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Research Paper

Phenotypically distinct subtypes of psychosis accompany novel or rare variants in four different signaling genes

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ABSTRACT

Background: Rare gene variants are important sources of schizophrenia vulnerability that likely interact with polygenic susceptibility loci. This study examined if novel or rare missense coding variants in any of four different signaling genes in sporadic schizophrenia cases were associated with clinical phenotypes in an exceptionally well-characterized sample.

Method: Structured interviews, cognition, symptoms and life course features were assessed in 48 ethnically-diverse cases with psychosis who underwent targeted exome sequencing of *PTPRG* (Protein Tyrosine Phosphatase, Receptor Type G), *SLC39A13* (Solute Carrier Family 39 (Zinc Transporter) Member 13), *TGM5* (transglutaminase 5) and *ARMS/KIDINS220* (Ankyrin repeat-rich membrane spanning protein or Kinase D-Interacting Substrate of 220 kDa). Cases harboring rare missense coding polymorphisms or novel mutations in one or more of these genes were compared to other cases not carrying any rare missense coding polymorphisms or novel mutations in these genes and healthy controls.

Findings: Fifteen of 48 cases (31.25%) carried rare or novel missense coding variants in one or more of these genes. The subgroups significantly differed in important features, including specific working memory deficits for *PTPRG* ($n = 5$); severe negative symptoms, global cognitive deficits and poor educational attainment, suggesting a developmental disorder, for *SLC39A13* ($n = 4$); slow processing speed, childhood attention deficit disorder and milder symptoms for *TGM5* ($n = 4$); and global cognitive deficits with good educational attainment suggesting neurodegeneration for *ARMS/KIDINS220* ($n = 5$). Case vignettes are included in the appendix.

Interpretation: Genes prone to missense coding polymorphisms and/or mutations in sporadic cases may highlight influential genes for psychosis and illuminate heterogeneous pathways to schizophrenia. Ethnicity appears less important at the level of genetic variability. The sequence variations that potentially alter the function of specific genes or their signaling partners may contribute to particular subtypes of psychosis. This approach may be applicable to other complex disorders.

1. Introduction

It is likely that the genetic heterogeneity of the schizophrenia-related psychoses will be pertinent to the development of optimal person-specific treatments. We tested if a set of genes that had harbored deleterious *de novo* mutations for schizophrenia in sporadic cases showed other rare missense coding variants in an independent sample that included familial and sporadic cases (Kranz et al., 2015a, 2015b). Next we examined if cases harboring novel or rare variants in these genes, independent of family history, differed in their clinical characteristics. This report describes the phenotypes of subsets of cases with missense coding polymorphisms or novel mutations ("carriers") in any of four genes that act in different signaling pathways, which have been previously identified and replicated in independent cohorts: These are *PTPRG* (Protein Tyrosine Phosphatase, Receptor Type G); *SLC39A13* (Solute Carrier Family 39 (Zinc Transporter) Member 13); *TGM5* (Transglutaminase 5); and *ARMS/KIDINS220* (Ankyrin Repeat-Rich Membrane-Spanning Protein or Kinase D-Interacting Substrate of 220 kDa). These genes are potentially relevant for psychosis. In addition to the presence of rare missense

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coding polymorphisms and/or novel mutations in a sporadic case in comparison to healthy parents, each gene is highly expressed in the central nervous system, involved in signaling pathways for neuronal network integration, stabilization, and connectivity. Almost a third of the cases in this sample carried rare missense coding polymorphisms or novel mutations in one or more of them (Kranz et al., 2015a, 2015b).

We hypothesized that these genetic variants, especially in known protein interaction domains within each gene, might differentially influence multilevel psychosis phenotypes. This report describes the phenotypes of the respective gene carrier subgroups and provides clinical vignettes on each case serving as a molecular-era case series with implications for treatment. The information is based on rigorous clinical research diagnostic and assessment procedures.

2. Materials and methods

2.1. Sample ascertainment and diagnosis

The study, a component of an NIMH Challenge Grant to examine multilevel phenotypes and genomics in a sample of cases with chronic psychosis, was approved by the Bellevue Hospital Center and NYU Medical Center Institutional Review Boards and all subjects provided written informed consent. Cases with chronic psychosis were recruited from clinical treatment settings if they were taking stable medication doses for at least one month. Healthy controls were recruited from Internet postings and university announcements. Research assessments were conducted by trained and reliable master's and doctoral level clinicians using the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994); Positive and Negative Syndrome Scale (PANSS), (Kay et al., 1987) which were scored using positive, negative and general psychopathology scales, the pentagonal five factor model (White et al., 1997) and as the sum of the positive minus the negative scale scores to indicate if the prominent symptom profile was positive (Type 1) or negative (Type 2) (Crow, 1997); Social Adjustment Scale (SAS) (Weissman and Bothwell, 1976); Chapman Scales for Physical and Social Anhedonia (Chapman et al., 1976); and Young Mania Rating Scale (Young et al., 1978). Wechsler Adult Intelligence Scale, Third Edition (WAIS-III) (Wechsler, 1997) results yielded Full Scale (FS), Verbal (VIQ) and Performance IQ (PIQ) scores and indices of Verbal Comprehension (VCI), Perceptual Organization (POI), Working Memory (WMI), and Processing Speed (PSI).

2.2. Targeted exome capture

Cases underwent targeted exome capture as described in detail in previous studies (Kranz et al., 2015a, 2015b). Briefly, 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 s (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 PCRMaster Mixwith HF Buffer Kit, in a 60 μl 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 sequencer using the 2 × 150 paired-end cycle protocol. The average coverage across all samples was 190× (133 × — 360×). Over 97% of the target region had coverage of over 50× in all samples. Reads were aligned to hg19 build of the human genome using BWA with duplicate removal using samtools as implemented by the IlluminaMiSeq Reporter. Variant detection and annotation were performed with GATK Unified Genotyper Charity annotator and cross-referenced against known dbSNP, 1000 Genomes, COSMIC mutations and Schizophrenia Genebook entries. Only previously reported rare missense coding variants (MAF < 0.01 in 1000g2012apr_all) and unreported novel mutations were considered in this study and were analyzed by Polyphen-2.

Brief clinical vignettes (see appendix) were prepared for cases with missense coding polymorphisms (minor allele frequency < .01) or novel mutations ("carriers") (Kranz et al., 2015a, 2015b), categorized as follows: "1" for *PTPRG* carriers; "2" for *SLC39A13*; "3" for *TGM5*; and "4" for *ARMS/KIDINS220* carriers. Cases carrying two of these genes were categorized in the above order for the first gene, an asterisk (*) indicating that they carried others of these genes. The sequenced cases with common variants in all of these genes were categorized as "non-carriers" for comparison, although they certainly have other genetic susceptibility. The carrier groups were statistically compared to groups of non-carrier cases and healthy controls. Each gene carrier group was then independently compared to the non-carrier case group by separate ANCOVA analyses utilizing a Bonferroni corrected significance level of $p < .01$ in light of the multiple testing. The mean age differs due to the different counts in particular analyses.

3. Results

Fifteen of the 48 cases (31.25%) carried missense coding ultra-rare polymorphisms or novel mutations: 5 in *PTPRG*, 4 in *SLC39A13*, 4 in *TGM5*, and 5 in *ARMS/KIDINS220*, as previously reported in Kranz et al., (2015a, 2015b). Three carried more than one rare missense coding polymorphism in different genes considered in this analysis: one case harbored *PTPRG* and *SLC39A13* polymorphisms; another harbored rare polymorphisms in both *SLC39A13* and *ARMS/KIDINS220*; and the third case had rare missense coding polymorphisms in *ARMS/KIDINS220* and *TGM5*. The latter case had chronic psychosis but did not meet strict DSM-IV schizophrenia or schizoaffective criteria based on confounding by continuous substance abuse. One *PTPRG* and one *ARMS/KIDINS220* variant carrier did not complete all assessments. As described in the vignettes (appendix) known risk factors for psychosis were common in the carrier cases, including premorbid brain injury, substance abuse, prematurity, and a family history of psychosis. Thyroid disorders were common to all carrier groups, despite none having received lithium pharmacotherapy. Comparing the four carrier groups to non-carrier cases showed no differences in sex, age, or ethnicity.

As shown in Table 2, *ARMS/KIDINS220* and *SLC39A13* variant carriers had lower mean verbal and full scale IQ scores and had more severe general psychopathology symptoms than the other groups. The *SLC39A13* variant carriers also had more severe negative symptoms. In addition the *PTPRG* and *SLC39A13* variant carriers cases had an early onset age shown in Table 1. Just one of the *SLC39A13* carriers graduated from high school (differing from the non-carriers), whereas all *PTPRG* carriers with onset after age 17 years attained some college education and employment. The *SLC39A13* carriers notably had significantly more suicide attempts, whereas no *PTPRG or TGM5* carrier made any suicide attempt. Childhood learning disorders, based on the clinical interview reports, were significantly more commonly reported for *PTPRG* carriers (100%) and significantly less common for *TGM5* carriers (0%). Conversely, half of the *TGM5* carriers had reported a history of attention deficit disorder, which significantly differed from non-carriers. Depression and substance abuse rates were high in all cases groups. *SLC39A13* variant carriers reported the greatest number of medical comorbidities and both they and the *ARMS/KIDINS220* variant carriers experienced significantly more degenerative joint disease.

Table 1
Descriptive measures by disrupted gene carrier groups.

	PTPRG (n = 5)	SLC39A13 (n = 4)	TGM5 (n = 4)	ARMS/KIDINS220 (n = 5)	Other cases (n = 32)
Demographics					
Sex: male	100%	50%	50%	60%	60.6%
Mean age	44.4 (7.1)	48.5 (6.4)	39.0 (14.5)	46.0 (11.3)	42.9 (8.9)
Mean age of onset	17.4 (7.9) †	18.0 (3.7) †	26.3 (9.5)	24.8 (8.9)	21.3 (6.8)
Ethnicity^	AA = 4, H = 1	AA = 3, H = 1	AA = 1, ME = 1, H = 1, C = 1	AA = 3, C = 2	AA = 17, H = 6, C = 8, A = 1
Education: < hs/hs/college	1/1/3	3/0/1 ††	0/2/2	1/1/2	2/10/21
Psychosis onset <16 years	40%	50%	0%	0%	29%
Psychiatric comorbidities					
Major depression	40%	75%	50%	40%	68%
Suicide attempt	0% †	75% †	0%	20%	48%
Substance abuse	100%	100%	100%	100%	68%
Medical Comorbidities:					
Thyroid disorder	40%	25%	25%	20%	<1%
Degenerative Joint disease	60%	100% †	25%	80% †	28%
Diabetes	20%	50%	25%	40%	19%
Migraines	40%	75%	75%	20%	22%
Birth complications	60%	0%	0%	0%	25%
Learning comorbidities:					
Learning disabilities	100% †	75%	0%	40%	25%
Attention deficit disorder	20%	25%	50 †	0%	<1%

Each carrier group independently compared to non-carrier group by separate ANCOVA analyses for continuous measures or by Fisher's exact tests for categorical measures: † = p < .050, †† = p < .01.
*Sex and ethnicity transposed for confidentiality, actual ethnicity and sex numbers maintained.
**Table includes 3 dual hit cases and 2 cases with incomplete assessments.
***The sum of case numbers by missense genes are >15 since several cases had rare missense coding variants in more than one of these genes.
(%) Rounded to nearest whole number.
^Ethnicity: AA: African American, ME: Middle Easters, C: Caucasian, H: Hispanic, A: Asian.
#Education: <hs/hs/college: Less than high school/High school diploma or GED/At least some college.
+Incomplete data on 1 case; ++ incomplete data on 2 cases.

Comparing cognitive and clinical measures across all case groups and controls (Table 2) showed group effects for all measures, except physical anhedonia, and WAIS Perceptual Organization, Verbal Comprehension, and Working Memory indexes. Examining symptoms among case groups showed significant effects for psychopathology (p < .008), with *SLC39A13* carriers having the most severe symptoms and being significantly more symptomatic than *TGM5* and non-carriers. *SLC39A13* had the highest mania and negative symptoms and it was noteworthy that it was the only group to show a preponderance of negative versus positive symptoms, although not statistically significant in these small samples. Scores for social and physical anhedonia and social adjustment did not differ significantly across these groups.

Multiple separate ANCOVA analyses comparing each mutation carrier group to the non-carriers, adjusting for sex, age, and age of onset showed *SLC39A13* had higher scores for general psychopathology (F[1,27] = 10·45, p = .003) and activation (F[1,27] = 8.79, p = .006) meeting the studies Bonferroni corrected significance level of <.01, with other consistent but only marginally significant results for autism (F[1,27] = 6.17, p = .02), PANSS negative scale scores (F[1,27] = 5.28, p = .03) and negative factor scores (F[1,27] = 5.52, p = .026). Findings for more social anhedonia (F[1,29] = 3.99, p = .055) and worse social function (F[1,29] = 3.94, p = .057) were also consistent with the negative symptoms but did not reach study criteria for significance. The *ARMS/KIDINS220* carriers demonstrated the highest autistic factor scores of any group (F[1,26] = 5.51, P = .027). By contrast, ratings for *TGM5* carriers were similar in all measures to the non-carrier group of cases and these two groups had the lowest positive symptoms scores.

PANSS symptoms configured into factors are shown in Fig. 1. The profiles are of interest although the differences did not achieve significance. The groups showed similar positive symptoms (F = 1.5 (4,35), p = .22), with marginal group effects for negative (F = 2.2 (4,35), p = .09), activation (F = 2.4 (4,35), p = .07), and dysphoric mood factors (F = 2.3 (4,35), p = .08). The severe symptoms of the *SLC39A13* carriers drove these effects (higher negative symptoms than *TGM5*, *ARMS/KIDINS220*, and non-carriers; higher activation scores than *TGM5*; and higher dysphonic mood scores than *TGM5* and non-carrier cases). The trend-level group difference in the autistic factor (F = 3.1 (4,35), p = .027) was driven by higher autistic scores for the *ARMS/KIDINS220* variant carriers.

Cognitive data is presented in Fig. 2a for IQ scores and 2b for the WAIS indices. The WAIS Processing Speed Index (PSI), a measure of one's ability to process simple visual information quickly and to efficiently perform tasks based on that information, significantly differed based on gene groups (p < .008). Other comparisons, while not significant, suggest diverse profiles of cognitive functioning. *TGM5* carriers were the slowest and this index was their weakest compared to all other indexes. *ARMS/KIDINS220* demonstrated the second slowest processing speed but they scored similarly on all of the cognitive measures. *PTPRG* carriers had the fastest PSI, but they (and *SLC39A13* carriers) showed weakness on the Working Memory index, which is a measure of short-term memory skills, concentration, and the ability to mentally manipulate information. As PSI was notably better than the other WAIS indices for *SLC39A13*, impairment in this domain is unlikely to have driven their pathology.

4. Discussion

Despite the relatively small numbers of cases with genetic alterations in these particular signaling genes, this study demonstrated different multilevel phenotypes for groups of cases with schizophrenia-related psychoses based on which of the sequenced genes harbored rare missense coding polymorphisms or novel mutations in *PTPRG*, *SLC39A13*, *TGM5*, and *ARMS/KIDINS220*. The allele frequencies of all rare missense coding polymorphisms was <2% in accordance to the respective ethnicity. Moreover, it is noteworthy that some of these rare

Table 2
Group measures and comparisons of mutation carriers, other cases and control groups.

	PTPRG Carriers	*SLC39A13* Carriers	*TGM5* Carriers	*ARMS/KIDINS220* Carriers	Cases without mutations in any of these four genes	Healthy Controls	ANOVA with all groups		ANOVA without control group	
							F (df)	P	F (df)	P
Age	N = 5	N = 4	N = 4	N = 5	N = 32	N = 20	2.75 (5,58)	.027	1.87 (4,39)	.136
	37.0(9.8)	47.7(7.5)	33.3(11.2)	49.0(3.6)	43.3(9.0)	36.8(8.7)				
Positive Scale	4	3	3	3	27	19	10.87 (5,53)	.001	1.66 (4,35)	.181
	16.3(4.9)	19.0(7.9)	13.7(2.9)	20.0(1.7)	14.2 (4.7)	7.6(1.8)				
Negative Scale	4	3	3	3	27	19	10.14 (5,52)	.001	2.36 (4,35)	.072
	15.5(2.6)	22.7(1.2)	13.3(6.7)	14.3(.58)	14.1(5.0)	8.2(1.5)				
General Psychopath-Ology	4	3	3	3	27	19	16.93 (5,53)	.001	4.07 (4,35)	.008
	33.5(5.4)	40.7(7.6)	25.3(3.1)	36.7(9.7)	28.1(6.4)	17.9(2.9)				
Positive minus Negative Scale	4	3	3	3	27	19	1.07 (5,53)	.385	0.86 (4,35)	.498
	.75(6.7)	−3.7(7.6)	.33(8.6)	5.7(1.2)	.11(6.1)	−.53(1.8)				
Mania Scale	4	3	3	3	28	19	5.63 (5,54)	.001	1.08 (4,36)	.383
	10.0(9.0)	14.7(11.9)	9.0(8.0)	11.0(5.6)	6.9(6.2)	.68(1.9)				
Physical Anhedonia	3	3	3	3	29	17	1.80 (5527)	.129	0.59 (4,36)	.672
	21.0(8.2)	16.3(6.7)	17.3(15.0)	13.3(9.1)	15.7(5.1)	11.2(6.4)				
Social Anhedonia	3	3	3	3	29	17	6.81 (5,52)	.001	1.10 (4,36)	.372
	15.0(7.6)	22.3(10.3)	13.3(7.6)	18.7(6.4)	15.0(6.1)	6.8(3.6)				
Social Adjustment	3	3	3	3	29	18	5.23 (5,53)	.001	1.05 (4,36)	.397
	2.7(.78)	2.6(.28)	1.9(.16)	2.1(.99)	2.3(.56)	1.6(.32)				
VIQ	4	3	3	3	27	18	3.39 (5,52)	.010	0.62 (4,35)	.651
	89.8(17.0)	74.0(4.4)	88.3(22.0)	81.7(5.9)	88.5(17.3)	103.7 (13.1)				
PIQ	4	3	3	3	27	18	3.57 (5,52)	.008	0.71 (4,35)	.593
	92.3(20.7)	75.7(3.1)	81.7(21.7)	78.3(9.3)	84.5(14.4)	100.2 (14.3)				
FSIQ	4	3	3	3	27	18	4.00 (5,52)	.004	0.76 (4,35)	.559
	90.0(19.8)	72.7(1.5)	84.0(22.6)	77.7(1.2)	86.4(16.1)	102.4 (13.3)				
VCI	4	3	3	3	27	18	2.93 (5,52)	.021	0.82 (4,35)	.520
	95.3(18.7)	77.3(7.6)	92.7(24.2)	83.3(6.4)	93.0(17.2)	105.6 (11.9)				
POI	4	3	3	3	27	18	2.25 (5,52)	.063	0.59 (4,35)	.674
	95.0(18.8)	78.0(5.3)	87.3(19.0)	80.3(11.2)	86.1(16.6)	99.1(14.0)				
WMI	4	3	3	3	27	18	3.16 (5,52)	.015	0.90 (4,35)	.474
	81.5(19.6)	70.3(4.6)	85.0(20.9)	88.3(7.8)	86.9(14.9)	100.9 (17.3)				
PSI	4	3	3	3	27	18	5.84 (5,52)	.001	0.52 (4,35)	.719
	88.8(24.0)	83.3(10.0)	75.0(15.9)	80.0(11.5)	83.0(11.0)	103.0 (14.9)				

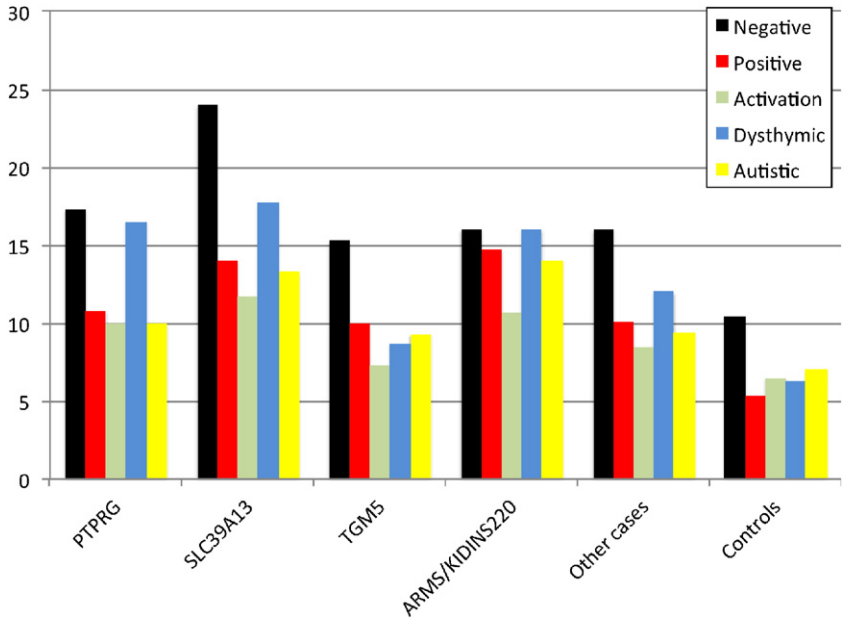

Fig. 1. PANSS pentagonal model factor scores. Significant group differences are demonstrated on all measures, at $p < .001$, with respective F values for negative = 9.6 (5,53), positive = 9.5 (5,53), activation = 6.7 (5,53), dysphoric mood = 9.0 (5,53) and autistic = 8.2 (5,53) factors.

polymorphisms have not been annotated yet in the respective ethnicity group in the 1000 Genomes project (Table 3). These results suggest that a meaningful proportion of the genetic complexity of the psychoses may be resolved by focussing on the CNS signaling genes that had been identified as harboring *de novo* missense coding variants in sporadic cases. Previous large-scale studies performing whole exome sequencing in schizophrenia cases (familial and non-related individuals) showed that rare polymorphisms and mutations are enriched in genes involved in synaptic plasticity and neuronal development (Purcell et al., 2014; Fromer et al., 2014) and our analysis of the multilevel phenotypes for several such genes show this body of work may be relevant for the development of precision treatment. In concordance with other large-scale analyses, these findings support the contention that schizophrenia is not a single disease, but is a group of distinct disorders for which major influential genes harboring rare disruptive variants may be discerned (Arnedo et al., 2015) and for which specific treatments may be developed.

As to the significant differences, *PTPRG* missense coding variant carriers had significantly earlier onset of psychosis and each had a history of learning disabilities. PTPRG is a protein tyrosine phosphatase highly expressed in the hippocampus, striatum and neocortex and is highly upregulated during neuroinflammatory processes (Lorenzetto et al., 2014). Despite their earlier onset, those without childhood psychosis attained some college education and employment. Their cognition was intact, with the exception of impaired working memory. Working memory is a neurocognitive domain that is a major target of treatment development in schizophrenia (Schwarz et al., 2016). None of the *PTPRG* variant carriers reported a suicide attempt despite their high rates of comorbid depression. While this is the first comprehensive phenotypic description for *PTPRG* missense coding variant carriers, a number of genome-wide association studies (GWAS) (Hamshere et al., 2009; Schizophrenia Psychiatric Genome-Wide Association Study C, 2011) have supported the relevance of *PTPRG* as risk loci for bipolar disorder, schizoaffective disorder, and major depression (Shi et al., 2011; Wellcome Trust Case Control C, 2007). A recent whole genome study accomplished in nine schizophrenia cases with the 22q11.2 deletion syndrome showed that *PTPRG* is enriched with deleterious missense coding variants (Merico et al., 2015). The current work in combination with previous genetic studies on neuropsychiatric cases supports the idea that the phenotype of such cases may add importantly to relevant genotypes and phenotypes in schizophrenia.

Cases with rare missense coding variants in *SLC39SA13*, a zinc transporter, also displayed an early onset but they showed globally disrupted cognition and the most severe psychopathology, including negative symptoms and severe suicide attempts. They had the lowest intelligence and least educational attainment. Working memory was impaired comparably to other cognitive domains in these cases and since processing speed was their strongest domain, it is also unlikely to be driving their pathology. They had the greatest medical morbidity, including degenerative joint diseases, consistent with the gene's role in connective tissue development (Fukada et al., 2008). The psychiatric findings are concordant with the *SLC39A13* animal knockout model (Hagmeyer et al., 2014), which shows analogous features to depression including anxiety, aggression, and impaired social functioning. Interestingly, these symptoms were alleviated with zinc supplements in rodents and rhesus monkeys (Hagmeyer et al., 2014), suggesting a possible treatment option. Furthermore, another study investigating a constitutive knockout mouse model of *SLC39A13* shows impairment of the BMP/TGFβ signaling pathway (Fukada et al., 2008). A recent study performed on the superior temporal gyrus in *post-mortem* schizophrenia ($n = 9$) and control brains ($n = 9$) yielded that mRNAs of the BMP/TGFβ signaling pathway were differentially expressed (Pietersen et al., 2014). Moreover, the severity of the zinc deficiency in the *SLC39A13* knockout mice appears to be associated with the severity of depressive symptoms (Maes et al., 1994; Amani et al., 2010).

TGM5 cases had less severe symptoms than the other gene carrier groups. *TGM5* is highly expressed in the epidermis and mutations are causative for acral peeling skin syndrome (Pigors et al., 2012; van der Velden et al., 2012). In addition, *TGM5* is expressed in fetal brain, substantia nigra, temporal lobe and the spinal cord (Grenard et al., 2001). We identified the rare missense coding polymorphisms in parent–child trios diagnosed with schizophrenia (Kranz et al., 2015a) and in an independent cohort of 48 schizophrenia cases presented in this study. All but one case attended college and their onset of psychosis occurred later in life. None reported suicide attempts or alcohol abuse and only half were cigarette smokers. Notably, childhood attention deficit disorder affected each of these cases, perhaps owing to particularly slow processing speed, which could also drive their pathophysiology.

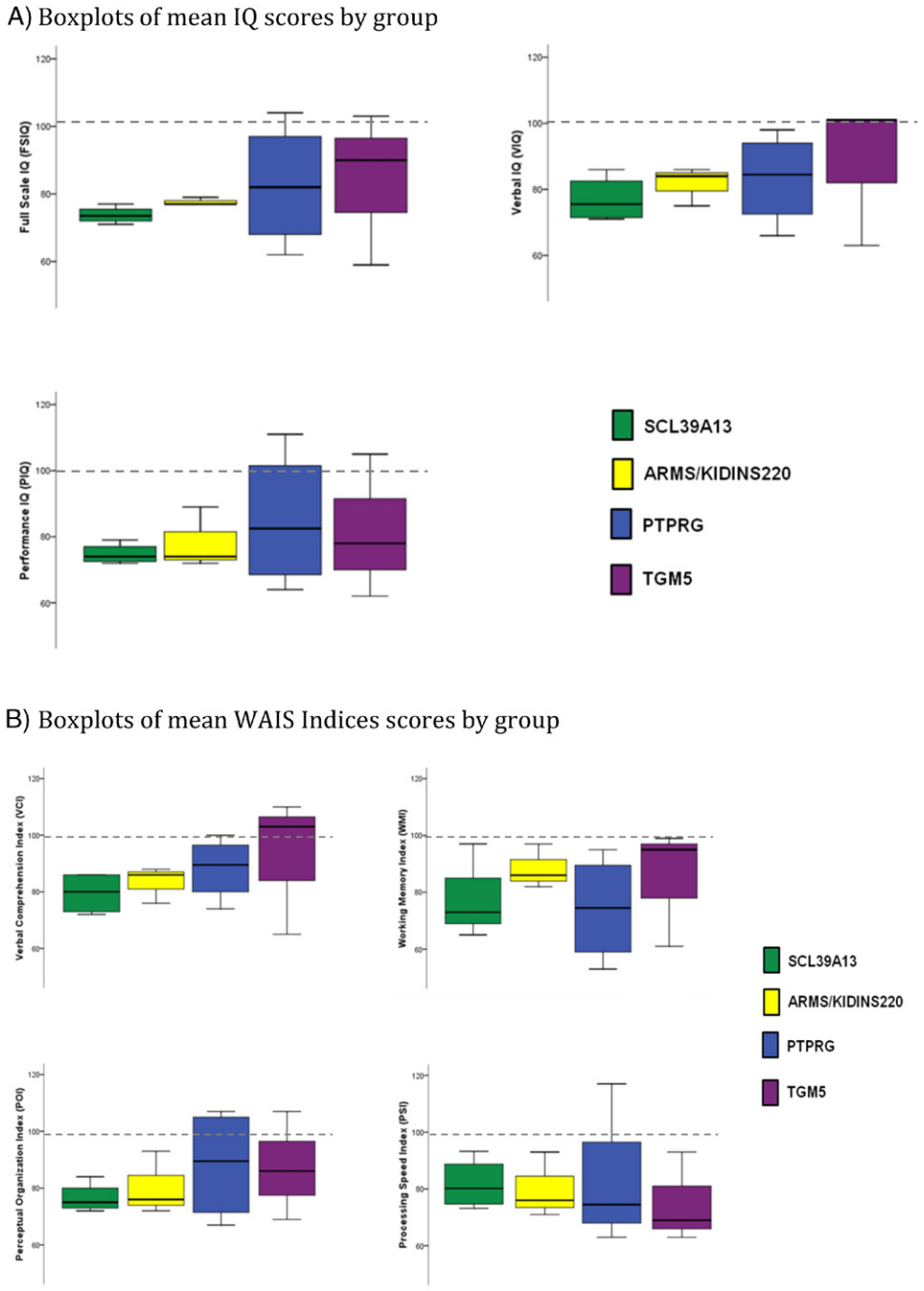

Fig. 2. a. Boxplots of mean IQ scores by group. b. Boxplots of mean WAIS Indices scores by group.

Given the fewer medical comorbidities and lower propensity for substance abuse, *TGM5* may convey a less debilitating form of schizophrenia.

ARMS/KIDINS220 carriers showed a picture consistent with low borderline intelligence and diminishing education and employment over earlier ages are inferred, since each completed at least one year of college (excluding dual-hit cases). While they showed the second slowest processing speeds after the *TGM5* carriers, all of their cognitive measures were similarly impaired. Ankyrin repeat-rich membrane spanning protein (*ARMS*) or *KIDINS220* (Kinase D-Interacting Substrate of 220 kDa) is a scaffold protein, which is a substrate of protein kinase D and neurotrophin receptors (Iglesias et al., 2000; Kong et al., 2001). In addition, it is a major signaling hub protein in the neurotrophin pathway as well as in ephrin signaling (Neubrand et al., 2012). It is important in the nerve growth factor-induced signaling (Rogers and Schor, 2013) and reveals dysregulated expression patterns in and Alzheimer's disease (Lopez-Menendez et al., 2013). In addition, mice with a constitutive heterozygous mutation of *ARMS/KIDINS220* display neuronal loss in the frontal cortex and entorhinal cortex resulting in a deficit in spatial memory (Duffy et al., 2011).

It is worth noting that the analyses of illness features accompanying particular affected genes were binary and not subjected to multiple comparison considerations, as each case either harbored a particular gene with missense coding polymorphisms or mutations or they did not. Adjustments for multiple comparisons were considered in defining the differences between the gene-based subgroups.

Table 3
Rare variants and mutations in four genes from two independent schizophrenia cohorts.

Gene	ID	Chr	Genomic Pos. (hg19)	Ref	Alt	Transcripts / AA exchange	SNP / novel mut.	1000g2012apr_all (%)	1000g_Case_Ethnicity (%)	Polyphen-2	
										Score	Prediction
PTPRG (736 kb)	1A	chr3	62153771	C	T	PTPRG:NM_002841:exon8:c.C967T:p.R323C	rs142366357	0.05	1 (AFR)	0.876	possibly damaging
	1B	chr3	62189036	G	A	PTPRG:NM_002841:exon12:c.G1567A:p.G523S	rs149885804	<0.01	0.3 (AFR)	1	probably damaging
	1D	chr3	62240843	G	A	PTPRG:NM_002841:exon16:c.G2512A:p.G838S	rs72878145	1	1.9 (AFR)	0	benign
	1E	chr3	62257194	G	A	PTPRG:NM_002841:exon21:c.G3146A:p.R1049Q	rs150212631	<0.01	0.02 (AFR)	0.498	possibly damaging
	1C*	chr3	62240841	T	G	PTPRG:NM_002841:exon16:c.T2510G:p.I837S	novel	.	. (AMR)	0.997	probably damaging
SLC39A13 (9 kb)	2A*	chr11	47434952	C	G	SLC39A13:NM_001128225:exon5:c.C539G:p.A180G,SLC39A13:NM_152264:exon5:c.C539G:p.A180G	rs147227015	0.14	0.9 (AFR)	0.703	possibly damaging
	1C*	chr11	47433573	C	T	SLC39A13:NM_001128225:exon3:c.C398T:p.T133M,SLC39A13:NM_152264:exon3:c.C398T:p.T133M	rs140574574	0.05	. (AMR)	1	probably damaging
	2B	chr11	47431764	G	A	SLC39A13:NM_001128225:exon2:c.G119A:p.R40Q,SLC39A13:NM_152264:exon2:c.G119A:p.R40Q	rs35741412	1	0.2 (AFR)	0.02	benign
	2C	chr11	47436707	C	T	SLC39A13:NM_001128225:exon9:c.C1037T:p.P346L,SLC39A13:NM_152264:exon9:c.C1016T:p.P339L	rs35978122	0.14	0.6 (AFR)	0.396	benign
TGM5 (34 kb)	3A	chr15	43525791	A	G	TGM5:NM_004245:exon11:c.T1724C:p.V575A,TGM5:NM_201631:exon12:c.T1970C:p.V657A	rs80058195	1	. (AJ)	0.137	benign
	3A	chr15	43527022	T	G	TGM5:NM_004245:exon10:c.A1574C:p.E525A,TGM5:NM_201631:exon11:c.A1820C:p.E607A	rs80192997		. (AJ)	0.984	probably damaging
	3D*	chr15	43525791	A	G	TGM5:NM_004245:exon11:c.T1724C:p.V575A,TGM5:NM_201631:exon12:c.T1970C:p.V657A	rs80058195		. (EUR)	0.137	benign
	3D*	chr15	43527022	T	G	TGM5:NM_004245:exon10:c.A1574C:p.E525A,TGM5:NM_201631:exon11:c.A1820C:p.E607A	rs80192997		. (EUR)	0.984	probably damaging
	3B	chr15	43527020	T	C	TGM5:NM_004245:exon10:c.A1576G:p.K526E,TGM5:NM_201631:exon11:c.A1822G:p.K608E	rs76456763		. (EUR)	0.745	possibly damaging
	3C	chr15	43527020	T	C	TGM5:NM_004245:exon10:c.A1576G:p.K526E,TGM5:NM_201631:exon11:c.A1822G:p.K608E	rs76456763		0.1 (AMR)	0.745	possibly damaging
ARMS/KIDINS220 (112 kb)	2A*	chr2	8873731	G	C	KIDINS220:NM_020738:exon29:c.C3896G:p.A1299G	rs76164009	0.27	1.7 (AFR)	0	benign
	4A	chr2	8890402	T	C	KIDINS220:NM_020738:exon24:c.A3254G:p.H1085R	novel	.	. (AFR)	0.011	
	4B	chr2	8873731	G	C	KIDINS220:NM_020738:exon29:c.C3896G:p.A1299G	rs76164009	0.27	. (EUR)	0	
	4C	chr2	8873731	G	C	KIDINS220:NM_020738:exon29:c.C3896G:p.A1299G	rs76164009	0.27	. (EUR)	0	
	3D*	chr2	8928894	G	A	KIDINS220:NM_020738:exon15:c.C1670T:p.A557V	rs201557833	0.05	. (EUR)	0.009	

Chr: chromosome, Ref: reference allele, Alt: alternative allele, AA exchange: amino acid exchange, 1000g2912apr_all (%): minor allele frequency of variant in 1000G project including across all investigated ethnicities, 1000g_Case_Ethnicity (%): minor allele frequency of variant with regard to case ethnicity

The patterns and severity of symptoms depicted by the factor score profile (Fig. 1) may be illuminating as to common and unique pathologies. *PTPRG* carriers uniquely showed comparable high levels of positive symptoms and dysphoric mood. *ARMS/KIDINS220* carriers showed similar high scores on most symptom factors, yielding the highest autistic factor score, but a lower factor score for activation, reflecting lesser hostility. *SLC39A13* carriers showed the greatest levels of all psychopathology and especially negative symptoms. Finally, *TGM5* carriers and the group of cases without rare missense coding genetic variants in any of these genes were almost indistinguishable in profiles and severity. Both groups showed roughly doubled scores of the healthy controls in the same distribution. Given their possibly slower processing speed of these *TGM5* carriers, slow processing speed may also be common in the cases that did not carry missense coding variants in any of these particular genes and may be an important target for treatment in these cases.

A recent study demonstrated three psychosis biotypes using a wide biomarker panel, which clearly corroborates the need for further and better alignment of endophenotypes with the underlying symptomatological complexes (Clementz et al., 2015). Given the complementary findings of our study, we propose that the biomarker panel can be anchored to rare gene variants in influential pathways. Only categorizing the psychosis endophenotype as a categorical disorder may inadvertently minimize finding some important risk variants. It is also notable that the respective potentially altered protein functions encoded by these rare missense coding polymorphisms and novel mutations may influence the clinical picture throughout the life course as the current findings suggest. The birth complications, brain injuries, and premorbid substance abuse common in the *PTPRG* carriers may reflect pleiotropic effects of altered protein function, rather than a causal chain of exposures. The findings also suggest that ethnicity may not be as large a roadblock as had been expected for research in schizophrenia. The initial missense coding polymorphisms and mutations of these genes were found in sporadic cases from Israeli and Afrikaner samples, yet different missense coding variants of each gene in the current study occurred in African Americans and, for all but one gene, in a Hispanic case. Ethnicity appears less important when gene function is considered, even if particular rare missense coding polymorphisms predominate in different ethnicities. It is of interest that at least half of the cases in each carrier group had a first-degree relative with psychosis. Genes prone to harbor missense coding *de novo* mutations, which typically occur in the male germ line and are transmitted to offspring in association with paternal age in schizophrenia, may thus afterwards be heritable to other generations and constitute important sources of population-wide psychosis vulnerability.

These results do not prove causal relationships between these genetic variations and phenotypes, but set the stage for replication and mechanistic research to inform precision medicine. Despite the limitation of this preliminary study (notably a small sample size), significant differences were found, even in conservative analyses, supporting different phenotypic profiles for rare missense coding variants in these genes. The authors are aware there can be limitations in studying a small sample size. Numerous symptom indices and cognitive measures were examined and described with minimum attention to the many comparisons. However, the strength of this study is the deep coverage of the target genes (>50× for 97% of exon sequence (Kranz et al., 2015a, 2015b)), which enables high confidence of the detected variants and even the ability to find extremely rare missense coding polymorphisms and mutations. In addition, we observed that these rare variants and novel mutations are located in protein interaction domains and that one of the variants (ARMS/KIDINS220 H1085R) leads to reduced total protein expression (Kranz T.M., unpublished). Although some of the Polyphen-2 predictions indicate that some of the polymorphisms are benign, this is not likely to be the case in each instance. For example biochemical analysis of some supposedly benign variants in a

gene have actually revealed significant effects on downstream signaling in the encoded protein, as demonstrated for the Y1096 variant in the rat ARMS/Kidins220, where the loss of tyrosine phosphorylation by mutation of this site leads to the loss of recruitment of the adaptor protein CrkL and of sustained MAPK signaling (Arevalo et al., 2004).

Although the inclusion of a control cohort would have been ideal for internal validation of these sequence variation frequencies, our findings of missense coding polymorphisms with allele frequencies of <1%, and even novel mutations, suggests that these findings are sufficiently supported to be of keen interest. A larger replication sample may more reliably draw connections between gene variant and observed phenotype and the stage will be set for a new approach to the disease as being partly constituted by multiple independent subtypes of illness.

The difficulty in finding high impact genetic variation for psychoses had lessened enthusiasm in the field over whether particular genes or phenotypes could be leveraged for person-specific interventions. Conversely, this report suggests that optimism may be appropriate for the substantial portion of cases harboring abnormalities in genes prone to *de novo* mutations and rare missense coding polymorphisms in earlier generations. As events in these genes were identified in more than 30% of the cases in this replication sample, these genes may influence a substantial proportion of cases with chronic psychosis and become targets for differential treatments. In particular, treatment approaches may usefully address processing speed in *TGM5* carriers, working memory in *PTPRG*, zinc augmentation in *SLC39A13*, and neuroprotection in *ARMS/KIDINS220* carriers. Precision approaches can be informed by knowledge of these genotypes to advance treatment for persons with psychoses. Treatments found to lack efficiency in heterogeneous groups of cases may be highly promising in selected groups of cases.

5. Conclusions

These findings support the contention that single influential genes, targeted for sequencing because they had harbored *de novo* mutations in sporadic cases compared to healthy parents in other studies, may be influential with respect to the phenotypes of psychosis in unselected cases and potentially relevant for treatment studies. Despite the relatively small-sized subgroups based on the harboring of rare missense polymorphisms in any of four different genes in this study, group differences were demonstrated on multiple levels of the phenotype. Knowledge about these genes may be useful to identify relevant genetic architectures for psychosis and in the development of person-specific treatments.

Authors' contributions

Study design and data collection (DM, MC, JWM, TMK), data analysis (RG, TMK, JWM), data interpretation (TMK, DM, JWM, RG, AB, KR), preparation and editing of manuscript (all authors).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.03.008.

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