Supplementary material

Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network

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Figure S1. Individual coverage and joint coverage of trios. On average across all samples, 93% of targeted regions were covered at ≥10X (dots with connecting lines). Joint coverage of each parent-parent-child trio was defined as fold-coverage for the least well covered individual in the trio (Iossifov et al., 2012). Proportions of trios with >10X joint coverage are shown as black dots and with >20X joint coverage are shown as open circles.

Figure S2. Interconnectedness of transcriptional co-expression networks, at various developmental stages and in different brain regions, based on genes harboring *de novo* benign mutations. Co-expression of genes harboring *de novo* benign mutations in cases and in controls was evaluated using RNASeq data from the BrainSpan Atlas. Gene pairs were defined as co-expressed if $|R| \geq 0.8$ for their RNASeq expression levels across all tissues from a given brain region and a given developmental stage. Networks were created for co-expressed gene pairs as described for Figure 2B. Dotted lines indicate numbers of connections (edges) in networks created using genes with *de novo* benign mutations in cases. Histograms represent distributions of the numbers of edges in 10,000 simulated networks using 247 genes with *de novo* benign mutations in controls.

Table S1: Participants.

Insert Excel file Table S1

Notes. For COGS participants, premorbid IQ was estimated using the Wide Range Achievement Test, Third edition (WRAT-3), reading subtest (Jastak and Wilkinson, 1993). For UW participants, IQ was assessed using the Wechsler Abbreviated Scoales of Intelligence (WASI) (Stano, 1999)

Table S2. Proportions of non-synonymous *de novo* mutations in six exome studies

Type	Present study	O'Roak et al. 2012	Neale et al. 2012	Iossifov et al. 2012	Sanders et al. 2012	Xu et al. 2012
Silent	50	84	50	148	67	30
Missense	92	186	101	414	207	126
Nonsense	13	18	10	28	15	6
Total coding	155	288	161	590	289	162
% non-synonymous	67.7	70.8	68.9	74.9	76.8	81.5

Notes: For each study, information is drawn from reported analyses, including data provided in supplemental information.

Table S3: De novo mutations in schizophrenia probands and unaffected siblings.

Insert excel file Table S3

Table S4. De novo mutations in unaffected siblings from other exome studies of autism and schizophrenia

Insert excel file Table S4

Table S5. Number of tissues by brain region and developmental stage used in network analyses (BrainSpan, 2013)

			Number of specimens		
Brain region		Tissue	13-26 pcw	4 months-11 years	13-23 years
SC	STR	Striatum	11	8	5
	MD	Mediodorsal nucleus of thalamus	9	9	5
	AMY	Amygdaloid complex	10	11	5
	HIP	Hippocampus	11	11	5
SM	A1C	Primary auditory cortex (core)	11	11	6
	M1C	Primary motor cortex (area M1, area 4)	7	10	6
	S1C	Primary somatosensory cortex (areas S1,3,1,2)	7	11	6
	V1C	Primary visual cortex (striate cortex, area V1/17)	12	12	6
FC	DFC	Dorsolateral prefrontal cortex	12	11	6
	MFC	Anterior (rostral) cingulate (medial prefrontal) cortex	11	11	6
	OFC	Orbital frontal cortex	8	11	6
	VFC	Ventrolateral prefrontal cortex	11	12	6
TP	ITC	Inferolateral temporal cortex (area TEv, area 20)	8	12	6
	STC	Posterior (caudal) superior temporal cortex (area TAc)	12	11	6
	IPC	Posteroinferior (ventral) parietal cortex	11	11	6

SC: sub-cortical regions, SM: sensory-motor regions, FC: frontal cortex, TP: temporal-parietal cortex. 13-16 pcw (post conception weeks): fetal, 4 months – 11 years: infancy to late childhood, 13 - 23 years: adolescence to adulthood

Table S6. Network analyses of four brain regions at three developmental periods

Abbreviations: pcw: post conception week, SC: sub-cortical regions, SM: sensory-motor regions, FC: frontal cortex, TP: temporal-parietal cortex

Stages	Tissues	Nodes	P	Corrected P	Edges	P	Corrected P
All periods (13pcw-23yrs)	All tissues	24	0.21	0.21	42	0.95	0.95
Fetal (13-26 pcw)	SC	23	0.009	0.10	25	0.06	0.50
	SM	19	0.28	0.98	21	0.17	0.89
	FC	25	0.03	0.33	50	0.00006	0.0007
	TP	30	0.0009	0.01	44	0.01	0.16
Infancy to late childhood (4 months – 11 years)	SC	39	0.10	0.70	180	0.19	0.92
	SM	38	0.36	0.99	261	0.03	0.30
	FC	36	0.67	1.00	244	0.05	0.47
	TP	36	0.69	1.00	249	0.04	0.41
Adolescence to adulthood (13 – 23 years)	SC	34	0.27	0.98	54	0.91	1.00
	SM	35	0.79	1.00	201	0.12	0.78
	FC	37	0.26	0.97	149	0.33	0.99
	TP	36	0.69	1.00	191	0.16	0.88

Table S7. Network analyses of subregions of prefrontal cortex

Tissue group	Nodes	P	Corrected P	Edges	P	Corrected P
DFC	36	0.03	0.11	67	0.0001	0.0005
VFC	41	0.06	0.23	74	0.0004	0.002
MFC	30	0.38	0.85	64	0.03	0.12
OFC	44	0.79	1.00	115	0.15	0.49

DFC; Dorsolateral prefrontal cortex, MFC; Anterior (rostral) cingulate (medial prefrontal) cortex, OFC; Orbital frontal cortex, VFC; Ventrolateral prefrontal cortex.

Table S8: Probands harboring two or more damaging *de novo* mutations

Family	Gene	Effect	Comments
NIMH000007	multiple	del 2 MB	Genomic hotspot associated with schizophrenia and autism (Moreno-De-Luca et al., 2010)
NIMH000007	SERPINI1	R393stop	Neuronal differentiation; postmortem expression differences in schizophrenia (Vawter et al., 2004)
NIMH000009	BLNK	R49stop	Signaling and immune function (Imamura et al., 2009)
NIMH000009	FILIP1	R1119W	Neuronal migration (Sato and Nagano, 2005)
NIMH000009	MKI67	K857stop	Cellular proliferation (OMIM 176741)
NIMH000026	FRY	T1538A	Neuronal dendritic patterning (Emoto et al., 2004)
NIMH000026	HIF1A	L386V	Neuroprotective factor (Umschweif et al., 2013)
NIMH000079	KIAA1109	Q2439stop	Cell growth and differentiation; neuronal synaptic transmission (Verstreken et al., 2009).
NIMH000079	NCAN	P1219L	Neuronal adhesion and migration, SNPs associated with schizophrenia and mania (Muhleisen et al., 2012; Miro et al., 2012).
NIMH000255	DTX1	L69V	Notch1 signaling, gliogenesis (Patten et al., 2006)
NIMH000255	INTS1	S295N	Integrator complex, snRNA transcription (OMIM 611345)
NIMH019055	RUNX3	R211H	Transcription factor, cell growth and differentiation in brain (Horsfield et al., 2007)
NIMH019055	SLIT2	N521T	SLIT-ROBO signaling pathway (Zhang et al., 2012)
NIMH063154	ITGA3	P680A	Neuronal migration (Kähler et al., 2008)
NIMH063154	MYH9	c.2838(+1)G>T	Cytokinesis, cell motility (Li et al., 2008)
NIMH070188	DCDC5	P27R	Doublecortin superfamily, neuronal migration (Liu, 2011)
NIMH070188	SLIT3	C1011Y	SLIT-ROBO signaling pathway (Zhang et al., 2012)
SZ502	LDLRAD4	G22S	SNPs associated with schizophrenia (Kikuchi et al., 2003)
SZ502	CRYBG3	del TACTA	βγ-crystallin (Rajanikanth et al., 2012)

PROCEDURES

PARTICIPANTS

Participants were 399 persons: 105 probands, 84 of their unaffected siblings, and their 210 unaffected parents (Table S1). Families were drawn from the Consortium on the Genetics of Schizophrenia (COGS) (Calkins et al., 2007) and Project Among African-Americans to Explore Risks of Schizophrenia (PAARTNERS) (Aliyu et al. 2006), with DNA samples provided by the Center for Collaborative Genomic Studies on Mental Disorders at Rutgers University (www.nimhgenetics.org); and from families from the University of Washington, who previously had been evaluated for *de novo* CNVs with no such events found (Walsh et al., 2008). All probands met DSM-IV criteria for schizophrenia. All parents and siblings were free of major psychiatric illness; including psychotic illness, bipolar disorder and major depressive disorder.

Participants recruited through COGS and PAARTNERS received a comprehensive evaluation including the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) and the Family Interview for Genetic Studies (FIGS) (Maxwell, 1996). Participants recruited at the University of Washington received a comprehensive evaluation by the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1996) and the FIGS, in addition to clinical diagnoses. For all cases, diagnostic assignment incorporated information from medical records, health professionals, and available school records. Subject recruitment, sampling, and genomic analyses were conducted with approvals of local institutional review boards.

GENOMICS

From genomic DNA of the 399 participants, library construction, exome capture, and sequencing were carried out as previously described (Walsh et al., 2010), with some modifications. Library construction and exome capture were performed on a Bravo liquid handling robot (Agilent) in 96-well format. Samples were prepared by subjecting 1 ug of genomic DNA to acoustic fragmentation (Covaris), end-polishing, A-tailing, ligation of sequencing barcoded adaptors, and PCR amplification (Illumina). Sample libraries were captured by solution-based exome enrichment using SeqCap EZ Exome v2.0 (Nimblegen), then 1µg of library was hybridized to biotinylated capture probes for 72 hours at 47C and recovered via binding to streptavidin beads. Unbound DNA was washed away, and the captured DNA PCR amplified for 17 cycles. DNA samples of related subjects were prepared in the same 96-well plate to avoid potential batch effects. Library concentration and flow-cell loading cluster densities were determined using concentration values from the high sensitivity assay on a TapeStation (Agilent). Equimolar concentrations of four barcoded exome libraries were cluster-amplified per flow-cell lane. Sequencing was performed on an Illumina HiSeq2000 using paired-end 101bp runs with a 7bp index read with v3 Sequence-by-Synthesis chemistry. All samples were sequenced to median depth of at least 100X and considered complete when exome targeted read coverage was >10x over >90% of the exome target.

Samples were processed from real-time base-calls (RTA1.8 software [Bustard]), converted to qseq.txt files, and aligned to a human reference (hg19) using BWAv0.6.1-r104 (Li and Durbin, 2009). Sites with at least 10x coverage were retained. SAM alignment files were converted into BAM format and PCR duplicates were removed from read pairs with identical coordinates using SAMtools v0.1.18 (Li et al., 2009). Quality score recalibration and indel realignments were done

with GATK v1.5-21 prior using recommended parameters (www.broadinstitute.org/gatk/) prior to variant calling on each family. Genotypes were called using MAQ0.7.1, SAMtools v0.1.18, and GATK v1.5-21 independently with default parameters. Exome-based calls of copy number variants were made by CoNIFER (Krumm et al., 2012).

In order to exclude artifacts and common SNPs, variants were compared to dbSNP v135, to publicly available variants from 6,500 exomes sequenced by the NHLBI exome sequencing project (evs.gs.washington.edu/EVS/), and to 800 exomes previously sequenced in our lab. Insertions and deletions of 1 to 23 bp in length were identified using the pipeline developed by Ming Lee. Candidate *de novo* variants were retained if at least 30% of total reads represented the variant, after adjusting by individual inspection for genomic complexities (e.g. multiple pseudogenes of *CHEK2*).

For each candidate *de novo* variant, primers were designed using Primer3 software (Rozen and Skaletsky, 2000), genomic DNA samples of the proband or sib and both parents were amplified, then PCR products were purified and Sanger sequenced in both directions. For some variants, sequence could be read in only one direction due to nearby repeats or indels. Candidate *de novo* CNVs were validated by custom TaqMan copy number assays. For candidate *de novo* CNVs not flanked by segmental duplications, exact genomic breakpoints were determined with diagnostic PCR primers and Sanger sequencing. *De novo* variants were defined as variants present in a proband or a sib and absent from both parents.

NETWORK ANALYSIS

Network analysis was in two parts: (1) assessment of protein-protein interactions based on the GeneMANIA PPI dataset (Mostafavi et al., 2008) and (2) assessment of co-expression of genes evaluated by RNASeq in the BrainSpan Atlas of Developing Human Brain (2013). For the co-expression analyses, tissues were included from four anatomic regions of the brain: frontal cortex (FC), temporal and parietal regions (TP), sensory-motor regions (SM), and sub-cortical regions (SC); and from three developmental stages: fetal (13 – 26 post-conception weeks, corresponding to the second trimester of pregnancy), early infancy to late childhood (4 months – 11 years), and adolescence to adulthood (13 – 23 years) (Supplementary Table 6). Co-expression networks were constructed using absolute values of Pearson correlation coefficients of genes evaluated pair-wise. Gene pairs with $|R| \geq 0.8$ were defined as "connected" in the network constructions for each brain region and each developmental stage.

In order to build an appropriate comparison group of networks from control genes, we pooled genes with damaging *de novo* mutations in unaffected siblings from our cohort with genes harboring damaging *de novo* mutations in unaffected siblings of autism and schizophrenia probands from recently published studies (Xu et al., 2012; Iossifov et al., 2012; O'Roak et al., 2012; Sanders et al., 2012).

We applied the same criteria to define damaging versus benign mutations that we used for the variants in our schizophrenia probands. In the published healthy sibling cohorts, after exclusion of variants observed in both proband and unaffected sibling (n=9), variants without a precise nucleotide change definition (n=2) and variants that failed validation (n=2); 547 de novo events were pooled and 276 events in 267 different genes were predicted as damaging. Of these 267

genes, 264 were represented in the GeneMania and Developing Human Brain datasets, and were used as the control gene pool (Table S4). Using this pool, 10,000 random control gene sets were created each containing 54 different genes; corresponding to the number of genes harboring predicted damaging events in our probands (excluding genes potentially impacted by the two large *de novo* CNVs on chromosomes 2q21 and 17q12, each detected in one patient). PPI and co-expression networks were constructed for each gene set.

By counting the numbers of nodes and edges of each network, we estimated the statistical significance of differences in interconnectedness of case gene networks versus control gene networks. The means and standard deviations of numbers of nodes and edges in the simulated networks were fit to normal distributions. The significance of networks for genes harboring damaging mutations in probands was estimated using Z-scores. For graphical representation in Figure 5, the protein-protein physical interaction network and the most significant co-expression network (DFC) were merged using Cytoscape 2.8.394.

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