Psychiatry Research ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Psychiatry Research

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

Beta-catenin in schizophrenia: Possibly deleterious novel mutation

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ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form
10 April 2015
Accepted 3 May 2015

Keywords:
Candidate gene analysis
DNA mutational analysis
Human protein CTNNB1
Psychiatry

ABSTRACT

Schizophrenia is a debilitating psychiatric disorder, affecting approximately 1% of the human population. Mostly genetic factors contribute to schizophrenia, but the genetics are complex and various aspects of brain functioning and structure, from development to synapse plasticity, seem to be involved in the pathogenesis. The goal of the study was to look for novel mutations in genes, implicated in molecular networks, important in schizophrenia. In the study four candidate genes taking part in the WNT signaling pathway were analyzed by sequencing in a cohort of 87 schizophrenia patients from Saint Petersburg, Russia. The gene list included CTNNB1 (beta-catenin), GSK3B, WNT2B and WNT7B. The impact of discovered variants on the protein function was analyzed in silico. We found three variants in the genes CTNNB1 and WNT7B, absent in healthy controls, including 212 controls from the same geographic area. The novel mutation c.1943A > G (p.N648S) in CTNNB1 seems to be the best candidate for disease-associated mutation in this study, as it damages the protein product in silico. This is the first study reporting mutations in CTNNB1 in schizophrenia.

1. Introduction

Schizophrenia is a debilitating mental disorder, affecting approximately 1% of the human population worldwide; it has a complex clinical picture that can additionally vary within the same individual during his/her life course (Freedman, 2003). The complex clinical picture is in accordance with the complex, non-Mendelian genetics of schizophrenia. Etiological factors, resulting in schizophrenia, are in fact mostly genetic, making up to 80% of all factors contributing to the pathogenesis (Sullivan et al., 2003). Apart from long-established linkage and association studies of schizophrenia genetics, more approaches have emerged, studying de novo copy number variations and point mutations by next-generation sequencing (reviewed in Rees et al., 2012; Rodriguez-Murillo et al., 2012; Schreiber et al., 2013; Escudero and Johnstone, 2014; Kavanagh et al., 2015). Despite the complexity of schizophrenia genetics, the genetic studies are advancing, leading to discovery of numerous candidate genes, whose role was confirmed by different studies, important mostly in neural development, neurotransmission and immune system (Rodriguez-Murillo et al., 2012; Fromer et al., 2014; Hall et al., 2015). A widely accepted view of schizophrenia is that of a neurodevelopmental disorder, affecting the normal brain development during embryogenesis and childhood (Fatemi and Folsom, 2009; Rapoport et al., 2012). Aberrant brain development apparently leads to the multiple structural and functional brain abnormalities, such as aberrant neuronal connections, observed in schizophrenia patients (Ross et al., 2006; Schmitt et al., 2011). One of the hallmarks of a schizophrenic brain is diminished or even reversed left–right (LR) asymmetry of the brain structures (Oertel-Knochel and Linden, 2011; Oertel-Knochel et al., 2012). This finding allowed to propose that genes important for the establishment of LR asymmetry of the human brain are implicated in schizophrenia (Crow et al., 1989). The present study, a follow-up of the previous investigation of five candidate genes (Levchenko et al., 2014), is based on published data regarding several players of the WNT (Wingless-Int) pathway in the context of cerebral asymmetry and schizophrenia. The WNT pathway plays, among other roles in development, a role in establishment of the LR axis of the body

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http://dx.doi.org/10.1016/j.psychres.2015.05.014
0165-1781/

(Kitajima et al., 2013) and of the brain, at least of the diencephalon (Husken and Carl, 2013). It is also important for the functioning of the adult brain (Wisniewska, 2013). Moreover, the pathway is implicated in the pathogenesis of schizophrenia (Panaccione et al., 2013; Singh, 2013; Peng et al., 2014). The goal of this study was to screen by sequencing four WNT pathway genes in a cohort of individuals with the diagnosis of schizophrenia.

2. Methods

2.1. Ethics statement

Written informed consent was obtained from all the participants. The participants with a diagnosis of schizophrenia have not had a compromised capacity/ability to consent, because they were outpatients at an ambulatory care clinic and were not psychotic. The experiments complied with the current laws of the country (The Russian Federation) in which they were performed, and were also approved by the responsible authorities at the institutes (Saint Petersburg State University and Federal Almazov Medical Research Centre) where the work has been carried out. In addition, the research was done in full compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Subjects

The participants with the diagnosis of schizophrenia were outpatients at the Saint Petersburg State University psychiatric ambulatory care clinic; all of them resided in Saint Petersburg, Russia. The cohort of 87 patients was selected, based on the diagnostic criteria of The International Statistical Classification of Diseases and Related Health Problems 10th Revision, Chapter V (ICD-10). See Levchenko et al. (2014) for details on each patient and overall statistics of the cohort (the schizotypal disorder patients, while described in the previous study, were not included in the present study). The patients were also characterized, using the expanded 24-item Brief Psychiatric Rating Scale (BPRS-24) (Ventura et al., 1993; Dingemans et al., 1995), which is used to evaluate severity and structure of symptoms. Each item of the scale is rated with a score and sum of scores was used to characterize each patient (Levchenko et al., 2014).

2.3. Healthy controls for genetic study

A cohort of 212 healthy controls for the genetic study consisted of individuals free of any serious illness, including psychiatric illnesses, aged 22 to 30, 65% of them males, taking part in a genetic study of cardiovascular diseases at Federal Almazov Medical Research Centre. The controls, same as patients, resided in Saint Petersburg, Russia.

2.4. Candidate genes

After literature reviewing, four candidate genes for LR asymmetry of the human brain, as well as other aspects of brain development and function, were identified. The list included: CTNNB1 (MIM # 116806; NM_001904.3), GSK3B (MIM # 605004; NM_002093.3), WNT2B (MIM # 601968; NM_004185.4) and WNT7B (MIM # 601967; NM_058238.2).

The catenin CTNNB1 is a major player in the WNT pathway and accomplishes two roles inside the cell: as a transcriptional activator for transcription factor families like lymphoid enhancer-binding factor 1/T-cell transcription factor (LEF1/TCF) family and as a protein that binds to cadherins at adherens junctions, thus stabilizing the cytoskeleton and taking part in the structure of synapses (Logan and Nusse, 2004; Wisniewska, 2013). CTNNB1 with the transcription factors LEF1/TCF initiate expression of genes important in many aspects of development, including neurogenesis and synaptic plasticity (Wisniewska, 2013). CTNNB1 protein levels were found to be decreased in the hippocampus of schizophrenic patients in a post-mortem study (Cotter et al., 1998). Antipsychotic drugs on their turn increase beta-catenin protein levels in several areas of the rat brain (Alimohamad et al., 2005a, 2005b). Numerous, protein-disrupting mutations were found in this gene in patients with autism and mental retardation (de Ligt et al., 2012; O'Roak et al., 2012; Tucci et al., 2014).

GSK3B negatively regulates the activity of CTNNB1 in the so-called canonical WNT pathway, by phosphorylating it; phosphorylated CTNNB1 undergoes degradation in the proteasome (Logan and Nusse, 2004; Wisniewska, 2013). This kinase is also implicated in regulation of neuronal cytoskeleton, by phosphorylating microtubule-associated proteins (Goold et al., 1999; Wood-Kaczmar et al., 2009). GSK3B is inhibited by a number of molecular factors, implicated in schizophrenia, including antipsychotic drugs acting on dopamine receptors, lithium and Disrupted in Schizophrenia 1 (Mao et al., 2009; Singh, 2013). Several SNPs in intronic and promoter regions of GSK3B were moreover found to be associated with schizophrenia (Souza et al., 2008; Li et al., 2011; Tang et al., 2013).

WNT2B and WNT7B are members of the WNT family of signaling factors, binding to the receptors Frizzled, therefore activating the canonical and non-canonical WNT pathways, as well as binding to some types of receptors tyrosine kinase; all these events are implicated in neurogenesis, synaptic plasticity and regulation of neuronal cytoskeleton (reviewed in Logan and Nusse, 2004; Singh, 2013; Wisniewska, 2013). These two ligands in particular are expressed in the dorsal midline structures of the developing human diencephalon (Abu-Khalil et al., 2004). Midline structures are implicated in establishment of LR asymmetry of the brain (Bisgrove et al., 2000; Concha et al., 2003).

2.5. Genetic screening

For each gene we selected one to four transcripts, i.e. entire mRNA, containing both coding sequence and non-coding 5′- and 3′-UTRs, so to cover all known exons, listed in the "UCSC genes" track of the UCSC genome browser (http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=199238144_oRYZMEYqwwPxpom85995Qroz2Z35&c=chr10&g=knownGene) (Karolchik et al., 2014).

These transcripts were used to design primers with the ExonPrimer script from Helmholtz Zentrum München (http://www.helmholtz-muenchen.de). At least 45 base pairs (bp) of intronic sequence bordering each exon were included in the PCR amplicons.

The transcripts' names in the UCSC genome browser, their boundaries within the hg19 human genome assembly, the number of amplicons, and the length of amplicons (ranging from smallest to largest values) are indicated for each gene:

CTNNB1: uc010hia.1, chr3:41235400–41281845; uc003ckr.2, chr3:41239941–41282939; uc011azf.1, chr3:41239941–41282939; uc003cks.2, chr3:41273899–41279937; 22 amplicons, 282–893 bp.
GSK3B: uc003edn.2, chr3:119539803–119814264; 21 amplicons, 333–790 bp.
WNT7B: uc003bgo.2, chr22:46315247–46374008; 7 amplicons, 455–716 bp.
WNT2B: uc001eca.2, chr1:113009039–113064908; uc001ecb.2, chr1:113050369–113064908; 9 amplicons, 424–590 bp.

Genomic DNA was extracted from peripheral blood leukocytes, using the FlexiGene DNA Kit (Qiagen, Germany), following procedures, recommended by the manufacturer.

Please cite this article as: Levchenko, A., et al., Beta-catenin in schizophrenia: Possibly deleterious novel mutation. Psychiatry Research (2015), http://dx.doi.org/10.1016/j.psychres.2015.05.014