or gain) in the gene-set (abbreviated as *SZ_g1n*, *SZ_g2n*, ... *CT_g1n*, ...)

Please note that, by performing simple simulation analyses, we realized that *Pvalue_glm* can be extremely over-conservative in presence of very few gene-set counts different than 0, while *Pvalue_dev* tends to be slightly under-conservative. While the two p-values tend to agree well for gene-set analysis, *Pvalue_glm* is systematically over-conservative for gene analysis since smaller counts are typically available for single genes.

Gene burden analysis: pre-processing

Subjects were restricted to the ones with at least one rare CNV. Only genes with at least a minimum number of subjects impacted by CNV were tested; this threshold was picked by comparing the BH-FDR to the permutation-based FDR and ensuring limited FDR inflation (permuted FDR < 1.65 * BH-FDR at BH-FDR threshold = 5%) while maximizing power. For gains the threshold was set to 12 counts, while for losses it was set to 8 counts.

Gene burden analysis: statistical test

For each gene, we fit the following logistic regression model (as implemented by the R function *glm* of the *stats* package), where subjects are statistical sampling units:

$$y \sim \text{covariates} + \text{gene}$$

Where:

- *y* is the dichotomous outcome variable (schizophrenia = 1, control = 0)
- *covariates* is the set of variables used as covariates also in the genome-wide burden and probe association analysis (sex, genotyping platform, CNV metric, and CNV associated principal components)

- *gene* is the binary indicator for the subject having or not having a CNV of the desired type (loss or gain) mapped to the gene

The gene burden was assessed by performing a chi-square deviance test (as implemented by the R function *anova.glm* of the *stats* package) comparing these two regression models:

- $y \sim \text{covariates}$
- $y \sim \text{covariates} + \text{gene}$

Gene burden analysis: multiple test correction

Multiple test correction was performed for loci rather than for genes, to avoid the strong correlation between test introduced by multi-genic CNVs; for the same reason, it is more useful to count false positives as loci rather than genes. We followed a greedy step-down procedure:

- start from gene with most significant deviance p-value G1, create locus L1
- remove from the gene list all genes that share at least 50% of their carrier subjects with G1, and add them to locus L1
- do the same for the next gene most significant gene in the list (thus creating a new locus L2), and proceed recursively until there is no gene left
- define locus p-value as the smallest deviance p-value of its genes

We computed permutation-based FDR by permuting subjects' condition labels (schizophrenia, control), but not covariates (as those are expected to correlate to CNV distribution), 1,000 times. The FDR was then defined as the ratio between the average number of tests passing a given p-value threshold across the 1,000 permutations and the number of tests passing the same p-value threshold for real data. FDRs were also generated counting only the subset of genes with positive and negative regression coefficients (i.e. risk and presumed protective). The p-value threshold for permutation-based FDR calculation was picked by choosing the maximum nominal p-value corresponding to a given BH-FDR threshold (e.g. 5%). BH-FDR is supposed to be slightly inflated because (i) the deviance test p-value is slightly under-conservative in presence

of very few gene indicators different than 0, (ii) we use the smallest gene p-value to define the locus p-value.

Methods References

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PGC Schizophrenia CNV analysis – Supplementary Information

Supplementary Results:

- **CNV burden between sexes**
- **Probe level power analysis**
- **Gene-based network analysis**
- **Follow up of significant CNV loci**
- **Proportion of variance in SCZ explained by top CNV loci**
- **NAHR enrichment in significant novel gene loci**

Consortium Membership

Acknowledgements

Supplementary Results

CNV burden between sexes

Following recent evidence that ostensibly healthy females carry an increased burden of rare CNVs¹, we examined whether this increased female burden existed in the current PGC dataset. We used a logistic regression model predicting sex using CNV burden and controlling for study covariates, as well as the Wilcoxon rank-sum test comparing male to female CNV burden¹. Focusing on the significant findings in the previous paper, we examined the burden in autosomal CNV count and genes affected among PGC controls (9856 males and 10371 females). We do see an elevated CNV count in control females (1.90 autosomal CNV rate) to males (1.87 autosomal CNV rate), however this difference is not significant in either the regression model (OR = 1.004, $p = 0.66$) or the Wilcoxon rank-sum test ($p = 0.1$). We do, however, observe a marginally significant enrichment when focusing on CNV loss count, where control females (0.99 autosomal CNV loss rate) show a higher burden than control males (0.94 autosomal CNV loss rate; logistic regression OR = 1.03, $p = 0.05$; Wilcoxon rank-sum test $p = 3e^{-3}$). No single genotyping platform seemed to drive the enrichment in females (data not shown), and we don't observe any difference in CNV count when looking at CNV gains (logistic regression OR = 0.98, $p = 0.18$; Wilcoxon rank-sum test $p = 0.56$). Finally, no significant differences between sexes were found using either test when examining the number of genes affected, or when we include SCZ cases and controls (all $p > .05$).

Probe level power analysis

By restricting analysis to rare CNV in the population ($MAF < 0.01$), many loci do not have enough CNV to surpass genome-wide correction for multiple testing, prompting pathway and gene level analyses to achieve sufficient statistical power. To use a specific example, the 3q29 deletion is fully penetrant in the current sample, with 16 SCZ carriers and 0 controls ($MAF = 3.8e^{-4}$) at the peak of association. Assuming no platform bias, this

leads to an uncorrected chi-square p -value of $8.9e^{-5}$, and a permuted p -value of $6.2e^{-5}$ testing association using SCZ phenotype residuals. Neither p -value, however, surpasses their respective genome-wide significance cutoff for CNV deletion. While permutation methods used to generate genome-wide cutoffs accurately reflect the testing space among observed CNVs (very rare CNVs have little to no contribution to the family-wise error rate), we wanted to estimate the proportion of CNV detectable at the probe level. Under our current analytical design and sample size, we calculated the power to detect associated CNV across various MAFs and effect sizes and determine the proportion of association tests capable of surpassing genome-wide correction.

We simulated CNVs within our dataset (21094 cases and 20227 controls) and regressed them using the same association design with SCZ residual phenotypes. We simulated various effect sizes by randomly sampling cases and controls at different probabilities as CNV carriers, and rounded to the nearest CNV count to reflect the MAF of each CNV in the sample. For each combination of effect size and MAF, we ran 1000 simulations, retrieving the t -test p -value of CNV carriers from the SCZ residual phenotype. Simulated p -values behaved in much the same way as the Z-score correction on permuted p -values used in the primary test (data not shown). In **Extended data figure 8**, we show the proportion of simulations for CNV losses surpassing genome-wide correction at each MAF and effect size parameter (gains perform similarly).

We define statistical power as the proportion of simulations surpassing genome-wide significance. For a fully penetrant risk CNV, we require a MAF of $\sim 6e^{-4}$ (or about 25 CNV) to achieve 80% detection power. For CNV with a genotype relative risk (GRR) of 10, we require a MAF of $1e^{-3}$ (or at least 41 CNV) to achieve 80% detection power. Looking across the landscape of CNVs tested, on the whole about 10% of deletion or duplication CNV breakpoints reach a frequency greater than $1e^{-3}$ in the sample. On the other extreme, a CNV with MAF of .005 (or at least 206 CNV) and a GRR of 2 will only be detected 58% of the time.

Gene-based network analysis

To identify a gene network enriched in schizophrenia risk genes, we queried GeneMANIA² using the 17 genes with deletion gene-test Benjamini-Hochberg FDR $\leq$ 25% and member of the “GO synaptic” or “ARC complex” sets. We thus created a synaptic protein interaction network of 136 genes, with the most densely connected network core corresponding to post-synaptic density organizers (DLGs, DLGAPs, SHANKs) and ionotropic glutamate receptors (GRIAs, GRIDs, GRINs). NRXN1 is connected to the network core via adhesion partners (NLGN1-3) and CASK. We tested this schizophrenia gene network, and found significant enrichment in genes with evidence of de novo coding variants in sequencing studies of schizophrenia trios³ (for frameshift, stop-gain and splice-site: Fisher’s Exact Test p-value 0.0023; missense and amino acid insertion/deletion: Fisher’s Exact Test p-value 0.0004); in addition, we found a greater enrichment for this network, compared to the larger set composed of all “GO synaptic” and “ARC complex” genes. No significant enrichment was found for de novo variants identified in controls.

Follow up of significant CNV loci

Both gene and probe level association follow a uniform testing framework across the genome, however risk loci may exhibit a more nuanced CNV architecture across the entirety of the association peak. All associated loci with FDR $<$.05 in the gene based test were followed up for further testing, along with a small number of candidate loci showing suggestive association in the probe-level association. We visually inspected each association peak and determined the bp coordinates that encapsulate the associated region and determine which CNV segment inclusion, be it covering exons or overlapping a minimum percentage of the total region, most appropriately reflect the association signal. To comprehensively examine the robustness and source of

association, we also ran additional tests controlling for individual dataset, splitting by sex, and examining a dosage model, whereby copy number is measured with one copy for deletion, two copies for no CNV, and three copies for duplication. We also examined significant CNV loci in an unfiltered CNV call set, using CNVs called prior to the removal of common CNVs (MAF > 1%) and CNV overlapping segmental duplications.

We further evaluated the associated regions by determining the concordance of calls within the call set with those determined by unsupervised clustering. Call set CNVs were defined as CNVs with at least a 50% overlap with regions in **Table 1**. We restricted this analysis to 26,959 samples across six cohorts (14,419 Affymetrix 6.0, 12,540 Illumina platforms; 1:1:1 case:control ratio). Features for clustering included the median logR ratio (mLRR) and the median logR ratio of the chromosome for which a locus resides in, controlling for large chromosomal abnormalities. We implemented Density-Based Spatial Clustering of Applications (DBSCAN) found in the python scikit-learn library (<http://scikit-learn.org>) because of high sensitivity to detect outliers in clusters. For each novel region and within each cohort, genotypes were assigned to every sample based on the DBSCAN defined cluster. The cluster with the highest number of samples was designated as reference and assumed to have a copy number of two. Other clusters were flagged as gain or loss based on the average regional mLRR and its relation to the reference regional mLRR. We removed clusters with average chromosomal mLRR outside 3 SD from the reference. CNVs were considered concordant if they were flagged non-reference by DBSCAN and present in the 41k call set, matching on CNV type. We applied a locus based call set concordance filter of $\geq 70\%$; one region, NPY4R, failed to meet this requirement with a concordance of 0.1%. In addition, both proximal and distal loci of ZNF600 were removed due to batch effects, which we defined as a significant deviation from a Poisson distribution of call set calls per plate. Regions that passed both concordance and batch effect filters are reported in **Table 1**.

Proportion of variance in SCZ explained by top CNV loci

To measure the proportion of variance explained on the liability scale of SCZ, we estimated the overall heritability of liability (or logRR genetic variance) explained by the eight CNV loci surpassing genome-wide significance. All eight loci were collapsed into a single signal. Two SCZ affected individuals were found to carry two CNVs in these loci, and their contribution was only counted once. In sum, we observed 298 SCZ patients with a CNV in these regions (1.4% of the total SCZ affected sample), and 29 controls (0.1%; CMH stratified OR = 10.1). To estimate the variance in SCZ liability explained by loci surpassing genome-wide correction, we calculated the heritability of liability using the INDI-V online tool (cnsgenomics.com/software) described in ⁴ using an overall disease risk of 1% and a sibling recurrence risk of 8.8 ⁵.

NAHR enrichment in significant novel gene loci

To test if novel significant loci (FDR<0.05; **Table 1**) were enriched for NAHR events, we performed a permutation test (n=10,000) simulating the null distribution of NAHR-mediated CNVs for a set of random loci. Each simulation randomly selected nine loci taken from CNVs overlapping at least 50% to genes in the gene-set burden analysis. These nine random loci were matched according to CNV call frequency to the nine novel significant loci in Table 1. We then created windows for each start and end position for every overlapping CNV to a random locus. Start positions were expanded -50kb and +5kb, and end positions were expanded -5kb and +50kb. We flagged CNVs as NAHR-mediated when both start and end expanded windows overlapped to 1kb segmental duplications obtained from the hg18 build of the UCSC table browser (<https://genome.ucsc.edu/cgi-bin/hgTables>). Every iteration reported the fraction of NAHR-mediated CNVs; that is the ratio of CNVs flagged as NAHR to the total number of overlapping CNVs. We found an enrichment of NAHR mediated CNVs in significant novel loci when compared to the null distribution (86% NAHR-mediated, 6 fold enrichment, p=0.008).

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