Schizophrenia Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Rare variants in the neurotrophin signaling pathway implicated in schizophrenia risk

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ARTICLE INFO

Article history:
Received 11 April 2015
Received in revised form 30 June 2015
Accepted 2 July 2015
Available online xxxx

Keywords:
Schizophrenia
Sporadic
De novo
ARMS
Kidins220
Rare variant
Neurotrophin
Exome sequencing

ABSTRACT

Multiple lines of evidence corroborate impaired signaling pathways as relevant to the underpinnings of schizophrenia. There has been an interest in neurotrophins, since they are crucial mediators of neurodevelopment and in synaptic connectivity in the adult brain. Neurotrophins and their receptors demonstrate aberrant expression patterns in cortical areas for schizophrenia cases in comparison to control subjects. There is little known about the contribution of neurotrophin genes in psychiatric disorders. To begin to address this issue, we conducted high-coverage targeted exome capture in a subset of neurotrophin genes in 48 comprehensively characterized cases with schizophrenia-related psychosis. We herein report rare missense polymorphisms and novel missense mutations in neurotrophin receptor signaling pathway genes. Furthermore, we observed that several genes have a higher propensity to harbor missense coding variants than others. Based on this initial analysis we suggest that rare variants and missense mutations in neurotrophin genes might represent genetic contributions involved across psychiatric disorders.

1. Introduction

Schizophrenia is a complex genetic disorder that affects roughly 7 in 1000 individuals worldwide (McGrath et al., 2003). Several different mechanisms have been shown to confer risk for schizophrenia. One genetic contribution to illness risk is copy number variations (CNV), which are detected in several neurodevelopmental and psychiatric conditions, such as autism spectrum disorder, bipolar disorder and schizophrenia (Doherty et al., 2012; King and Lord, 2011; Sullivan et al., 2012). Further support for shared genetic architecture among psychiatric diseases was demonstrated by a genome-wide association analysis, which found variations in calcium-channel activity genes, with obvious pleiotropic effects, across five different psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics, 2013).

Despite several comprehensive efforts to define overlapping genetic susceptibilities, the signaling pathways that are associated with many psychiatric dysfunctions have not been fully defined. Several coexisting hypotheses have been proposed. Recent large exome sequencing studies of the genomes of autism spectrum disorder and schizophrenia trios have revealed a number of *de novo* mutations (Dong et al., 2014; Fromer et al., 2014). These findings, accomplished in different populations and samples, converge to the conclusion that *de novo* mutations may play a significant role in the pathophysiology of these disorders. These studies also point to the enrichment of rare occurring *de novo* mutations in genes encoding for pre- and postsynaptic proteins. In addition to genetic contributions, schizophrenia etiology is significantly influenced by environmental factors, especially early life stress during pre- and postnatal life (Caspi and Moffitt, 2006; Fumagalli et al., 2007).

A very important family of proteins responsible for correct neurodevelopment and maintenance of functional neuronal networks are neurotrophins and their receptors. In particular, the neurotrophin brain-derived neurotrophic factor (BDNF) has been shown to be important for both the integrity and plasticity of neuronal connections in the

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http://dx.doi.org/10.1016/j.schres.2015.07.002
0920-9964/

central nervous system and is the best studied of all neurotrophins (Bekinschtein et al., 2014; Callaghan and Kelly, 2012; Park and Poo, 2013). Neurotrophins facilitate a variety of functions, including cell survival, differentiation, axonal and dendritic growth as well as synaptic plasticity, arborization and synapse formation (Chao, 2003; Park and Poo, 2013).

The actions of trophic factors entail two different transmembrane receptors, the Trk and the NGFR (p75) receptor (Chao, 2003; Huang and Reichardt, 2003). Upon binding of neurotrophins to their corresponding Trk receptors, adapter molecules such as Src homology 2-containing protein (Shc) and fibroblast growth factor receptor substrate 2 (FRS2) are recruited to specific docking sites within the Trk receptors (Zampieri and Chao, 2006). Neurotrophin binding to Trk receptors activates multiple intracellular pathways, including MAPK, PI3K-AKT and PLCγ-PKC, whereas p75 can engage the apoptotic machinery and induce morphological changes, such as growth cone arrest (Deinhardt et al., 2011; Gehler et al., 2004). Activation of any of the three before mentioned signaling pathways upon Trk receptor activation influences synaptic plasticity in a specific manner. MAPK signaling preferably acts on neuronal differentiation (Nakamura et al., 1996; Ortega and Alcantara, 2010). The Akt and PLCγ pathways are responsible for neuronal survival and plasticity (Chao, 2003; Minichiello et al., 1999). 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 tyrosine phosphorylation (Iglesias et al., 2000; Kong et al., 2001).

Gene expression studies on neurotrophin genes indicated decreased expression patterns in the human brain. Altered BDNF expression is described in *post-mortem* cortices of schizophrenia cases (Issa et al., 2010; Ray et al., 2014; Weickert et al., 2003). Both BDNF and NT-3 protein expression levels were increased in the cortical areas and decreased in the hippocampus (Durany et al., 2001). Another factor that points to the importance of neurotrophic factors like BDNF and NGF and their signaling pathways is their occurrence in brain regions that are found impaired in cases diagnosed with neurodegenerative and psychiatric conditions such as the cortex, hippocampus and pituitary gland (Goedert et al., 1986; Hefti and Weiner, 1986; Maisonpierre et al., 1991; Mariga et al., 2015).

Several genetic association studies and reports have elucidated the potential impact of the TrkB–BDNF system on the etiogenesis of neurodevelopmental and psychiatric disorders. A mutation in the tyrosine kinase domain of TrkB (Y722C) was identified in a young male case with extreme obesity and developmental delay. The mutation decreases the autophosphorylation ability of TrkB and thus especially MAPK signaling (Yeo et al., 2004). Further corroboration of the importance of the neurotrophin signaling pathway for correct neurodevelopment is provided by a clinical report about four patients, each diagnosed with a variable degree of developmental delay and obesity with a deletion encompassing the BDNF gene (Shinawi et al., 2011). Most studies on BDNF have emphasized a SNP in the prodomain Val66Met (rs6265), with ambiguous results among psychiatric disorders and related endophenotypes (Jiang et al., 2005; Schumacher et al., 2005; Soliman et al., 2010; Tsai et al., 2003; Verhagen et al., 2010; Zou et al., 2010). An interaction of BDNF and TrkB SNPs was found to be involved in the susceptibility of paranoid schizophrenia in Han Chinese, although single SNPs in these genes did not confirm this finding (Lin et al., 2013).

Based on the neurodevelopmental impact of neurotrophin signaling pathways on neuronal networks and the ambiguity of the current findings on neurotrophins and schizophrenia etiology, we performed targeted exome capture of selected neurotrophin genes in 48 schizophrenia related psychosis cases. The authors are aware there can be limitations in studying a small sample size. However, the strength of this study is the deep coverage of the target genes (>50× for 99% of exon sequence) and the ability to assess a well-defined pathway of interacting genes (see Supplementary Fig. 1). The detection of ultra rare genetic variants and *de novo* mutations support the neurotrophin signaling pathway as a potential contributor to schizophrenia etiogenesis.

2. Material and methods

2.1. Ascertainment and characterization of cases

The study subjects were recruited from Bellevue Hospital Center and New York University and IRB approvals were obtained from both institutions. Cases with schizophrenia or schizoaffective disorder, age 18–55 years, were recruited from treatment settings and all provided informed consent for the study. Diagnosis was determined by best estimate diagnostic procedures that included the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) conducted by reliable master's level diagnosticians and family history assessed with the Family Interview for Genetic Study (FIGS) (Maxwell, 1992). The inter-rater reliability was $\kappa = .95$ for DSM-IV diagnosis and $\kappa = .80$ for individual symptoms. Symptoms were assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and expressed as factors for the severity of positive, negative, dysthymia, activation and autism symptoms (White et al., 1997). The Wechsler Adult Intelligence Scale (WAIS-III)(Wechsler, 1999) was used to assess Verbal, Performance, and Full Scale IQ. Early trauma exposure was assessed using the Early Trauma Inventory (ETI) (Bremner et al., 2000).

2.2. Sample size and DNA source

Targeted exome capture of 48 non-related schizophrenia-affected individuals of diverse ethnicity was conducted on DNA derived from peripheral leukocytes.

2.3. Targeted exome capture and variant calling

Genomic DNA was isolated from leukocytes derived from whole blood using a simple salting out procedure (Miller et al., 1988). The DNA (500 ng) was sheared to an average of 150 bp. Barcoded libraries were prepared using the Kapa Low-Throughput Library Preparation Kit Standard Libraries and amplified using the KAPA HiFi Library Amplification Kit (Kapa Biosystems) (8 cycles). Quantification was performed using Qubit Fluorimetric Quantitation (Invitrogen) and Agilent Bioanalyzer. An equimolar pool of the 4 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 (see Supplementary Fig. 1). Capture by hybridization was performed according to the manufacturer's protocols with the following modifications: 1 nmol of a pool of blocker oligonucleotides and post-capture PCR amplification were done using the KAPA HiFi Library Amplification kit in a 60 μl volume. Pooled capture library was quantified by Qubit (Invitrogen) and Bioanalyzer (Agilent) and sequenced in an Illumina MiSeq sequencer using the 2 × 150 paired-end cycle protocol, or on an Illumina HiSeq 2500 using a 2 × 100 run. Reads were aligned to the hg19 build of the human genome using BWA with duplicate removal using samtools as implemented by the Illumina MiSeq Reporter. Variant detection was performed using GATK UnifiedGenotyper. Variants were annotated with ANNOVAR annotator to cross-reference against known dbSNP, 1000 Genomes, ESP6500,COSMIC mutations and Schizophrenia Exome Sequencing Genebook entries.

3. Results

3.1. Neurotrophin signaling pathway genes harbor rare missense coding mutations

The goal was the detection of rare missense coding polymorphisms and novel missense variants in the Trk receptors (TrkA, TrkB,

Please cite this article as: Kranz, T.M., et al., Rare variants in the neurotrophin signaling pathway implicated in schizophrenia risk, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.07.002