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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.