Progress in Neuro-Psychopharmacology & Biological Psychiatry 62 (2015) 7–13

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

The *NVL* gene confers risk for both major depressive disorder and schizophrenia in the Han Chinese population

Meng Wang [a,1], Jianhua Chen [a,b,1], Kuanjun He [c], Qingzhong Wang [b], Zhiqiang Li [a], Jiawei Shen [a], Zujia Wen [a], Zhijian Song [a], Yifeng Xu [b], Yongyong Shi [a,d,e,*]

[a] Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200030, PR China
[b] Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, PR China
[c] College of Life Science, Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia 028000, PR China
[d] Shanghai Changning Mental Health Center, 299 Xiehe Road, Shanghai 200042, PR China
[e] Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, Shanghai 200042, PR China

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 31 March 2015
Accepted 7 April 2015
Available online 17 April 2015

Keywords:
Association
Major depressive disorder
NVL gene
Schizophrenia
Telomerase activity

ABSTRACT

NVL (nuclear VCP (valosin containing protein)/p97-Like), a member of the AAA-ATPase (ATPases associated with various cellular activities) family, encodes a novel hTERT (human telomerase reverse transcriptase)-interacting protein NVL2 which is a telomerase component essential for holoenzyme assembly. Previous researches have reported the impacts of telomerase activity on mental illness and the potential association between *NVL* and major depressive disorder. To validate the susceptibility of *NVL* to major depressive disorder, and to investigate the overlapping risk conferred by *NVL* for both major depressive disorder and schizophrenia, we analyzed 9 tag single nucleotide polymorphisms (tag SNPs) using TaqMan® technology, in 1045 major depressive disorder patients, 1235 schizophrenia patients and 1235 normal controls of Han Chinese origin. We found that rs10916583 ($P_{allele} = 0.020$, $P_{genotype} = 0.028$, OR = 1.156) and rs16846649 (adjusted $P_{allele} = 0.014$, $P_{genotype} = 0.007$, OR = 0.718) were associated with major depressive disorder, while rs10916583 (adjusted $P_{allele} = 1.08E-02$, OR = 1.213), rs16846649 (adjusted $P_{allele} = 7.40E-06$, adjusted $P_{genotype} = 8.07E-05$, OR = 0.598) and rs10799541 (adjusted $P_{allele} = 8.10E-03$, adjusted $P_{genotype} = 0.049$, OR = 0.826) showed statistically significant association with schizophrenia after Bonferroni correction. Furthermore, rs10916583 (adjusted $P_{allele} = 9.00E-03$, adjusted $P_{genotype} = 3.15E-02$, OR = 1.187) and rs16846649 (adjusted $P_{allele} = 8.92E-06$, adjusted $P_{genotype} = 8.84E-05$, OR = 0.653) remained strongly associated with the analysis of combined cases of major depressive disorder and schizophrenia after Bonferroni correction. Our results indicated that the *NVL* gene may contain overlapping common genetic risk factors for major depressive disorder and schizophrenia in the Han Chinese population. The roles of *NVL* in telomerase biogenesis were also highlighted in psychiatric pathogenesis. The study on variants conferring overlapping risk for multiple psychiatric disorders could be tangible pathogenesis support and clinical or diagnostic references.

1. Introduction

Major depressive disorder (MDD) and schizophrenia (SCZ) are common and debilitating mental diseases with pervasive impacts on the quality of life for both sufferers and their families. Patients with MDD or SCZ may suffer from such symptoms as loss of interests, sad mood, insomnia, energy and cognitive impairment (Craddock and Forty, 2006). MDD affects about 8–12% of the world population and has approximate 40–50% individual heritability (O'Donovan et al., 2009), while SCZ is a severe disorder with a prevalence of 1% worldwide and an individual heritability of 60–85% (Burmeister et al., 2008). So far, a large number of studies including linkage analysis (Ng et al., 2008), candidate gene association studies (Luo et al., 2014) and genome-wide association studies (GWAS) (Ripke et al., 2013) have identified number of susceptibility loci responsible for the occurrence of MDD or SCZ. Specially, a multi-

Abbreviations: *NVL*, nuclear VCP/p97-Like; GWAS, the genome-wide association study; MDD, major depressive disorder; SCZ, schizophrenia; LD, linkage disequilibrium; OR, odds ratio; AAA-ATPase, ATPases associated with various cellular activities; hTERT, human telomerase reverse transcriptase; SNPs, single nucleotide polymorphisms; HWE, Hardy–Weinberg equilibrium test; CHB, Han Chinese in Beijing; VCP, valosin containing protein; PGC, Psychiatric Genetic Consortium; DOB1, a human homologue of yeast Dob1p/Mtr4p; MDDWG, Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium; *FoxP2*, Forkhead-box P2; ITIH, the homologous heavy chains of the inter-alpha-trypsin inhibitor; CEU, northern and western Europe in the United States; YRI, Yoruba in Ibadan, Nigeria.

* Corresponding author at: Bio-X Institutes, Shanghai Jiao Tong University, Central Little White House, 1954 Huashan Road, Shanghai 200030, PR China.
E-mail address: shiyongyong@gmail.com (Y. Shi).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.pnpbp.2015.04.001
0278-5846/

stage genome-wide association study recently identified 108 conservatively defined loci linked with schizophrenia, which further provided support to the research of genetic factors and mental disorders (Psychiatric Genetic Consortium, 2014).

When compared with general population, apart from typical psychological symptoms, individuals with major mental illnesses, such as MDD and SCZ, show other features like higher rates of chronic medical conditions and reduced life expectancies (Colton and Manderscheid, 2006; Kupfer et al., 2012). In the meantime, there has been suggestive potential association between MDD and accelerated biological or functional decline due to cellular aging. The mainstream method used to examine the accelerated aging hypothesis is to study leukocyte telomeres, which measures the corresponding telomere length and telomerase activity (Kinser and Lyon, 2013). Telomeres, known as the distal part of chromosomes in somatic cells, gradually condense during the process of incomplete replication of linear chromosomes (Blackburn, 2005). Telomerase is a ribonucleoprotein enzyme which could protect and repair telomeres by elongating telomeric DNA, counteracts telomere shortening and insures cellular viability (Calado and Young, 2009). Telomerase is expressed at insufficient levels to completely restore telomeres in most somatic cells, while high levels of telomerase exist in neural progenitor cells both in the developing (Klapper et al., 2001) and adult brain (Caporaso et al., 2003). Various factors including sex and genetic variation could affect the efficiency of telomere length maintenance (Babizhayev et al., 2011; Barrett and Richardson, 2011), and telomere shortening was reported to be associated with some aging and somatic diseases like cancers, diabetes and cognitive decline (Wolkowitz et al., 2011). Recently, research has demonstrated that decreased telomerase activity and accelerated telomere shortening are involved in the processing of psychiatric conditions like SCZ (Kao et al., 2008), mood disorders (Simon et al., 2006) and chronically stressed individuals (Epel et al., 2004), which suggested that telomerase may be associated with adult neurogenesis and the regulation of mood behaviors.

The core enzyme of telomerase consists of a telomerase RNA component (TERC), and a telomere reverse transcriptase (TERT) (Collins, 2006). In the search of factors capable of interacting with human telomerase, NVL2 is reported to be an hTERT-interacting protein, which co-localizes and interacts with hTERT in the nucleolus, and associates with catalytically competent telomerase (Her and Chung, 2012). *NVL*, also known as nuclear VCP-like, was first reported in 1997 by Emily L. Germain-Lee as a gene encoding a member of AAA family (ATPases associated with diverse cellular activities) (Germain-Lee et al., 1997). These kinds of proteins normally contain one or two conserved AAA modules (ATP Binding Modules) (Neuwald et al., 1999). The AAA-ATPases associate with various cellular activities and functions, such as membrane fusion, protein folding, cytoskeletal regulation, DNA replication and proteolysis (Neuwald et al., 1999, Ogura and Wilkinson, 2001). Two alternatively encoded and spliced forms of NVL exist in cells as the short form, NVL1, and the long form, NVL2. The difference in lengths of N-terminal extensions is due to the different methionine of the translation initiation sites (Germain-Lee et al., 1997). These isoforms of *NVL* have been reported to exist in different locations in the nucleolus by previous studies (Nagahama et al., 2004). The NVL1 exists in the nucleolar while NVL2 is present mainly in the nucleolus as the major form. Previous studies have proved that it is NVL2 rather than NVL1 that is involved in ribosome biogenesis (Nagahama et al., 2006). Moreover, NVL2 was found to participate in the biogenesis of the 60S ribosome subunit by associating specifically with ribosome protein L5 (Nagahama et al., 2004) and modulating the function of DOB1 (a human homologue of yeast Dob1p/Mtr4p) (Nagahama et al., 2006). In addition to its role in ribosome biogenesis, it is also required for telomerase assembly and the regulation of telomerase activity (Her and Chung, 2012). Therefore, NVL2 was supposed to play a new role in modulating telomerase activity in cancer and psychiatric disorders.

A genome-wide association mega-analysis performed by MDDWG (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium) towards MDD suggested a possible association of rs11579964 within *NVL* ($P = 1.0E-07$) in Europeans, although it did not reach the genome-wide significance level (Ripke et al., 2012). Interestingly, this result coincides with the fact that *NVL* may regulate the telomerase activity, which is reported to be associated with mood disorders and SCZ (Porton et al., 2008). To validate the presumed impacts of altered activity of telomerase on mental illness, and to verify the potential association of *NVL* with MDD and the shared risk for both MDD and SCZ, we included nine SNPs for genotyping which were screened for a good coverage of this region by using DNA samples of 3515 individuals of Han Chinese origin (1045 MDD cases, 1235 SCZ cases and 1235 normal controls).

2. Materials and methods

2.1. Subjects

The sample set consists of 1045 unrelated MDD cases (729 males and 316 females), 1235 unrelated SCZ cases (805 males and 430 females) and 1235 normal controls (665 males and 570 females) recruited from Han Chinese population in Shanghai China. The mean age is (mean age ± S.D., 34.4 ± 12.1), (mean age ± S.D., 36.4 ± 9.0) and (mean age ± S.D., 30.4 ± 11.4), corresponding to MDD cases, SCZ cases and normal controls, respectively. All included cases of MDD and SCZ were outpatients or stable in-patients. The process of interview and diagnosis was strictly done according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, the fourth edition) criteria based on SCID-I (Structured Clinical Interview for DSM-IV Axis I Disorders) by two independent psychiatrists. The selection of MDD cases was based on the fact that all of the patients have suffered at least two distinct MDD episodes and with no signs of manic, and the SCZ cases were all paranoid schizophrenics with no presence of depressive symptoms and never had suffered from the episode of mania. The normal controls were selected from the general public of the Han Chinese population in Shanghai. Volunteers participated in the evaluation of medical history through a written questionnaire with auxiliary questions like psychosis or other major complex diseases. Physical examinations concerning weight, height, blood pressure and face to face interviews were conducted before the process of blood collection. The study was scrutinized and approved by the local ethical committee with all informed consent being accessible to subjects.

2.2. DNA extraction, SNP selection and genotyping

QuickGene DNA whole blood kit L (FUJIFILM) protocol was used to execute the genomic DNA extraction from peripheral blood samples. Nine SNPs (rs12565875, rs16846649, rs4653581, rs6664230, rs10916544, rs10799541, rs10799551, rs10916583, rs3767732) were selected using tag SNPs selecting strategies performed by online software: Tagger (http://www.broadinstitute.org/mpg/tagger/server.html). We chose 9 SNPs in total and the coverage was 89% (de Bakker et al., 2005, 2006). Subsequently, rs12565875 was excluded from further analysis due to its failure to meet Hardy–Weinberg equilibrium both in cases and controls. Table 1 shows the detailed information of the nine SNPs, while Fig. 1 demonstrates relative positions in gene *NVL* of each SNP being selected. The genotyping was performed with TaqMan SNP Genotyping Assays on the Fluidigm EP1 platform. All probes and primers were designed by the Assay-by-Design™ or Assay-on-Demand™ service of Life Technologies. SNPs are determined by the genotype calls of each sample with a call rate better than 95.0%.

2.3. Statistical analysis

Hardy–Weinberg equilibrium analysis, allele and genotype frequencies, association tests, linkage disequilibrium and combined association analysis of cases of MDD and SCZ were carried out through online