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De novo mutations from sporadic schizophrenia cases highlight important signaling genes in an independent sample

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Abstract

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The authors declare no conflict of interest.

Schizophrenia is a debilitating syndrome with high heritability. Genomic studies reveal more than a hundred genetic variants, largely nonspecific and of small effect size, and not accounting for its high heritability. *De novo* mutations are one mechanism whereby disease related alleles may be introduced into the population, although these have not been leveraged to explore the disease in general samples. This paper describes a framework to find high impact genes for schizophrenia. This study consists of two different datasets. First, whole exome sequencing was conducted to identify disruptive *de novo* mutations in 14 complete parent–offspring trios with sporadic schizophrenia from Jerusalem, which identified 5 sporadic cases with *de novo* gene mutations in 5 different genes (*PTPRG, TGM5, SLC39A13, BTK, CDKN3*). Next, targeted exome capture of these genes was conducted in 48 well-characterized, unrelated, ethnically diverse schizophrenia cases, recruited and characterized by the same research team in New York (NY sample), which demonstrated extremely rare and potentially damaging variants in three of the five genes (MAF < 0.01) in 12/48 cases (25%); including *PTPRG* (5 cases), SCL39A13 (4 cases) and *TGM5* (4 cases), a higher number than usually identified by whole exome sequencing. Cases differed in cognition and illness features based on which mutation-enriched gene they carried. Functional *de novo* mutations in protein-interaction domains in sporadic schizophrenia can illuminate risk genes that increase the propensity to develop schizophrenia across ethnicities.

Keywords

schizophrenia; *de novo*; PTPRG: rare variant; paternal age; exome sequencing

1. Introduction

The heritability of schizophrenia is established from a century of family, twin and adoption studies, but genes of major effect for the devastating psychotic disorder remain elusive. Genome Wide Association Studies (GWAS) show a multitude of risk alleles of small effect sizes that are related to diverse psychopathologies (McCarroll et al., 2014). It is postulated that major genes for schizophrenia arise *de novo* to replenish the population risk and counteract the reduced reproductive fitness of the disease (Malaspina, 2001). If so, *de novo* mutations from sporadic cases may point to genes whose variation is more specifically associated with schizophrenia. There is a large body of evidence that *de novo* mutations are enriched in diseases cases versus controls, as e.g. in autism (De Rubeis et al., 2014; Dong et al., 2014) and schizophrenia (Fromer et al., 2014; Purcell et al., 2014b; Takata et al., 2014; Xu et al., 2011). Methodological rigor in examining family history and localization of these mutations within protein interaction networks as well as functional domains may enhance the probability that particular *de novo* mutations are relevant to the disease.

Penrose (1995) first proposed that advancing paternal age was the major source of *de novo* mutations, which was further explicated by Crow (2000). We hypothesized that gene variants associated with the risk for schizophrenia could be introduced into the population in association with paternal age *de novo* and then persist in the gene pool to contribute to familial illness, perhaps individually influencing the specific disease phenotype. We tested this hypothesis in a two-stage approach.

The first epidemiological demonstration that steadily increasing schizophrenia risk accompanies advancing paternal age was in the Jerusalem Perinatal Schizophrenia Study (JPSS) (Malaspina et al., 2001), wherein each decade increased schizophrenia risk by 1.4-fold, with the relative risk for offspring of fathers >45 years being 3-fold greater than for 20–24 year-old fathers. After controlling for maternal age and other factors, advancing paternal age, beginning at 25 years, explained 26% of schizophrenia risk, comparable to estimates in other cohorts (Brown et al., 2002; Sipos et al., 2004; Tsuchiya et al., 2005; Zammit et al., 2003).

In our clinical research, we cast a net for genes associated with schizophrenia, sequencing 14 sporadic offspring-parent trios from the JPSS sample. Our findings identified a handful of *de novo* mutations in genes that we subsequently sequenced in 48 non-related individuals from our New York sample. These candidate genes all represent important central nervous system relevant signaling proteins, which may belong to pathways associated with schizophrenia. The samples are smaller than in many studies because our hypotheses are specific: genes showing functional *de novo* mutations in sporadic cases can shine a window on high impact genes for the illness *per se*.

2. Materials and Methods

2.1. Recruitment/Ascertainment of schizophrenia trios and individuals

Subjects for the discovery sample trios were recruited as an independent clinical research component of the JPSS. This birth cohort had been initiated to study hypertension in pregnancy (Davies et al., 1969), but later expanded to capture other obstetric information and health outcomes, including our linkage of the data to the Psychiatric Case Registry within the Ministry of Health (Harlap et al., 2007).

Cases with schizophrenia-related-psychosis (SRP), schizophrenia or schizoaffective disorder, with no family history of psychosis and living parents, were recruited from psychiatric treatment settings in Israel to sequence the whole exomes in cases and unaffected parents. IRB and Helsinki committees from NYU, the Washington University School of Medicine and Hebrew University approved the genetic study arm of the JPSS. All subjects provided informed consent and had psychiatric assessments with a Hebrew version of the NIMH Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994). All interviewers had graduate or medical degrees, were fluent in English and Hebrew and participated in ongoing face-to-face internet-based reliability conferences between the Israeli and the New York teams.

Next, a series of unrelated cases with SRP were recruited from treatment settings at Bellevue Hospital Center in New York City, with the approval of the NYU and Bellevue Hospital Center IRB committees. Subjects provided informed consent and underwent diagnostic procedures using the same DIGS interviews, which includes sections on psychiatric and medical comorbidity, traumatic brain injury and substance abuse. Cases were given symptom assessments with the Positive and Negative Symptom Scale (PANSS), scored based on specific symptom factors (White et al., 1997). Cognition was tested with the WAIS

III (Wechsler, 1999) and olfaction was tested using the University of Pennsylvania Smell Identification Test (Doty et al., 1984).

Whole exome sequencing of JPSS trios—Exons were captured by NimbleGen Se Cap EZ SR v2 and Illumina sequencing technology and run Multiplex, 4 libraries per lane on v3 HiSeq flowcell. Median depth of coverage was >100×, with 93% of base pairs covered at ≥ 10× per sample. Sequences were aligned and SNVs and indels were identified with in-house scripts, filtered for variants present in the proband but neither parent, which were potentially disruptive and were frameshift or nonsense mutations, splice mutations ± 3 bp, or missense mutations with a Polyphen-2 score >0.5. The events were validated based on filtering set of >800 in-house unrelated exomes for point mutations that were novel.

Targeted exome capture in replication sample—All annotated exons of the *de novo* JPSS genes were sequenced using the following methodology. DNA (500 ng) from each sample was sheared to an average of 150 bp in a Covaris instrument for 360 seconds (Duty cycle-10%; intensity-5; cycles/Burst-200). Barcoded libraries were prepared using the Kapa Low-Throughput Library Preparation Kit Standard (Kapa Biosystems). Libraries were amplified using the KAPA HiFi Library Amplification kit (Kapa Biosystems) (8 cycles) and quantified using Qubit Fluorimetric Quantitation (Invitrogen) and Agilent Bioanalyzer. An equimolar pool of the four barcoded libraries (300 ng each) was used as input to exon capture using one reaction tube of the custom Nimblegen SeqCap EZ (Roche) with custom probes targeting the coding exons of the genes of interest. Capture by hybridization was performed according to the manufacturer's protocols with the following modifications: 1 nmol of a pool of blocker oligonucleotides and (B) post-capture PCR amplification was done using the KAPA HiFi Library Amplification kit instead of the Phusion High-Fidelity PCR Master Mix with HF Buffer Kit, in a 60 ul volume, since we found a greatly reduced or eliminated the bias against GC-rich regions. The pooled capture library was quantified by Qubit (Invitrogen) and Bioanalyzer (Agilent) and sequenced on a Illumina MiSeq or HiSeq 2500 sequencerusing the 2×150 paired-end cycle protocol. The average coverage across all samples was 190X (133X–360X). Over 97% of the target region had coverage of over 50X in all samples. Reads were aligned to hg19 build of the human genome using BWA with duplicate removal using samtools as implemented by the Illumina MiSeq Reporter. Variant detection and annotation was performed with GATK UnifiedGenotyper Charity annotator and cross-referenced against known dbSNP, 1000 Genomes, COSMIC mutations and Schizophrenia Genebook entries. Only rare variants (MAF < 0.01 in 1000Genomes) and novel mutations, which were not described in any reported database were considered to be positive findings in this study and were analyzed by Polyphen-2.

3. Results

Five of the sporadic cases of 14 in the JPSS trios had *de novo* mutations in five different genes that had not been reported in any accessible schizophrenia database (Table 1). The mean paternal age for the five trios with potentially disruptive *de novo* mutations was five years older than in the other 9 trios (36.60 vs. 31.78, t=− 1.27, p=.22), although this difference was not significant in this small study.

One *de novo* nonsense mutation produced a premature stop codon in *PTPRG* in Exon 10 instead of encoding arginine (R) at amino acid position 422, which generates a truncated protein lacking the catalytic phosphatase domains. The other four detected mutations all caused amino acid changes in functional or protein-interaction domains (Table 1).

In the NY sample, a quarter of the 48 cases (25%) showed missense variants in any of three of the five genes that had harbored *de novo* mutations in the JPSS sample, although not the identical sequences: *PTPRG* variants in five cases; *SLC39A13* variants in four cases and *TGM5* variants in four cases (Table 2). One case had rare variants in both PTRPRG and *SLC39A13*. For *PTPRG* and *SLC39A13* the variants were rare and one of them was consistent with a previously unidentified mutation in *PTPRG* (I837S).

Polyphen-2 predictions of all of the rare variants in the replication cohort demonstrate that 2/3 of them are possibly or even probably damaging and in each replicated gene, at least two variants are possibly or probably damaging (Table 2). Of interest, all rare *PTPRG* variant except one were predicted to be possibly or probably damaging (rs142366357, Polyphen: .876; rs149885804, Polyphen: 1; rs150212631, Polyphen: .498). The new mutation (I837S) was predicted to be probably damaging (Polyphen: .997).

The phenotypic features of variant carriers in the three replicated genes are summarized in Table 3. Childhood disorder was diagnosed in 75% of the cases irrespective of their genetic background. Anxiety disorder was only observed in 25% of the cases, but all 3 case groups shared high PANSS score values for the dysthymia factor symptoms. A difference between the groups, even with such small sample sizes, concerns their intelligence scores (WAIS III). The PTPRG group had the highest performance IQ, while the SLC39A13 group displayed the lowest. The TGM5 carriers however had a medium performance IQ. Only the TGM5 variant carriers had a normal smell identification score among all three groups.

4. Discussion

In the NY sample, 25% of the cases had rare missense coding variants in one or more of three genes, for which we previously discovered *de novo* mutations for sporadic schizophrenia from the JPSS sample. Rare variants (MAF ≤ 0.01) and one novel missense mutation were replicated for *PTPRG*, *SLC39A13* and *TGM5*, but not *BTK* or *CDKN3/KAP*, suggesting that rare variants in the former genes might be related to psychosis across ethnically diverse populations and be more common in the disease.

These findings are consistent with the origin of *de novo* mutations arising with increases in paternal age (Brown et al., 2002; Crow, 2000; Malaspina, 2001; Malaspina et al., 2001; Penrose, 1955; Sipos et al., 2004; Tsuchiya et al., 2005; Zammit et al., 2003). For each decade of paternal age, Sipos found no increase in familial schizophrenia risk, whereas there was a 60% increase/decade in sporadic cases, with > 5 fold increase for offspring of oldest than youngest fathers. Some rare gene variants from the paternal germ line may produce an advantage to replication rates and expansion of spermatogonial stem cells (Goriely et al., 2013). Such oncogenic associations of paternal age related mutations are consistent with the rare exonic *PTPRG* variations in 6 of the replication cases.

PTPRG is a protein tyrosine phosphatase regulating cellular processes of growth, differentiation, mitosis, and oncogenic transformation as well as response to neuroinflammation (Lorenzetto et al., 2014). It is highly expressed in the male germ line and its highest CNS expression is in hippocampal and sensory neurons with further upregulation by inflammation (Lorenzetto et al., 2014). Here we also report a newly identified missense coding mutation in *PTPRG* (I837S, Polyphen: 0.994, probably damaging) in the tyrosine phosphatase domain, in addition to the JPSS *de novo* nonsense mutation (R422Stop), both which have not been described before. Already four missense mutations were identified in the tyrosine phosphatase domain in *PTPRG* through whole exome sequencing in schizophrenia: Y1070YC, L1102LF and R1312W (n = 5,091) and T1105S (n = 1,869) (Fromer et al., 2014; Purcell et al., 2014b). All four missense mutations, like the one we discovered in a sporadic case (I837S) are located in the tyrosine phosphatase domain or lead to a truncated protein lacking the actual tyrosine phosphatase domain (R422Stop). *PTPRG* may be a major candidate gene for schizophrenia acting through balanced protein kinase to phosphatase activity. PTPRG global knockout mice reveal significant connectivity aberrations between the hippocampus and the prefrontal cortex and they also reveal significantly lower baseline activity levels in contextual fear conditioning tests (Lamprianou et al., 2006).

One sporadic JPSS case demonstrated an unknown *de novo* missense mutation in the zinc transporter, *SLC39A13*, (D228N, Polyphen 1.0: probably damaging). The Swedish exome-sequencing study identified three other missense mutations in schizophrenia cases located near our mutation (P260S, R271W and A297T) (Purcell et al., 2014a). Four of the 48 replication cases had rare missense variants (MAF < 0.01) in *SLC39A13*. The zinc transporter is involved in BMP/TGFβ signaling pathways (Fukada et al., 2008). Pyramidal neurons from *post-mortem* examinations of schizophrenia cases impressively differ in mRNA expression of genes belonging to TGFβ and BMPs signaling pathway (Pietersen et al., 2014). These neurons are implicated in cognitive abnormalities and symptoms in the disease, so it is of interest that the cases with *SLC39A13* variants had the lowest intelligence, and most severe symptoms of these subgroups.

Transglutaminase 5 (*TGM5*) showed an unreported *de novo* missense mutation in a Jerusalem sporadic trio case (L139I Polyphen: 1.0: probably damaging). *TGM5* is ubiquitously expressed and responsible for cross-linking and conjugation of polyamines to proteins. Recessive mutations produce acral peeling skin syndrome, a skin fragility disorder (Bienvenu et al., 2000) wherein patients harm their own skin in a repetitive manner (Gyuris et al., 1993). Apart from the unreported *de novo* mutation in one of the JPSS trios, we discovered four more cases with three rare missense variants in *TGM5* in the NY sample, which were characterized by low normal intelligence and mild symptoms of psychosis. Rare missense variants in the two genes identified in the JPSS trios but not the NY sample may be associated with schizophrenia in larger studies or in cases of a different ethnicity.

The cyclin-dependent kinase inhibitor 3 (*CDKN3*) belongs to the family of dual specificity protein phosphatases, which interact with CDK2 and dephosphorylate it, thus preventing its activation. Mutations in this gene have been reported in several cancers (Gyuris et al., 1993; Lee et al., 2000). The previously unreported *de novo* missense mutation from the JPSS

(C79F, Polyphen: 1.0 probably damaging) might potentially alter dephosphorylation activity.

The other *de novo* mutation from a single case from the JPSS trios is not reported in any publicly accessible genomic database before and not observed in our New York sample is Bruton's tyrosine kinase (*BTK*, R255Q, Polyphen: 1.0 probably damaging). Located on the X chromosome, *BTK* is critical for the development of B cells and includes a tyrosine kinase as well as a pleckstrin homology (PH) domain. Mutations are associated with agammaglobulinemia, an X-linked primary immunodeficiency disorder (Hyvonen and Saraste, 1997; Vetrie et al., 2012). This offers a potential causative link with the reported association of toxoplasma gondii and the disease (Alipour et al., 2011).

The proportion of affected cases with a family history (Yang 2009), substance abuse, psychiatric and medical comorbidity, and cognitive deficits are not unexpected in the disease (Jeste 1996), nor are findings of prematurity (Byrne et al 2007), learning problems (Doody et al 1998) or seizures (Cascella et al 2009). Schizophrenia is acknowledged to be a syndrome. However, the suggestion that diverse aspects of the phenotype could segregate with particular haplotypes is intriguing and consistent with epistasis. While no functional mutations were identified from 9 of the 14 sporadic discovery cases, epigenetic changes also accompany paternal aging and these mechanisms or their impairments may influence gene expression and behavioral functioning (Milekic et al., 2014).

In summary, the replication of genes revealing novel or rare missense variants initially identified as *de novo* missense mutations in sporadic schizophrenia is an alternate way forward to resolve its complexity. This approach may be applicable to other genetic conditions that commonly present as sporadic cases. It is important to identify individually rare events, since genes harboring one disease-predisposing allele appear likely to harbor many more rare missense variants associated with the disease. Thus, finding a rare mutation in one case holds the promise that there may be other mutations or rare variants in that gene in others that are relevant to the etiopathophysiology of schizophrenia. This type of approach represents a starting point to identify signal transduction components and pathways that are associated with schizophrenia. Aberrations in neuronal and immunological pathways were among the top hits in a recent psychiatric GWAS (Network Pathway Analysis Subgroup of the Psychiatric Genomics, 2015).

Another supporting fact is that the discovered genes in this study reveal the highest expression levels during early embryonic development. Since our investigated cohort is rather small scaled, larger samples, ideally ascertained by the same group of clinical experts, are needed to replicate our findings and to define subgroups based on personal genomics and phenotypes for optimized personalized treatments. In addition, the subsequent biochemical characterization of the identified missense coding mutations and rare polymorphisms in the protein interaction domains in signaling genes is crucial in order to receive the experimental evidence for their clinical impact and also their confirmation as potential pharmacological target sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

De novo mutations in JPSS trios

Gene	Genomic position	AA exchange	Exon	Gene function	Polyphen-2		Phenotype
					score	prediction	
PTPRG	Chr3: 62.180.781 C>T	R422Stop	10	protein tyrosine phosphatase regulating cellular processes of growth, differentiation, mitosis and response to neuroinflammation, missense mutation creates loss-of-function protein lacking the tyrosine phosphatase domain	x	x	male schizoaffective case with polydrug dependence
SLC39A13	Chr11: 47.435.184 G>A	D228N	6	Golgi Apparatus membrane, between TM4 and 5, lumenal, mediates Zinc influx, connective tissue development, Ehlers-Danlos syndrome	1	probably damaging	male with paranoid schizophrenia and cannabis abuse
CDKN3	Chr14: 54.878.244 G>A	C79F	5	dual specifity phosphatase, interacts with cyclin-dependent kinases, implicated in cell cycle regulation, associated with somatic hepatoblastoma	1	probably damaging	male with disorganized schizophrenia
TGM5	Chr15: 44.552.271 G>T	L139I	3	transglutaminase, peeling skin syndrom, conjugation of poiyamines to proteins, close to PLCgamma SH2 domain	1	probably damaging	male with schizoaffective disorder
BTK	ChrX: 100.615.568 C>T	R255Q	8	Non-RTK, B cell development, Morbus Bruton, Agammaglobunemia, SH3 domain	0.549	possibly damaging	female with schizoaffective disorder

Table 2

Rare Variants and new mutation (PTPRG: I837S) in JPSS genes replicated in NY sample

Gene	Genomic location	AA exchange	TranscriptID	Exon	1000Genomes	Ethnicity	Subjects	Allele frequency	Polyphen-2	
								1000Genomes	Score	Prediction
PTPRG	chr3:62,153,771	R323C	NM_002841	8	rs142366357	AfricanAm (AFR)	1	0.01	0.8 76	possibly damaging
	chr3:62,189,036	G523S		12	rs149885804	AfricanAm (AFR)	1	0.003	1	probably damaging
	chr3: 62,240,841	I837S		16	not reported	Hispanic (AMR)	1	not reported	0.9 97	probably damaging
	chr3:62,240,843	G838S		16	rs72878145	AfricanAm (AFR)	1	0.019	0	benign
	chr3:62,257,194	R1049Q		21	rs150212631	AfricanAm (AFR)	1	0.0002	0.4 98	possibly damaging
SLC39A 13	chr11:47,431,764	R40Q	NM_001128 225	2	rs35741412	AfricanAm (AFR)	1	0.002	0.0 2	benign
	chr11:47,433,573	T133M		3	rs140574574	Hispanic (AMR)	1	not reported	1	probably damaging
	chr11:47,434,952	A180G		5	rs147227015	AfricanAm (AFR)	1	0.009	0.7 03	possibly damaging
	chr11:47,436,707	P346L		9	rs35978122	AfricanAm (AFR)	1	0.006	0.3 96	benign
TGM5	chr15:43,527,020	E607A V657A	NM_201631	11 12	rs80192997 rs80058195	Ashkenazi Jew / Caucasian (EUR)	1 / 1	not reported (both)	0.9 84	probably damaging
	chr15:43,525,791							not reported (both)	0.1 37	benign
	chr15:43,527,022	K608E		11	rs76456763	Caucasian (EUR) / Hispanic (AMR)	1 / 1	not reported / 0.001	0.7 45	possibly damaging

Table 3

Rare Variants of and Phenotypic Features of the Replication Cases

	PTPRG*		
5 male	**SLC39A13***		
3 male, 1 female	**TGM5**		
2 male, 2 female			
Ethnicity: European: African: Hispanic	E=0, A=4, H=1	E=0, A=3, H=1	E=3, A=0, H=1
Family History of Psychosis: (FIGS)	1 /5	2 /4	0 /4
Paternal Age: Mean (Standard deviation)	35.0 (12.7) years n= 2	22.3 (5.6) years n=3	33.7 (5.7) years n=3
Premorbid Risk Factors: (DIGS)			
Premature Birth: proportion	2 /5 each 1 month	0 /4	0 /4
Premorbid Traumatic Brain Injury: proportion	1 /5	1 /4	1 /4
Premorbid Cannabis Abuse: proportion	2 /5	1 /4	2 /4
Psychiatric Comorbidity: (DIGS)			
Childhood learning disorder: proportion	4 /5	3 /4	3 /4
Depression Symptoms: proportion	2 /5	3 /4	2 /4
Mania Symptoms: proportion	1 /5	2 /4	1 /4
Suicide Attempts: proportion	1 /4	3 /4	0 /4
Anxiety Symptoms: proportion	0/4	2 /4	1 /4
Medical Comorbidity: (DIGS)			
Seizures: proportion	1 /5	0 /4	0 /4
Joint complaints: proportion	3 /5	4 /4	1 /4
GI disorder: proportion	0 /5	2 /4	0 /4
Hypothyroid: proportion*	1 /5	1 /4	0 /4
Diabetes: proportion	1 /5	1 /4	1 /4
Intelligence: (WAIS III)			
Full Scale IQ: Mean (Standard deviation)	97.0 (9.9) n=2	73.7 (3.1) n=3	89.3 (14.0) n=3
Verbal IQ: Mean (Standard deviation)	94.0 (5.7) n=2	76.3 (8.4) n=3	92.7 (14.4) n=3
Performance IQ: Mean (Standard deviation)	101.5 (13.4) n=2	75.3 (3.5) n=3	87.0 (15.6) n=3
Smell Identification Score:			
UPSIT: Mean (Standard deviation)	34.5 (2.12) n=2	33.3 (1.15) n=3	36.5 (1.0) **+ n=2**
PANSS Scores: Mean (Standard deviation)	N=2:	N=3:	N=3
Positive Factor Symptoms	12.0 (4.2)	13.0 (2.0)	11.7 (3.5)
Negative Factor Symptoms	17.5 (2.1)	21.0 (4.4)	12.7 (2.5)
Activation Factor Symptoms	10.0 (2.8)	11.0 (3.0)	7.3 (1.2)
Autistic Preoccupation	11.5 (0.7)	14.0 (1.7)	8.7 (3.1)
Factor Symptoms			
PANSS Dysthymia Factor Symptoms	13.0 (0.0)	17.7 (4.2)	12.0 (3.0)

[*] A single male of Hispanic ethnicity had rare variants in both PTPRG and SLC39A13. He had no family history of psychosis, premorbid TBI or cannabis exposure. He was the only PTPRG case with mania and the only case with hypothyroidism, notably with no history of lithium treatment. He did not complete IQ testing or symptom assessments.

[+] excludes one cases with anosmia (UPSIT = 22).