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# Genetic heterogeneity shapes brain connectivity in psychiatry

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**Keywords:** Genetic heterogeneity, Copy number variant, Functional connectivity, Autism spectrum disorder, Polygenic score, transdiagnostic approach.

## **Abstract**

### *Background*

Polygenicity and genetic heterogeneity pose great challenges for studying psychiatric conditions. Genetically-informed approaches have been implemented in neuroimaging studies to address this issue. However, the effects on functional connectivity of rare and common genetic risks for psychiatric disorders are largely unknown. Our objectives were to estimate and compare the effect-sizes on brain connectivity of psychiatric genomic risk factors with various levels of complexity: oligo-, multi-genic copy number variants (CNVs), and polygenic risk scores (PRS) as well as idiopathic psychiatric conditions and traits.

### *Methods*

Resting-state functional-MRI data were processed using the same pipeline across nine datasets. Twenty-nine connectome-wide association studies were performed to characterize the effects of 15 CNVs (1003 carriers), 7 PRS, 4 idiopathic psychiatric conditions (1022 individuals with either autism, schizophrenia, bipolar conditions, or ADHD), and 2 traits (31424 unaffected controls).

### *Results*

Effect sizes on connectivity were largest for psychiatric CNVs (estimates: 0.2 to 0.65 z-score) followed by psychiatric conditions (0.15 to 0.42), neuroticism and fluid intelligence (0.02 to 0.03), and PRS (0.01 to 0.02). Effect-sizes of CNVs on connectivity were correlated to their effects on cognition and risk for disease ( $r=0.9$ ,  $p=5.93e-06$ ). However, effect sizes of CNVs adjusted for the number of genes significantly decreased from small oligogenic to large multigenic CNVs ( $r=-0.88$ ,  $p=8.78e-06$ ). PRS had disproportionately low effect sizes on connectivity compared to CNVs conferring similar risk for disease.

### *Conclusion*

Heterogeneity and polygenicity impact our ability to detect brain connectivity alterations underlying psychiatric manifestations.

## Introduction

Polygenicity and genetic heterogeneity pose great challenges for studying mechanisms and risk underlying psychiatric conditions (1). Rare copy number variants (CNVs), as well as common variants, confer risk for neurodevelopmental and psychiatric disorders such as autism spectrum disorder and schizophrenia (SZ). CNVs that increase risk for autism and-or SZ also decrease intelligence quotient (IQ)(2,3). Their effect sizes range from large to mild (eg. 22q11.2 and 15q11.2 deletions decrease IQ by 29 and 3 points respectively and increase risk for SZ with odd-ratios (ORs) = 23 and 1.9 respectively, **Table 1**) (4,5). Effect sizes of CNVs on cognition and risk for neurodevelopmental and psychiatric disorders are positively correlated to the number of genes they contain. To account for the fact that not all genes contribute equally to the impact of CNVs, we developed a CNV severity score, which is the sum of genes encompassed in CNVs, weighted by the sensitivity of each gene to loss of function (6). The number of genes included in a CNV and its severity score are both measures of the level of multigenicity of a CNV. This severity score can predict the effect size of CNVs on cognition with close to 80% accuracy (7,8). Furthermore, the mean effect size on cognitive ability of one point of this severity score is similar for benign oligogenic and deleterious multigenic CNVs, which suggests that effect sizes of large CNVs are the additive effects of many individual genes with small effects.

Similarly, for common variants, polygenic risk scores (PRS) are additive models developed to estimate the aggregate effects of thousands of single nucleotide polymorphisms (SNPs) with very small individual effects (9,10). The risk for SZ ranges from OR=3.3 to 4.6 (9) when comparing individuals in the bottom and top deciles of PRS-SZ; similar or higher than the risk conferred by some oligogenic CNVs such as 1q21.1 and 15q11.2 deletions. For PRS-IQ, contrasting bottom and top deciles shows moderate to large effect sizes around 9 to 12 points of IQ (11) which is similar to several CNVs associated with neurodevelopmental and psychiatric disorders (eg. 16p11.2 duplication) (**Table 1**).

Because cognition is thought to be subserved by large-scale brain networks (12), it is reasonable to hypothesize that the effects of genetic variants on cognition and behavior (8,13) are mediated by brain

structure and networks (14,15). The organization of such networks can be inferred using resting-state functional MRI (rs-fMRI)(16,17). Functional connectivity (FC) has gained traction in the last decade, characterizing increasingly reproducible patterns of alterations associated with psychiatric conditions (18). However, these studies reported small effect sizes, which appeared discordant with the severity of autism and attention-deficit/hyperactivity disorders (ADHD) (19,20). Genetically-informed approaches have been introduced with the hope to focus on a specific biological risk. Effects of CNVs on FC have been investigated at only 2 genomic loci in humans (16p11.2 and 22q11.2) (14,21) demonstrating robust effects. Little is known about the effect sizes on FC of psychiatric PRS(22,23). While PRS and CNVs can have similar effect sizes on psychiatric risk, the effects on connectivity of these 2 classes of variants with vastly different levels of genomic complexity, have never been compared.

**Our aims were to** 1) Estimate and compare the effects of oligogenic (e.g. 4 protein-coding genes for 15q11.2 CNVs), multigenic (e.g. 49 protein-coding genes for 22q11.2 CNVs) (24), and PRS for psychiatric conditions on brain connectivity, 2) Characterize the relationship between effect sizes of genomic variants on cognition/behavior and connectivity, 3) Test the relationship between the level of multigenicity (measured by the number of genes and the severity score) and effect sizes of CNVs on connectivity.

To this end, we analyzed rs-fMRI data in n=33,452 individuals and performed 29 connectome-wide association studies for 15 CNVs, 7 PRS, 4 idiopathic conditions, inflammatory bowel disease (IBD), and 2 traits (fluid intelligence and neuroticism).

## Methods

The selection process for the CNVs, PRS, psychiatric conditions and traits are detailed in the supplemental methods.

### Cohorts

Our analysis included 33,452 individuals from nine datasets (**Table 1, Figure 1**). Each study of the corresponding dataset was approved by the research ethics review boards of the respective institutions. This project was approved by the research ethics review board at the Centre Hospitalier Universitaire Sainte Justine.

#### *Clinical genetic datasets*

We used 4 ‘genetically-informed’ CNV datasets, which were recruited based on the presence of a CNV associated with neurodevelopmental and psychiatric disorders, regardless of symptomatology (detailed in eMethod in Supplement). Of note, the term oligogenic refers to CNVs containing: 1 gene  $< \text{CNV} \leq 5$  genes, while multigenic CNVs contain more than 5 genes. These categories are descriptive, and cutoffs are descriptive. None of the analyses rely on these categories.

These four datasets included the Simons Variation in Individuals Project (SVIP for 16p11.2 and 1q21.1 CNVs) (25) and the University of California, Los Angeles 22q11.2 CNV project (UCLA). fMRI data have not yet been published for the Montreal rare genomic disorder family project (MRG, Canada)(8), and the Define Neuropsychiatric-CNVs Project (Cardiff, UK).

#### *Unselected population*

CNVs associated with neurodevelopmental and psychiatric disorders and non-psychiatric CNVs were also identified in the UK-Biobank dataset (UKBB)(25) (eMethods in the [Supplement](#)). Non-psychiatric CNVs were defined as variants without any previous association with a psychiatric condition in large case-control studies (4,26–28).

### *Idiopathic psychiatric conditions cohorts*

We used the ABIDE1 (29), ABIDE2 (30), ADHD-200 (31), the Consortium for Neuropsychiatric Phenomics (CNP) (32), and an aggregate dataset of 10 SZ studies (14,33); collectively these datasets include individuals with idiopathic autism, ADHD, SZ, and BIP, as well as their respective controls. Psychiatric assessments are detailed in eMethods in [Supplement](#).

### **CNV calling and Polygenic scores (PRS) computation**

CNVs were identified in the UKBB using PennCNV(34) and QuantiSNP (35) following previously published methods (7) (eMethods in [Supplement](#)).

We computed 7 PRS for individuals of European ancestry in the UKBB using Bayesian regression and continuous shrinkage priors (36) (**Table1**, eMethods and **eTable1** in [Supplement](#)).

### **Resting-state functional MRI preprocessing**

All datasets were preprocessed using the same parameters of Neuroimaging Analysis Kit (NIAK) (37). Preprocessed data were visually controlled for quality of the coregistration, head motion, and related artifacts (eMethods in [Supplement](#)).

### **Computing connectomes**

We segmented the brain into 64 functional seed-based regions and 12 networks defined by the multi-resolution MIST brain parcellation (38). FC was computed as the temporal pairwise Pearson's correlation between the average time series of the 64 seed-based regions, and then Fisher-z transformed. The connectome of each individual encompassed 2,080 connectivity values:  $(63 \times 64) / 2 = 2016$  region-to-region connectivity + 64 within seed-based region connectivity. We chose the 64 parcel atlas of the multi-resolution MIST parcellation ([https://simexp.github.io/multiscale\\_dashboard/index.html](https://simexp.github.io/multiscale_dashboard/index.html)) as it falls within the range of network resolution previously identified to be maximally sensitive to FC alterations in neurodevelopmental and psychiatric disorders such as autism (39). We corrected for multiple comparisons using a false discovery rate strategy (40).

## Connectome-wide association studies

We performed 29 connectome-wide association studies by either contrasting cases and their respective controls for:

- 7 CNVs associated with neurodevelopmental and psychiatric disorders and 8 non-psychiatric CNVs (**Table1**), 4 idiopathic psychiatric disorder cohorts (autism, SZ, BIP, and ADHD) and 1 non-brain related condition (IBD). Controls refer 1) to individuals without a CNV for analysis investigating the effect of CNVs and 2) individuals without a diagnosis in analyses investigating effects of psychiatric conditions.
- or by investigating the linear effects of 7 continuous PRS: autism, BIP, SZ, Cross-disorder (ADHD, autism, BIP, SZ, anorexia nervosa, major depression, obsessive-compulsive disorder, and Tourette syndrome), IQ as well as two non-brain related control traits: Low-Density Lipoprotein (LDL) and Chronic Kidney Disease (CKD), as well as 2 continuous traits provided by UKBB: Neuroticism, and Fluid intelligence.

FC was z-scored based on the variance of the pooled controls used for each connectome-wide association study (column ‘Cohorts’ in Table 1). They were conducted by linear regression, in which z-scored FC values were the dependent variables and genetic or diagnostic status or traits were the explanatory variables. PRS and traits were normalized within the UKBB sample.

It was previously demonstrated that global signal adjusted (GSA)-FC profiles show stronger correlations with cognition (41), and reduce confounding effects in multi-site studies (42). We, therefore, used GSA FC profiles for this study. Global effect sizes obtained without GSA are available in **eTable5** in the supplement.

Models were adjusted for sex, scanning site, head motion, age, and global signal (=‘GSA’) defined as the mean of all 2,080 Fisher’s Z values (42). FC profiles were defined as the 2,080  $\beta$  values of 2,080 connections.

$$Z\text{-score}_{\text{Connection [i, \dots, 2080]}} \sim \beta_0 + \beta_{\text{genetic status}} + \beta_{\text{age}} + \beta_{\text{motion}} + \beta_{\text{sex}} + \beta_{\text{site}} + \beta_{\text{global signal}}$$

This linear regression was applied for each of the 2,080 functional connections. Since all raw connectomes were normalized on the variance of the controls, regression estimates (beta) can be

interpreted as z-scores. We corrected for multiple testing using FDR ( $q < 0.05$ ) as well as a permutation procedure (see eMethods, in [Supplement](#)). Effect-size of genetic risk, conditions, and traits on connectivity was defined as the top decile of the 2080 absolute  $\beta$  values. Sensitivity analyses using a cross-validation approach (43) ensured that effect sizes were stable across the different sample sizes investigated in the study.

### **Multiple testing**

Within each independent variable (15 CNVs, 7 PRS, 4 conditions and 2 traits), we corrected for the number of tests (2,080 connections) using the Benjamini-Hochberg FDR correction at a threshold of  $q < 0.05$ <sup>(40,44)</sup>. We also computed an empirical p-value by conducting a permutation test, shuffling the genetic or clinical status labels of the individuals included in each connectome-wide association study (5,000 permutations). We estimated the empirical p-value by calculating the frequency of obtaining an effect size equal to or greater than the original observation (45).

### **Estimating effect sizes using cross-validation**

We generated effect sizes for each sample using K-fold cross-validation (CV) with 2, 5, and 10 folds CV (43). For each genetic risk, condition and trait, we split the sample into K segments (for case-control analyses segments are stratified accordingly), then for K iterations we held out a segment as a training sample to generate a betamap and identify the connections with effect size estimates in the top decile. On the remaining independent test group, we extracted the top decile connections, and computed their mean effect sizes. The overall effect size was computed as the mean of K estimates (eFigure1, Supplement).

### **Bootstrap procedure to estimate 95th Confidence Intervals of effect size ratios:**

We identified the 95% confidence intervals for the ratios of effect sizes using a bootstrap procedure (46). First, for each sample we generated the actual betamap, and identified the top decile connections and their mean X. Then, for 5000 iterations we resampled with replacement the same number of subjects (for case-control analyses the resampling was performed separately in each group), generated

a resampled betamap and took the mean of the identified connections to form a distribution ( $x_1, x_2, \dots, x_{5000}$ ). To generate a distribution of ratios for a given pair  $X_1, X_2$  (where  $X_1 > X_2$ ) we take the ratios of the bootstrap distributions ( $x_{1_1}/x_{2_1}, x_{1_2}/x_{2_2}, \dots, x_{1_{5000}}/x_{2_{5000}}$ ).

### **Sum of genes and CNV severity score**

The CNV severity score was previously published and is an additive model (7,8). It is the sum of genes included in a CNV, and each gene is weighted by its sensitivity to loss function, which is measured by the LOEUF score which is available for each coding gene (47). Smaller values of LOEUF represent genes with highest sensitivity to loss of function (more severe genes), therefore the inverse of LOEUF is used in the additive model:

$$\text{CNV Severity score} = (\text{gene1} \times 1/\text{LOEUF}_{\text{gene1}}) + (\text{gene2} \times 1/\text{LOEUF}_{\text{gene2}}) + \dots$$

This severity score is predictive of a CNVs effect size on cognition(7,48) and risk for psychiatric conditions (3,49).

As a sensitivity analysis, we computed a CNV severity score based on the pLI (**probability of being Loss of function Intolerant** (50), which is another constraint score with a binary distribution (>0.8 for intolerant genes and close to 0 for all other genes). As a result, this score only takes into account the contribution of intolerant genes.

## **Results**

### **Effects of genetic risk factors and psychiatric conditions on brain connectivity**

All 7 CNVs associated with neurodevelopmental and psychiatric disorders and none of the nine non-psychiatric CNVs significantly altered functional connections (FDR, 2080 connections,  $q < 0.05$ , **Table 2**). Empirical p-value analyses ('pval effect', Table 2) - performing contrasts in 5,000 randomly sampled groups - found the same level of significance compared to the FDR procedure (**Table 2**).

The previously published 22q11.2 deletion FC profile showed the largest effects (mean of brain-wide estimates in the top decile= 0.65) followed by the 16p11.2 deletion showed large effects on FC profiles (0.57). The 22q11.2 and 16p11.2 FC profiles were robust and correlated ( $r=0.7$  and  $0.83$ ) to previously

published profiles that were based on smaller samples (14). 1q21.1 deletion and duplication FC profiles showed moderate to large effects on FC. 15q11.2 deletion showed the mildest effects among CNVs associated with neurodevelopmental and psychiatric disorders. Individual CNV FC profiles in 3D maps showing effect sizes for each of the 64 functional regions are available at: [https://claramoreau9.github.io/Braimaps\\_Github.html](https://claramoreau9.github.io/Braimaps_Github.html).

All brain-related PRS (SZ, BIP, autism, cross disorder, IQ) altered FC profiles. The non-brain-related PRS (LDL, CKD) showed no significant effects (**Table 2**).

Individuals diagnosed with idiopathic SZ, BIP, autism but not ADHD had significantly altered FC compared to controls. SZ, ADHD, and autism FC-profiles were previously published (14) but we recomputed them with additional individuals. Correlations between new and previously published profiles were 0.95, 0.70 and 0.86 respectively.

Effect sizes were largest for CNVs associated with neurodevelopmental and psychiatric disorders followed by psychiatric conditions, fluid intelligence, neuroticism, and PRS (Figure 2A-D). Effect sizes of deletions were on average 1.3 fold larger than their reciprocal duplications. Effect sizes for a change in 1 SD of a cognitive trait or a PRS were on average one order of magnitude smaller than those associated with CNVs (Figure 2E). To test the relationship between GWAS sample size and the effect of PRS on FC, we compared the PRS-SZ based on the most recent GWAS in 76,755 subjects with SZ (51) to the one based on an older GWAS computed with 23,585 subjects with SZ (52). The 2 FC profiles associated with the 2 PRS-SZ were correlated ( $r=0.89$ ), but the number of significant connections was higher for the FC profile based on the larger SZ-GWAS. The effect size (for 1SD of PRS) was also larger, the top decile of beta values increasing from 0.0138, CI95 (0.011-0.016) to 0.016 CI95 (0.013-0.019).

Sensitivity analyses showed that effect size estimates were robust to several cross-validations as well as the effects of sex, pooled or matched controls, clinical or non-clinical ascertainment, and medication (eResults in [Supplement](#)).

## **Relationship between effect sizes of CNVs on connectivity and cognition or risk for neurodevelopmental and psychiatric disorders**

We observed a correlation between the effect size of CNVs on FC and their previously reported effect size on cognitive ability(7) ( $r=0.9$ ,  $p=5.93e^{06}$ ), but effects of CNVs on FC were systematically smaller than their effect on cognitive ability (**Figure 2F**). Effect size on FC was also correlated with previously reported, general risk for neurodevelopmental and psychiatric disorders; i.e. the highest risk conferred by each CNV for either autism (26,28) or SZ (4,53) ( $r=0.79$ ,  $p=0.001$ , **Figure 2H**). As expected, this correlation was weaker for autism ( $r=0.75$ ) and SZ-risk ( $r=0.6$ ) separately since some CNVs confer high risk for autism but not SZ and vice versa (**eFigure 4** in supplement). The correlation with cognitive ability was similar across all 12 networks (**Figure 2G**, **eFigure 5** in supplement).

## **Most networks are affected by genetic risk and conditions**

Genetic risk, conditions, and traits affected connections that were distributed across all functional brain networks (**Figure 3**). However, basal ganglia-thalamus and somatomotor networks exhibited over-connectivity across most genetic risk and conditions (sum beta values: 2.1 and 1.1 respectively). In contrast, limbic and auditory networks were predominantly under-connected (sum beta values: -2.7, -1.3 respectively).

## **Effect sizes of individual genes within CNVs decrease as CNVs increase in number of genes.**

We first asked if there was a relationship between the number of genes in a CNV (**Figure 4A**) and its effect size on functional connectivity. This was the case ( $r=0.72$ ,  $p=0.002$ , **Figure 4B**) and the relationship was similar when genes were weighted by their sensitivity to gene dosage (severity score,  $r=0.76$ ,  $p=0.0009$ , **Figure 4C**).

To investigate the effects of multigenicity on connectivity, we computed for each CNV an effect size adjusted for gene content (effect-size divided by the number of genes included in the CNV). We observed that the adjusted effect-size of CNVs significantly decreased as CNVs increased in number of genes ( $r= -0.85$ ,  $p= 3e^{05}$ , **Figure 4D**). Using the severity score showed the same phenomenon ( $r= -0.88$ ,  $p= 8.8e^{06}$ , **Figure 4E**). In other words, compared to small oligogenic CNVs, large multigenic

CNVs have smaller effects on FC than expected based on the number of genes they contain. We performed the same analysis using pLI (instead of LOEUF) to test the assumption that only a few intolerant genes contribute to the CNV-associated FC alterations (**eFigure 6**). The same decrease in effect size was observed as the number of intolerant genes increased in CNVs.

In contrast, there was no relationship between the severity score and its adjusted effect size on IQ ( $r=0.30$ ,  $p=0.25$ , **eFigure 7, *Supplement***).

To further investigate the effect of multigenicity on FC, we examined PRS and CNVs with similar effects (previously published, **Table2**) on cognitive ability and risk for autism and SZ. PRS effect sizes on connectivity were disproportionately lower (between 38 and 13-fold lower) than those observed for the selected CNVs (1q21.1 deletions, 16p11.2, and 22q11.2 duplications and 15q11.2 deletions) (**Figure 2E**).

## **Discussion**

### **Main findings**

In this large rsfMRI dataset, we demonstrated that most rare and common genetic risks for neurodevelopmental and psychiatric disorders affect FC but effect sizes vary over an order of magnitude across variants. We showed that the effect sizes of CNVs on FC were correlated with their previously reported effects on cognitive ability and risk for autism and SZ connectome-wide. This relationship was observed across all brain networks which is consistent with the fact that fluid intelligence is thought to be subserved by networks widely distributed across the brain (54). Whether these associations across all networks are causal of IQ decrease in CNV carriers remains an open question.

Multigenicity had a profound impact on FC signals: As CNVs increased in size and number of genes, effect size of CNVs adjusted for gene content (number of genes and severity score), rapidly decreased. In line with this observation, PRS had minute effects on FC, and the latter were disproportionately low compared to those observed for CNVs with similar effect sizes on IQ and risk for autism or SZ.

### **Similar effect sizes across functional and structural MRI**

Effect sizes on functional connectivity across neurodevelopmental CNVs and psychiatric conditions are consistent with those reported for structural MRI measures (55,56) even when much larger samples are investigated (Cohen's  $d=1$  and  $d=0.6$  for cortical surface and thickness respectively in  $n=475$  carriers of the 22q11.2 deletion (57)). For autism (58) and SZ (59) previously reported effect sizes for cortical thickness (Cohen's  $d=0.21$  and  $0.5$  respectively) were also similar to those observed in our study for functional connectivity (55). To date, the only effect sizes reported for PRS were for SZ (beta  $=0.02$  for cortical surface and thickness) and are consistent with the very small effects in the current study (60).

### **Even small levels of multigenicity increase heterogeneity at the functional connectivity level**

We observed that the effect size on FC of one gene (the CNV adjusted effect size) declines (by an order of magnitude) for increasingly multigenic CNVs. In other words, a gene would contribute to a smaller FC effect in a multigenic CNV compared to a gene with the same severity score (sensitivity to gene dosage) encompassed in a small oligogenic one. Multigenic CNVs may therefore represent heterogeneous combinations of relatively distinct FC profiles associated with each dosage-sensitive gene (**Figure 4F**). This suggests that genes within a CNV or a polygenic score may cancel out each other's effects on FC, leading to weaker effect sizes.

This effect of multigenicity may not be restricted to FC. As an example, Down syndrome, which encompasses more than 200 protein-coding genes (61), has an extreme effect size on cognition (a mean decrease of 3.3 SD(48,62)) but has been associated with smaller effects sizes on MRI structural measures (below 1.65 Cohen's  $d$  (63,64)). Based on our observations, genetic effects on rsfMRI would be best observed (with the largest effects) in the context of monogenic variants such as FMR1, NRXN1, or CHD8. This would likely apply to other brain modalities.

### **Why are polygenic scores associated with such small effect sizes?**

The microscopic effect sizes associated with autism-PRS, SZ-PRS, and intelligence-PRS are possibly related to extreme levels of heterogeneity. Our comparison of two PRS-SZ based on GWAS of

different sample sizes suggests that further increasing the GWAS sample size will improve the detection of significant connections altered by PRS, but will not substantially increase the effect size of psychiatric PRS on rsfMRI.

The CNV-PRS discordance is striking for PRS-IQ which has been associated with moderate to large effects on cognitive ability. There are infinite combinations of different common variants that would lead to the same PRS score. This may explain why PRS shows minimal convergence on a particular connectivity pattern. Of note, both traits (fluid intelligence and neuroticism) also showed similar effect sizes suggesting comparable levels of heterogeneity. However, alternative interpretations are possible. Current PRS may be vastly improved when larger GWAS will be available. Some of the CNVs investigated have small sample sizes which leads to inflated effect sizes. Functional connectivity may not represent a relevant intermediate phenotype for genetic risk or cognitive traits.

### **Limitations**

Genetic heterogeneity is only one of the plausible interpretations that may explain these observations such as the survivor effect where large deleterious multigenic CNVs are only observed in resilient individuals with disproportionately low alterations at the brain connectivity level. Similarly, bias toward less individuals, which have a higher probability of completing the MRI scan coils contributes as well to this drop-in effect size. An alternative interpretation that may explain why effect sizes of CNVs on FC < Cognition is that FC is a noisy metric and noise could be increased in individuals with neurodevelopmental conditions (i.e., head movement despite being carefully adjusted for).

For PRS, the portability across populations is poor which may contribute to the small effects of PRS on FC. Finally, CNVs including intolerant genes are under negative selection, whereas this is likely not the case for psychiatric PRS which are the sum of many variants that are individually frequent. This fundamental difference may contribute to differences in FC effect sizes observed for a PRS and a CNV matched for the level of disease risk.

Confounding factors include sex bias and age differences may have influenced some of the results. However, carefully conducted sensitivity analyses provided similar results (Supplemental results). Larger samples will be required to detect potential interactions between genetic risk and age or sex.

## **Conclusion**

Polygenicity may predominantly result in “poly-connectivity”, a scenario where thousands of autism or SZ genomic risk variants lead to a diverse set of connectivity patterns associated with the conditions. Future studies will require both in-depth partitionings of polygenic scores as well as clustering of rare variants - based on relevant gene functions - to obtain mechanistically coherent subgroups of individuals.

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This paper has been posted on medRxiv (65).

## **Author contributions**

C.A.M., S.J., and P.B. designed the overall study and drafted the manuscript.

## **Analyses**

C.A.M., A.H., and S.U. processed 90% of all the fMRI data and performed all imaging analyses.

G.H., J-L. M., and E. D. performed the CNVs calling.

P.O. preprocessed the SZ data.

H.S. performed the UKBB fMRI preprocessing.

L.M.S. and L.A. computed the polygenic scores.

A.L. gave feedback on the statistics used in this manuscript.

T.B. and T.R. gave feedback on the analyses using polygenic risk and LOEUF scores.

T.B., P.M.T., D.C.G., and C.E.B. contributed to the interpretation of the data and reviewed the manuscript.

## Data collection

KK, K.J., C-O.M, P.T., N.Y., E.D., S.L., recruited/scanned patients for the Montreal rare genomic disorder (MRG) family dataset.

C.E.B. provided the UCLA 22q.11.2 fMRI data.

D.E.J.L., M.J.O., M.V.B., J.H., and A.I.S. provided the Cardiff CNV fMRI data

All authors provided feedback on the manuscript.

## Competing interests

P.M.T. received partial research grant support from Biogen, Inc., for research unrelated to this study.

Other authors did not have a conflict of interest.

M.J.O., J.H., and M.V.B. have a research grant from Takeda Pharmaceuticals outside the scope of the present work.

J.H. is a founding director of the company Meomics (unrelated to this work).

## Data and materials availability

Data from UK Biobank was downloaded under the application 40980, and can be accessed via their standard data access procedure (see <http://www.ukbiobank.ac.uk/register-apply>). UK Biobank CNVs were called using the pipeline developed in Jacquemont Lab, and described in:

<https://github.com/labjacquemont/MIND-GENESPARALLELCNV>.

The final CNV calls are available from UK-Biobank returned datasets (Return ID: 3104, <https://biobank.ndph.ox.ac.uk/ukb/dset.cgi?id=3104> ).

ABIDE1, ABIDE2, COBRE, ADHD200, CNP, 16p11.2 SVIP data are publicly available:

[http://fcon\\_1000.projects.nitrc.org/indi/abide/abide\\_I.html](http://fcon_1000.projects.nitrc.org/indi/abide/abide_I.html),

[http://fcon\\_1000.projects.nitrc.org/indi/abide/abide\\_II.html](http://fcon_1000.projects.nitrc.org/indi/abide/abide_II.html), <http://schizconnect.org/queries/new>,

[http://fcon\\_1000.projects.nitrc.org/indi/adhd200/](http://fcon_1000.projects.nitrc.org/indi/adhd200/), <https://www.openfmri.org/dataset/ds000030/>,

<https://www.sfari.org/funded-project/simons-variation-in-individuals-project-simons-vip/>. The

22q11.2 UCLA raw data are currently available by request from the PI. Raw imaging data for the

Montreal rare genomic disorder family dataset is going to be available on the LORIS platform in 2023. The Cardiff raw data is not publicly available yet, contact the PI for further information. All processed connectomes are available through a request to the corresponding authors. Code for all analyses and visualizations, beta values, and p-values for the 29 FC profiles are available online through the GitHub platform with Jupyter notebook:

[https://github.com/claramoreau9/NeuropsychiatricCNVs\\_Connectivity](https://github.com/claramoreau9/NeuropsychiatricCNVs_Connectivity)

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## **Titles and legends for figures and tables**

### **Table 1 Demographics**

*Legend: CNV carriers (DEL: deletion; DUP: duplication), individuals with idiopathic psychiatric conditions (SZ: schizophrenia, ASD: Autism Spectrum Disorder; ADHD: Attention-Deficit / Hyperactivity-Disorder, BIP: Bipolar disorder), and controls after MRI quality control. Chr: chromosome number, coordinates are presented in Megabases (Mb, Hg19).*

*Age (in years); M: male, F: Female. Quantitative variables are expressed as the mean  $\pm$  SD (standard deviation). The cohort column provides the cohorts used to perform case-control studies for each of the 29 CWAS (CNP: Consortium for Neuropsychiatric Phenomics, MRG: Montreal rare genomic*

disorder). IQ loss: mean decrease in IQ points associated with each CNV (7,8). Odd-ratios (OR) for the enrichment of CNVs and PRS in autism and schizophrenia were previously published (4,5,9,11,26,27,53,66–71). OR for the enrichment of CNVs in ADHD were not available. ‘Control PRS’ = non-brain-related PRS.

The 8 non-psychiatric CNVs were defined as variants without any previous association with psychiatric conditions in large cases control studies (4,26–28), and detailed information relative to diagnosis, IQ, and motion, are available in eMaterial, [Supplement](#). Information relative to scanning sites, motion and diagnoses are also available in **eTables2-4**, [Supplement](#). All sites scanned controls. Abbreviations: PRS: polygenic risk score; CrossD: Cross-disorder, LDL: Low-Density Lipoprotein, CKD: Chronic Kidney Disease; IQ: intelligence quotient.

## **Table 2 Connectome-wide association study summary**

Legend: The number of significantly altered connections (FDR corrected) for each connectome-wide association study ( $n=29$ ) (**eTable 5** in supplement). ‘Control PRS’ = non-brain-related PRS; min-max: minimum-maximum of z-scored beta values; Top-dec beta values: Effect-size of genetic risk, conditions, and traits on connectivity defined as the top decile of the 2080 absolute  $\beta$  values. pval effect= empirical p-value obtained by conducting a permutation test, shuffling the genetic or clinical status labels of the individuals included in each connectome-wide association study (5,000 permutations). We estimated the empirical p-value by calculating the frequency of obtaining an effect size equal or greater than the original observation.

Abbreviations: DEL: deletion; DUP: duplication; SZ: schizophrenia, ASD: Autism Spectrum Disorder; ADHD: Attention-Deficit / Hyperactivity-Disorder, BIP: Bipolar disorder, CrossD: Cross-disorder, LDL: Low-Density Lipoprotein, CKD: Chronic Kidney Disease; IQ: intelligence quotient, IBD: Inflammatory bowel disease, PRS: polygenic risk score; Connection pos: number of positive connections surviving FDR; Connection neg: number of negative connections surviving.

## **Figure 1: Method flowchart**

GP: General population, QC: Quality Control, ROI: region of interest, MIST: Multiresolution Intrinsic Segmentation Template

## **Figure 2. Relationship between effect sizes of CNVs on cognition & connectivity**

(A-D) Effect size of genetic risk, conditions and traits on functional connectivity. *Effect sizes of (A) CNVs, (B) idiopathic psychiatric conditions, (C) traits, (D) Polygenic scores (PRS) on functional connectivity. Each dot (or diamond) is the mean of estimates in the top decile (the 208 connections with the highest beta estimates). X-axis values represent the effect sizes z-scored on the variance of the control group. Full dots represent significant effect sizes (the intersection of FC profiles with altered connections surviving FDR and empirical p values using 5000 permutation tests  $q < 0.05$ , Table 2) and empty diamonds are non-significant effect sizes. CI: confidence interval.*

### **(E) Ratio of effect sizes between genetic risk, conditions, and traits**

*Ratios are only computed for groups that have significant effect sizes on functional connectivity. The ratio is the line (numerator) divided by the column (denominator). CI95 for each ratio was computed using a bootstrap procedure (43,46) (Method). Boxes with black borders highlight CNVs and PRS that should have similar effects on connectivity because they are matched for effect size on cognition or risk for disease. We also highlight CNVs that have effect sizes on cognitive ability equal or smaller than 1 z-score to highlight the discordance with effect of 1 z-score of fluid intelligence on connectivity.*

### **(F) Effect sizes of CNVs on IQ and FC**

*We used previously published effect sizes of CNVs on IQ (7). X-axis: decrease in IQ associated with each CNV. Y-axis: effect sizes of CNVs on FC (top decile of estimates).*

### **(G) Effect sizes of CNVs on IQ and FC for 12 functional networks**

*X-axis: decrease in IQ associated with each CNV. (7) Y-axis: effect sizes of CNVs on FC for 12 functional networks (mean of the top decile of network-wide estimates) (For a representation of each network individually, see **eFigure 5** in supplement).*

### **(H) Effect sizes of CNVs on FC and risk for autism or SZ**

*Correlation between previously published effect sizes of CNVs on autism or SZ risk (4,26,28,53) and their effect sizes on FC. We used the highest risk conferred by each CNV for either autism (26,28) or SZ (4,53). X-axis: Odd ratios for autism (▲) or SZ (■). Y-axis: effect sizes on FC (top decile of estimates).*

*Abbreviations: ASD: autism spectrum disorder, SZ: schizophrenia, NT: neuroticism, Del: deletion, Dup: duplication, Fluid intel: fluid intelligence, IQ: intelligence quotient, ADHD: Attention-Deficit / Hyperactivity-Disorder, LDL: Low-Density Lipoprotein; CKD: Chronic Kidney Disease, DMN: Default Mode Network.*

### **Figure 3. Similarities at the network level across genetic risks, psychiatric conditions, and traits**

*Legend: Sankey plot shows effect sizes across 12 networks for genetic risk (left), conditions, and traits (right). The thickness of the connecting lines represents these effect sizes, which were defined as the mean beta value of all significant connections within the network and between the network and the other 11 networks. The length and color of rectangles on either side of each network in the middle of the Sankey plot represents the sum of effect sizes across all genetic risks, conditions and traits for that particular network. For each network, effect sizes values are summarized in the 12 boxes (bottom of the figure). Brain maps represent the max estimate value for each functional region (Table 2). Red = overconnectivity ; blue underconnectivity. Color bars represent the beta value.*

*Abbreviations: BG Thal: Basal Ganglia Thalamus; MOT: Somatomotor network; FP: Frontoparietal network; VIS: Visual network; DMN pm: Default mode network posteromedial; CER: Cerebellum; DM l: Default mode network lateral; VVIS DVIS: Ventral and dorsal visual network; AUD PINS: auditory network and posterior insula; DM am: Default mode network anteromedial; VATT SAL: Ventral attentional and Salience network; LIM: Limbic network; ASD: autism spectrum disorder, SZ: schizophrenia, BIP: bipolar disorder, NT: Neuroticism, PRS: Polygenic score, Del: deletion, Dup: duplication, Fluid intel: fluid intelligence, CrossD: Cross-Disorder.*

### **Figure 4. Relationship between multigenicity and connectivity**

**(A) Number of genes and severity score**

Bar plot showing for each CNV, the number of genes encompassed, and the sum of genes weighted by their intolerance score (sum of  $1/LOEUF$ ). The sum of  $1/LOEUF$  values of all genes encompassed in a CNV is highly predictive of the effect size of CNVs on cognitive ability.

**(B) Relationship between effect sizes on FC and number of genes.**

Y-axis: Effect size of CNVs on FC. X-axis: Number of genes in each CNV.

**(C) Relationship between effect sizes on FC and severity score.**

Y-axis: Effect size of CNVs on FC. X-axis: Severity score for each CNV.

**(D) Adjusted effect sizes on FC and multigenicity (genes)**

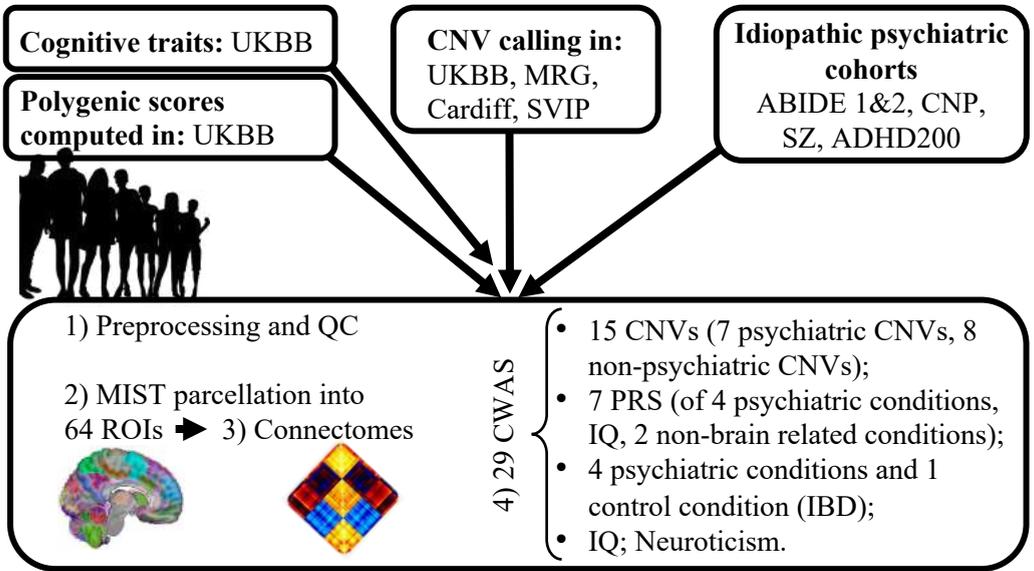
Y-axis: mean effect of one gene on FC (CNV effect sizes on FC adjusted for number of genes). X-axis: Number of genes in each CNV.

**(E) Adjusted effect sizes on FC and multigenicity (severity score)**

Y-axis: mean effect on FC of one point of severity score (CNV effect sizes adjusted by the severity score). X-axis: Severity score for each CNV.

**(F) Genetic heterogeneity within a CNV**

Genes encompassed in the 16p11.2 CNV color-coded based on their LOEUF score. Two scenarios are represented: Right, genes converge on shared brain patterns: the effect size of the CNV increases linearly with the number of intolerant genes and is large. Left, genes within a CNV are associated with distinct patterns: the resulting effect size is weaker.



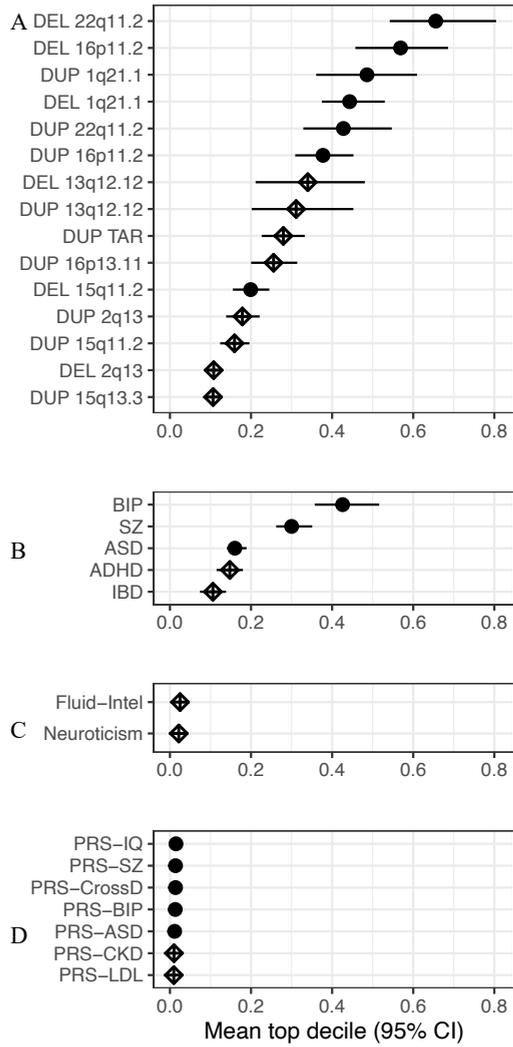
**Aim 1:** Estimate and compare the effects of oligogenic, multigenic and polygenic risk for psychiatric conditions on brain connectivity

**Aim 2:** Characterize the relationship between effects sizes of genomic variants on brain connectivity vs. on cognition/behaviour.

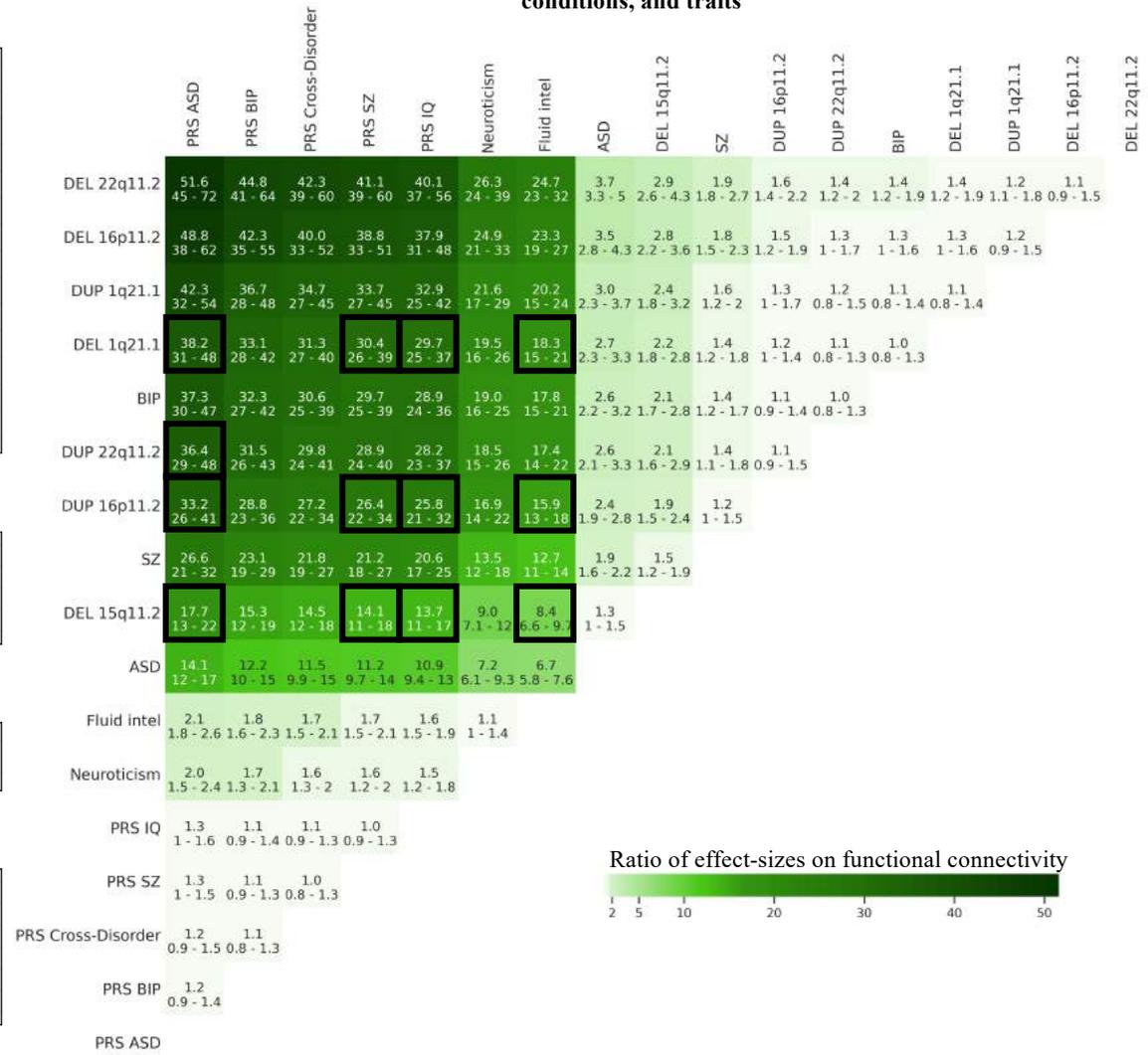
**Aim 3:** Test the relationship between the level of multigenicity and effect sizes of genomic variants on connectivity

Datasets		Aim 1 Figure 2.A-D	Aim 1 Figure 3	Aim 2 Figure 2.E-H	Aim 3 Figure 4
GP	UKBB	✓	✓	✓	✓
	Idiopathic psychiatric conditions				
Idiopathic psychiatric conditions	ABIDE1-2	✓	✓		
	ADHD200	✓	✓		
	CNP	✓	✓		
	Cobre	✓	✓		
	Clinical genetic datasets				
Clinical genetic datasets	SVIP	✓	✓	✓	✓
	MRG	✓	✓	✓	✓
	UCLA	✓	✓	✓	✓
	Cardiff	✓	✓	✓	✓

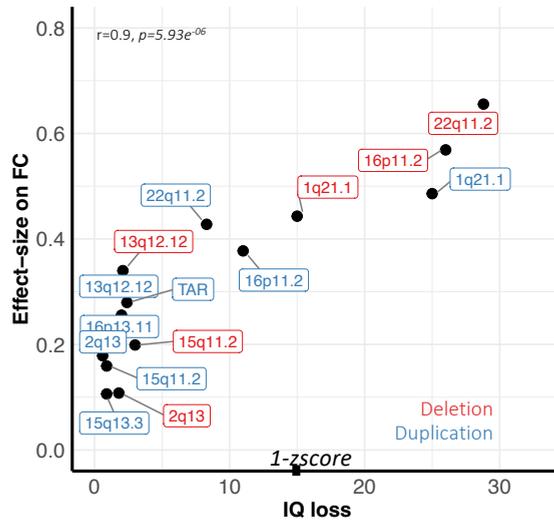
**A-D. Effect sizes of genetic risk, conditions and traits on connectivity**



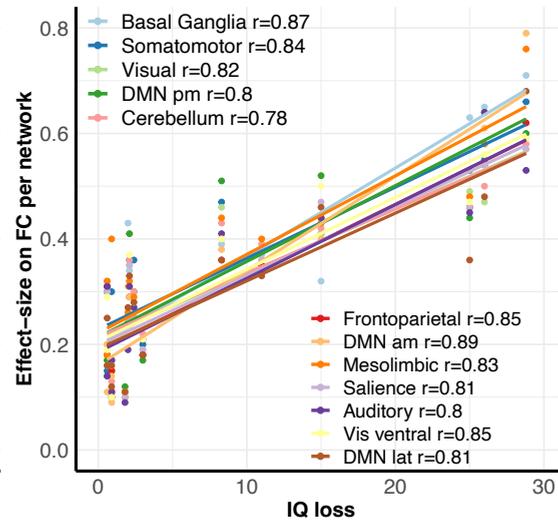
**E. Ratio of effect sizes between genetic risk, conditions, and traits**



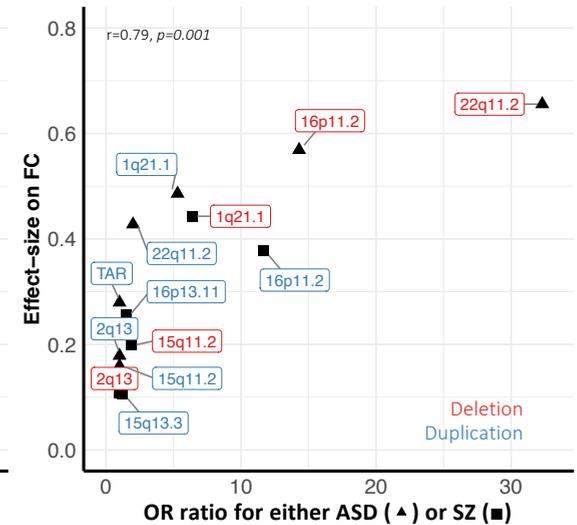
**F. Effect sizes of CNVs on IQ and FC**

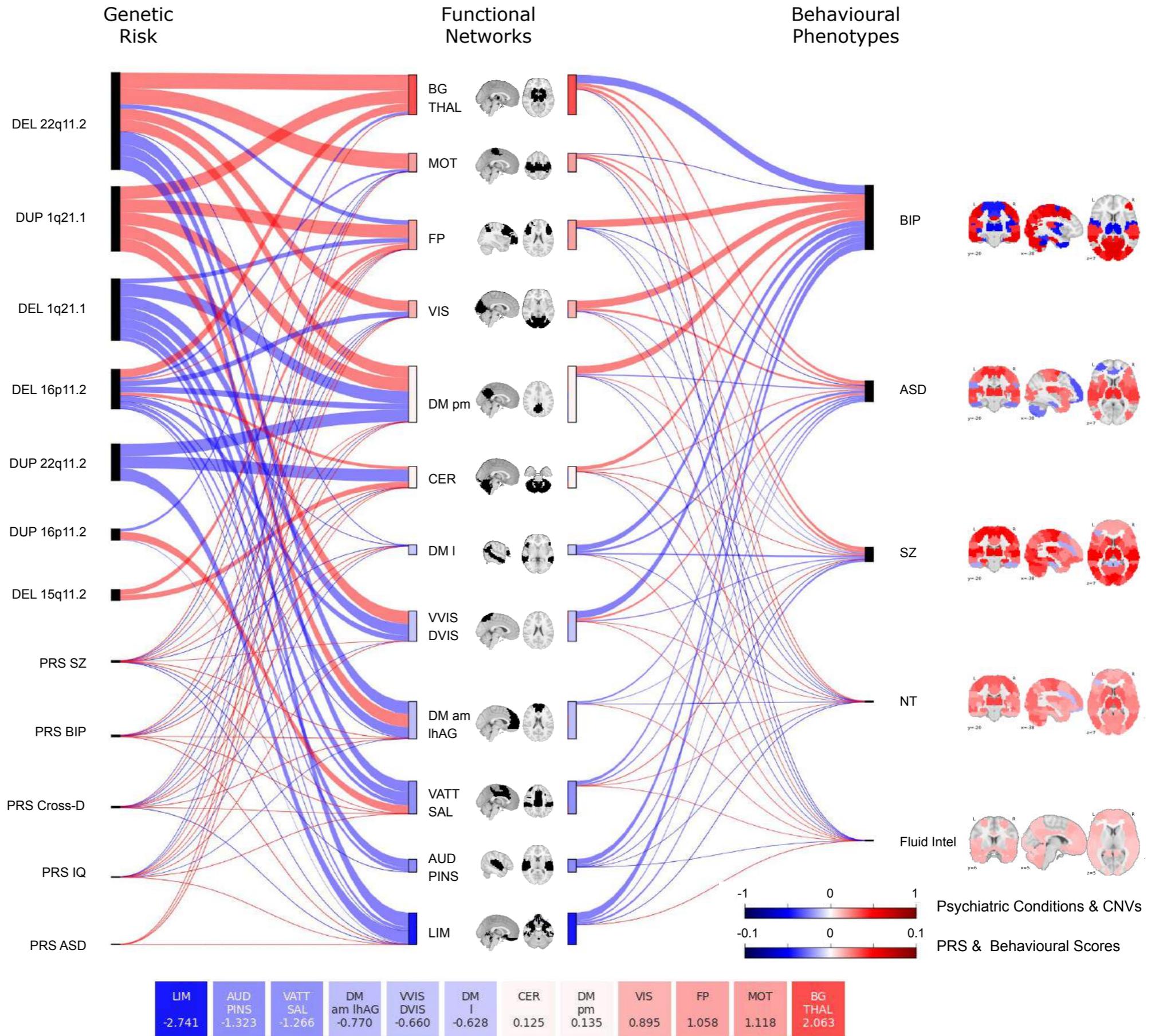
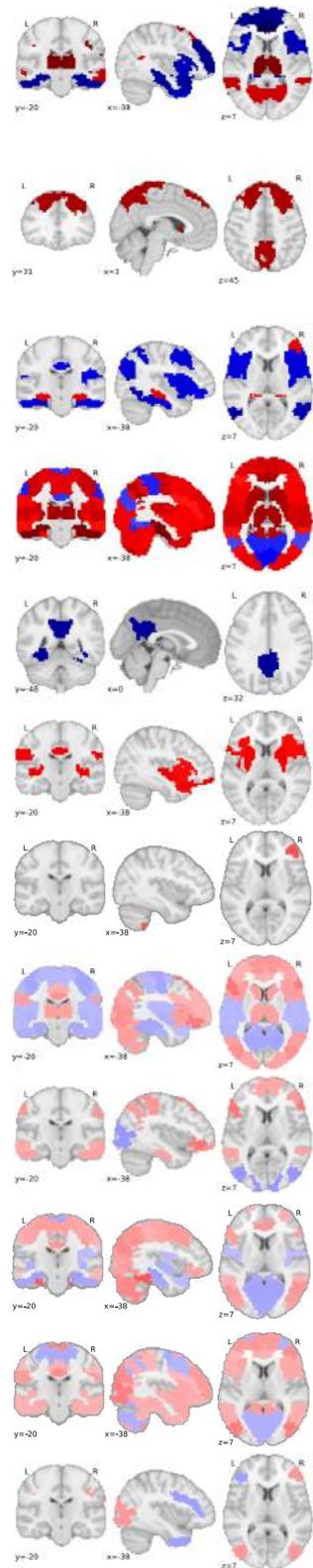


**G. Effect sizes of CNVs on IQ and FC for 12 functional networks**

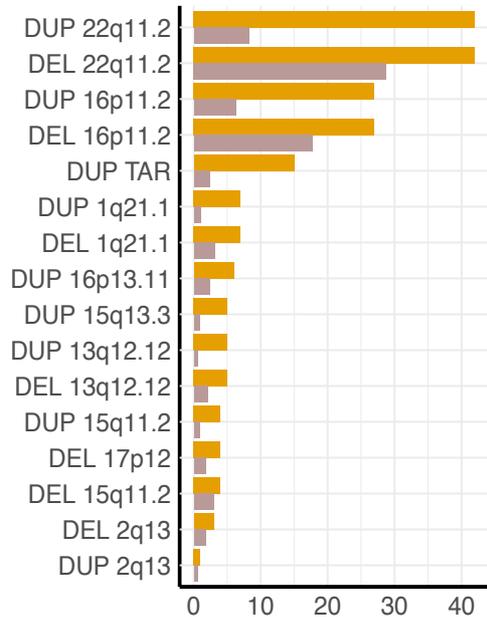


**H. Effect sizes of CNVs on FC and risk for ASD or SZ**





### A. Number of genes and severity score

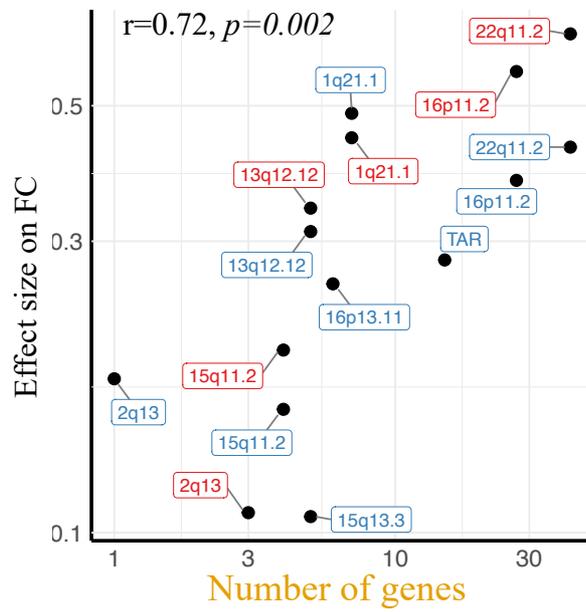


■ Severity score

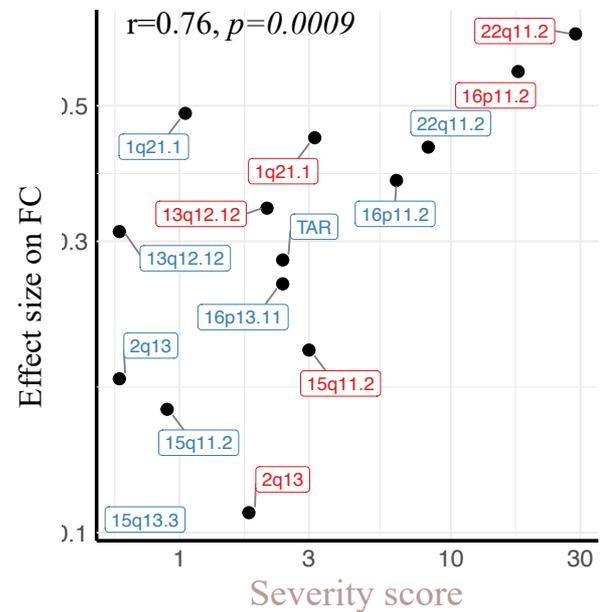
$$= \sum (\text{LOEUF scores for all genes in a CNV})$$

■ Number of genes

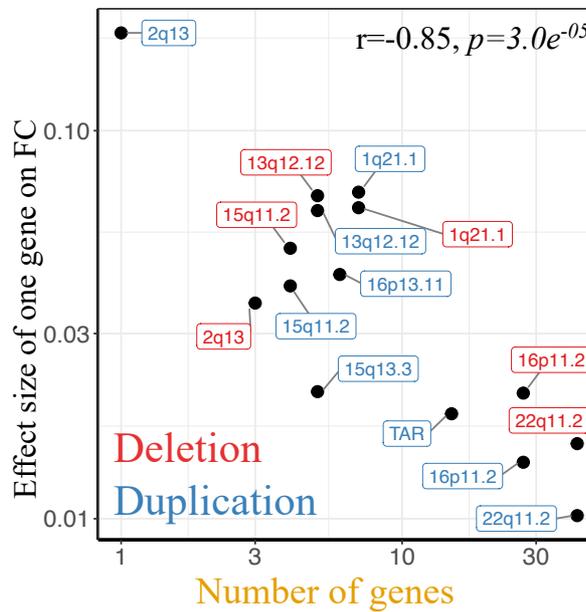
### B. Relationship between effect sizes on FC and number of genes



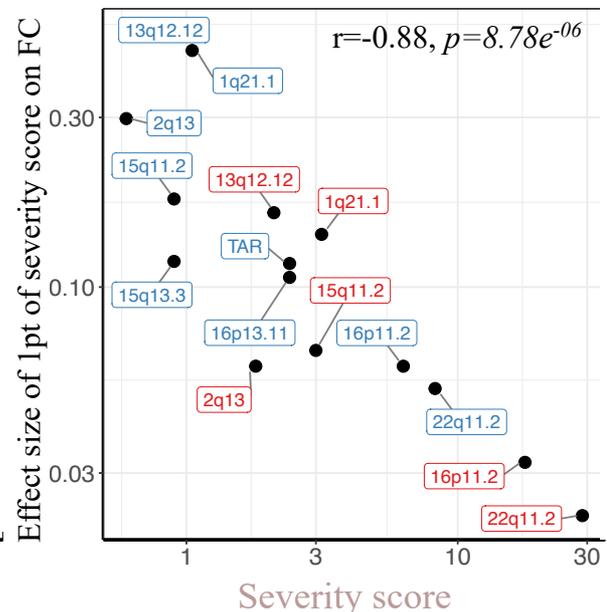
### C. Relationship between effect sizes on FC and severity score



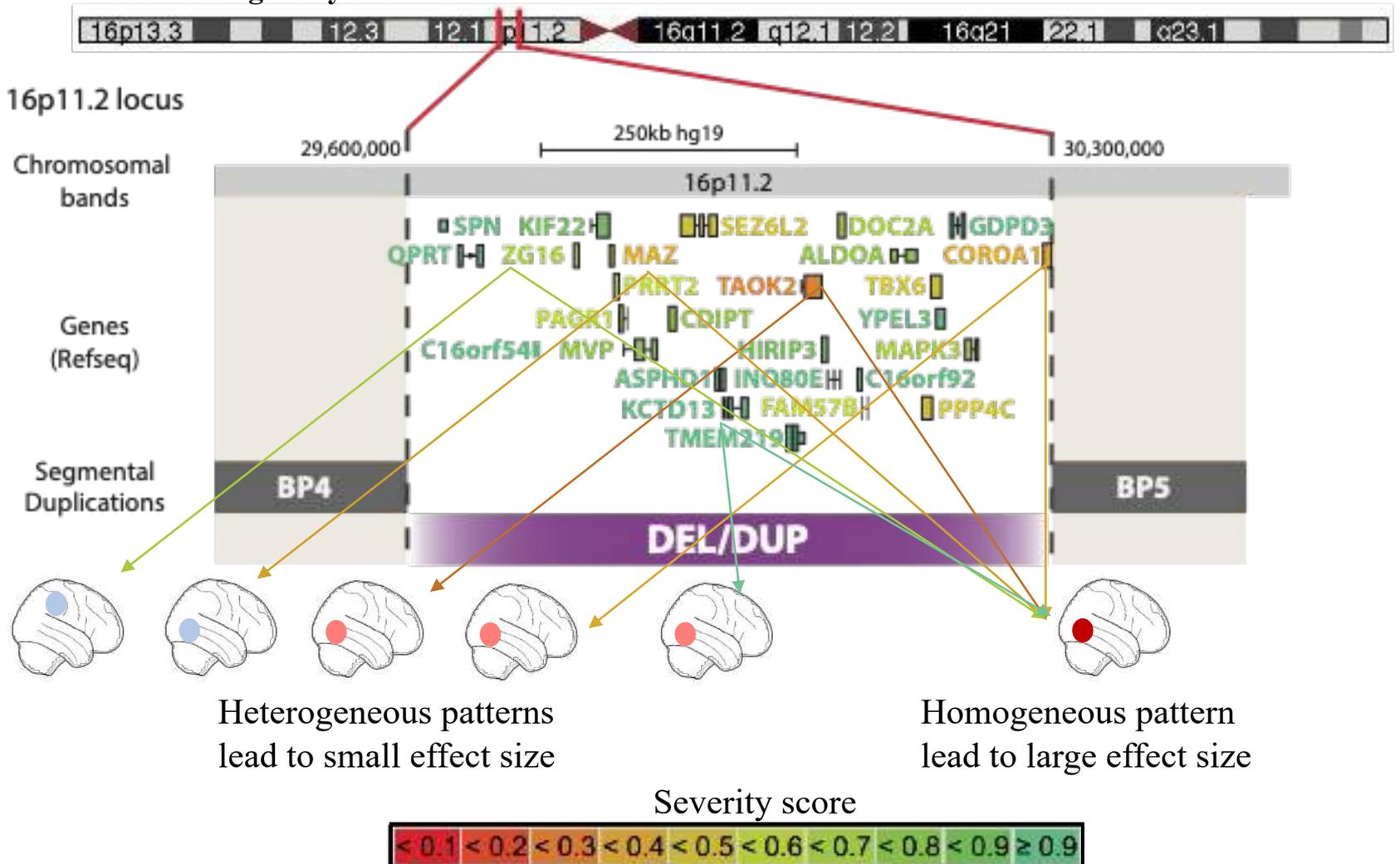
### D. Adjusted effect sizes on FC and polygenicity (genes)



### E. Adjusted effect sizes on FC and polygenicity (severity score)



### F. Genetic heterogeneity within a CNV



CNV (hg19)	n (genes) / Well known gene	Status	Ascertainment		Age	Sex (F/M)	Cohorts	IQ loss	OR ASD	OR SZ	
			Clinic	Un- selected							Previously published
<b>Psychiatric CNVs</b>	<b>1q21.1</b> 1: 146.53-147.39	7 <i>CHDIL</i>	DEL	15	10	44.4 (19)	12/13	UKBB- MRG- Cardiff- SFARI	15	3.2	6.4
			DUP	16	13	50.9 (19)	13/6		25	5.3	2.9
	<b>22q11.2</b> 22: 19.04-21.47	49 <i>TBX1</i>	DEL	43	0	16.9 (7)	19/24	UCLA	28.8	32.3	23
			DUP	12	10	39.4 (23)	12/10	UCLA-UKBB Cardiff- MRG	8.3	2	0.2
<b>16p11.2</b> 16: 29.65-30.20	27 <i>KCTD13</i>	DEL	28	4	21.7 (20)	13/19	SFARI - MRG - UKBB	26	14.3	1.1	
		DUP	29	6	34.1 (19)	14/21		11	10.5	11.7	
<b>Non-psychiatric CNVs</b>	<b>15q11.2</b> 15: 22.81-23.09	4 <i>CYFIP1</i>	DEL	0	103	64.3 (7)	55/48	UKBB	3	1.3	1.9
			DUP	0	136	63.7 (7)	76/60		0.9	1	1
	<b>15q13.3</b> 15: 31.08-32.46	5 <i>CHRNA7</i>	DUP	0	190	64.4 (7)	100/90		0.9	0.7	1.24
			DEL	0	183	63.1 (7)	110/73		1.8	-	1
	<b>2q13</b> 2: 110.86-110.98	3 <i>NPHP1</i>	DUP	0	88	64.7 (8)	43/45		0.6	-	1
			DEL	0	22	63.5 (6)	12/9		2.1	-	-
	<b>16p13.11</b> 16: 15.51-16.29	6 <i>MYH11</i>	DUP	0	40	64.7 (6)	21/19		2	1	1.5
			DEL	0	20	60.8 (7)	10/10		0.6	-	-
	<b>13q12.12</b> 13: 23.56-24.88	5 <i>SPATA13</i>	DUP	0	29	59.8 (7)	14/15		2.4	-	1
			DEL	0	22	63.5 (6)	12/9		2.1	-	-
<b>TAR</b> 1: 145.39-147.39	15 <i>RBM8</i>	DUP	0	29	59.8 (7)	14/15	2.4	-	1		
<b>Idiopathic Psychiatric Conditions</b>		<b>SZ</b>	283	0	33.9 (9.2)	73/210	Montreal-SZ CNP	-	-	-	
		<b>BIP</b>	44	0	35 (9)	20/24	CNP	-	-	-	
		<b>ASD</b>	472	0	14.9 (6)	0/472	ABIDE1, ABIDE2	-	-	-	
		<b>ADHD</b>	223	0	14.8 (9.5)	66/157	ADHD-200 CNP	-	-	-	
<b>Non-psychiatric condition</b>		<b>IBD</b>	0	287	64.7(7.5)	144/143	UKBB				
<b>Polygenic scores</b>		Autism	0	29460	64.2 (7.5)	15840/ 13620	UKBB	-	2.7	-	
		Schizophrenia						-	-	<b>3.5</b>	
		Bipolar						-	-	-	
		Cross-Disorder						-	-	-	
		IQ						9-12	-	-	
<b>“Control” PRS</b>	LDL, CKD							-	-	-	
<b>Traits</b>		Fluid intelligence	0	27522	64 (7.5)	14777/ 12745		-	-	-	
		Neuroticism	0	24025	64 (7.5)	12723/ 11302		-	-	-	
<b>Controls</b>		UKBB	0	30185	64.1 (7.5)	16260/ 3925	UKBB	-	-	-	
		SFARI	0	84	26.7 (15)	35/49	SFARI	-	-	-	
		MRG	0	39	34 (16)	25/14	MRG	-	-	-	
		Cardiff	0	8	39.8 (4)	4/4	Cardiff	-	-	-	
		UCLA	0	43	13 (4.6)	22/21	UCLA	-	-	-	
		Psychiatric cohorts	0	1066	20 [11]	244/822	-	-	-	-	

CNV/Conditions/Traits		Global signal adjustment					
		Connections		Beta values		Top-dec $\beta$ values	pval effect
		<i>pos</i>	<i>neg</i>	<i>min</i>	<i>max</i>		
Psychiatric CNVs	1q21.1 DEL	1	11	-1.07	0.62	0.44	0.002
	1q21.1 DUP	4	0	-0.62	0.84	0.48	0.002
	15q11.2 DEL	1	0	-0.29	0.36	0.2	0.01
	16p11.2 DEL	124	149	-0.98	1.67	0.57	<2e-4
	16p11.2 DUP	4	3	-1.04	0.55	0.38	0.002
	22q11.2 DEL	4	13	-1.48	1	0.65	<2e-4
	22q11.2 DUP	0	2	-0.78	0.69	0.43	0.04
Non-psychiatric CNVs	TAR DUP	0	0	-0.48	0.51	0.28	ns
	2q13 DEL	0	0	-0.15	0.19	0.11	ns
	2q13 DUP	0	0	-0.34	0.26	0.18	ns
	13q12.12 DEL	0	0	-0.54	0.5	0.34	ns
	13q12.12 DUP	0	0	-0.53	0.48	0.31	ns
	15q11.2 DUP	0	0	-0.24	0.24	0.16	0.04
	15q13.3 DUP	0	0	-0.20	0.18	0.11	ns
	16p13.11 DUP	0	0	-0.42	0.40	0.26	ns
Polygenic score	Cross Dis	23	22	-.02	.03	0.01	<2e-4
	Autism	3	1	-.02	.02	0.01	0.04
	Schizophrenia	30	27	-.02	.03	0.01	<2e-4
	Bipolar	16	2	-.02	.03	0.01	0.002
	IQ	74	42	-.02	.02	0.01	<3e-4
Control PRS	LDL	0	0	-.02	.02	0.009	ns
	CKD	0	0	-.02	.02	0.01	ns
Psychiatric conditions	Autism	51	55	-0.26	0.36	0.16	<2e-4
	Schizophrenia	221	258	-0.41	0.51	0.30	<2e-4
	Bipolar	33	24	-0.66	0.65	0.43	<2e-4
	ADHD	0	0	-0.22	0.22	0.15	<2e-4
	IBD	0	0	-0.16	0.16	0.11	ns
Traits	Fluid Intel	311	281	-.04	.04	0.02	<2e-4
	Neuroticism	208	208	-.03	.04	0.02	<2e-4