Progress in Neuro-Psychopharmacology & Biological Psychiatry 44 (2013) 226–232

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Association study between genes in Reelin signaling pathway and autism identifies *DAB1* as a susceptibility gene in a Chinese Han population

Jun Li [a,b], Jing Liu [a,b], Linnan Zhao [a,b], Yuanlin Ma [a,b], Meixiang Jia [a,b], Tianlan Lu [a,b], Yanyan Ruan [a,b], Qizhai Li [c], Weihua Yue [a,b], Dai Zhang [a,b,d,*], Lifang Wang [a,b,*]

[a] *Key Laboratory of Mental Health, Ministry of Health (Peking University), Beijing, PR China*
[b] *Institute of Mental Health, Peking University, Beijing, PR China*
[c] *Academy of Mathematics and Systems Science, Chinese Academy of Sciences, Beijing, PR China*
[d] *Peking-Tsinghua Center for Life Sciences, Beijing, PR China*

ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form 26 December 2012
Accepted 5 January 2013
Available online 17 January 2013

Keywords:
Autism
Family-based association test (FBAT)
Pathway
Reelin (*RELN*)

ABSTRACT

Autism is a pervasive neurodevelopmental disorder diagnosed in early childhood. The genetic factors might play an important role in its pathogenesis. Previous studies revealed that Reelin (*RELN*) polymorphisms were associated with autism. However, the roles of genes in Reelin signaling pathway for autism are largely unknown. As several knockout mice models in which the Reelin pathway genes (i.e. *DAB1*, *VLDLR*/*APOER2*, *FYN*/*SRC* and *CRK*/*CRKL*) are deficient have the similar phenotype as the *reeler* mice (*Reelin*$^{-/-}$), we hypothesized that the Reelin signaling pathway genes might play roles in the etiology of autism. Therefore, we conducted a family-based association study. Sixty-two tagged single nucleotide polymorphisms (SNPs) covering 15 genes in Reelin pathway were genotyped in 239 trios, and 14 significant SNPs were further investigated in the additional 188 trios. In the total 427 trios, we found significant genetic association between autism and four SNPs in *DAB1* (rs12035887 G: $p=0.0006$; rs3738556 G: $p=0.0044$; rs1202773 A: $p=0.0048$; rs12740765 T: $p=0.0196$). After the Bonferroni correction, SNP rs12035887 remained significant. Furthermore, the haplotype constructed with rs1202773 and rs12023109 in *DAB1* showed significant excess transmission in both individual and global haplotype analyses ($p=0.0052$ and 0.0279, respectively). Our findings suggested that variations in *DAB1* involved in the Reelin signaling pathway might contribute to genetic susceptibility to autism with Chinese Han decent, supporting the defect in the Reelin signaling pathway as a predisposition factor for autism.

1. Introduction

Autism is a pervasive developmental disorder with childhood onset that is characterized mainly by impairments in social interaction, communication, and repetitive and stereotyped patterns of behaviors or interests (Cooper, 1995) occurring within the first 3 years of life (Lord et al., 2000). The prevalence of the disorder was estimated to be ~1% in the United States of America (Kogan et al., 2009). The male to female ratio of autism was reported 6.58:1 in China (Wong and Hui, 2008). Although the etiology of autism is unknown, family and twin studies have conclusively revealed that autism is a highly heritable neuropsychiatry disorder with the heritability estimated to be more than 90% (Folstein and Rosen-Sheidley, 2001; Folstein and Rutter, 1977; Ronald et al., 2006; Steffenburg et al., 1989). There is compelling evidence that genetic factors are involved in the etiology of autism. However, the involvement of specific genes and variants remains elusive.

Due to the heterogeneous and multigenic nature of autism, it is unlikely that any single SNP or gene would be sufficient to explain the etiology of this disease. Recently, genome-wide association studies (GWASs) followed by targeted replications have been widely used to investigate the genetic susceptibility to autism. Besides, the pathway-based approach, which focuses on evaluating the cumulative contribution of the functionally related genes, may help to pinpoint the associations between a single disease and susceptibility genes within some clearly defined biological pathways (Holmans et al., 2009; Pedroso, 2010).

Abbreviations: DAB1, Dab, reelin signal transducer, homolog 1 (Drosophila); FYN, FYN oncogene related to SRC, FGR, YES; SRC, v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian); CRK, v-crk sarcoma virus CT10 oncogene homolog (avian); CRKL, v-crk sarcoma virus CT10 oncogene homolog (avian)-like; Lis1, lissencephaly-1; Cdk5, cyclin-dependent kinase 5; Cul5, cullin 5; SOCS3, suppressor of cytokine signaling 3; SOCS1, suppressor of cytokine signaling 1; SOCS2, suppressor of cytokine signaling 2; ITGA3, integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor); ITGB1, integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); PCR, polymerase chain reaction; MALDI-TOF, matrix-assisted laser desorption/ ionization time-of-flight.

* Corresponding authors at: Institute of Mental Health, Peking University, 51, Hua Yuan Bei Road, Beijing, 100191, PR China.
E-mail addresses: daizhang@hsc.pku.edu.cn (D. Zhang), lifangwang@bjmu.edu.cn (L. Wang).

0278-5846/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pnpbp.2013.01.004

The human Reelin gene (*RELN*) maps to chromosome 7 (DeSilva et al., 1997). The protein product codes for an extracellular secreted matrix protein (D'Arcangelo et al., 1995, 1997), which is made by Cajal–Retzius neurons in the marginal zones of neocortex and hippocampus and external granule layer neurons of cerebellum (D'Arcangelo et al., 1995; Rice et al., 1998). It serves important functions during brain development (Tissir and Goffinet, 2003). Recent reports implicated that *RELN* is a susceptibility gene in the causation of several neurodevelopmental disorders such as schizophrenia (Fatemi et al., 2000; Guidotti et al., 2000), bipolar disorders (Fatemi et al., 2000; Guidotti et al., 2000), lissencephaly (Hong et al., 2000), and autism (Fatemi, 2005; Fatemi et al., 2001). The reductions of Reelin levels in the cerebellum were associated with autistic subjects. Some of the observed structural and behavioral abnormalities in *reeler* mice are similar to the defects observed in patients affected with autism (Fatemi, 2001; Salinger et al., 2003). These lines of evidence bolster the notion that the Reelin protein might play an important role in the pathology of autism (Fatemi, 2002).

The Reelin signaling pathway is initiated by Reelin, and requires several receptors such as very low-density lipoprotein receptor (VLDLR), apolipoprotein E receptor 2 (ApoER2) (Hiesberger et al., 1999) and α3β1 integrins (Dulabon et al., 2000). Binding of Reelin to these receptors causes the activation of Fyn and Src, two src-family kinases (SFKs) that phosphorylate the adaptor protein Dab1 on specific tyrosine residues (Arnaud et al., 2003; Bock and Herz, 2003; Howell et al., 1999). PhosphoDab1 binds a variety of intracellular proteins including Crk/CrkL (Park and Curran, 2008) and Lis1 (Assadi et al., 2003) for regulating downstream cytoskeleton, and is then targeted for degradation by an E3 ubiquitin ligase containing Cullin5 and Socs proteins (Feng et al., 2007). The homozygous *reeler* mutant mice exhibit many brain histologic abnormalities such as inverted cortical lamination, abnormal positioning of neurons, cerebellar hypoplasia, and aberrant orientation of cell bodies and nerve fibers (Caviness and Sidman, 1973; Goffinet, 1984). The Vldlr/Apoer2 (Trommsdorff et al., 1999), Fyn/Src (Kuo et al., 2005), and Crk/CrkL (Park and Curran, 2008) double deficient, or Dab1 deficient (Howell et al., 1997; Sheldon et al., 1997) mice present brain developmental defects which resemble those in *reeler* mice. The absence of other proteins in the Reelin pathway such as alpha3/beta1 integrins (Schmid et al., 2005), Cdk5 (Ohshima and Mikoshiba, 2002), p35/p39 (Ko et al., 2001), Cul5 (Feng et al., 2007; Simo et al., 2010), Socs proteins (Feng et al., 2007), and Lis1 (Assadi et al., 2003) also leads to the distinctive abnormalities in neuron migration during cortical development. Therefore, we speculated that the deficiency of genes in Reelin signaling pathway would lead to autism-like phenotype which is similar to *RELN* deletion.

Many researches focused on the association between *RELN* and autism (Bonora et al., 2003; Dutta et al., 2008; He et al., 2011; Holt et al., 2010; Li et al., 2008; Serajee et al., 2006; Skaar et al., 2005). However, the roles of other genes in Reelin signaling pathway for autism are rarely known. To investigate the association between genes in Reelin signaling pathway and autism, we performed a family-based associated study in Chinese Han population.

2. Methods

2.1. Subjects

Two hundred and thirty nine Chinese Han family trios (singleton autistic disorder patients and their unaffected biological parents) were recruited for the present study at the Institute of Mental Health, Peking University, China. Of the 239 autistic child probands, 226 were male and 13 were female. The mean age of the children at the time of testing was 7.5 years. To further determine the association between *DAB1*, *FYN*, *CDK5*, *SOCS3* and autism, we expanded our sample to 427 autism trios (1281 individuals) by adding another 188 male autism trios (median age of autism probands was 6.0 years).

The assessments of autism were established by two senior psychiatrists. All patients fulfilled the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) criteria for autistic disorder. To assess the cases, childhood autism rating scale (CARS) (Schopler et al., 1980) and autism behavior checklist (ABC) (Krug et al., 1980) were used. Children with phenylketonuria, fragile X syndrome, or tuberous sclerosis, and previously identified chromosomal abnormalities were excluded. To decrease the heterogeneity of the cases, children affected with Asperger disorder and Rett syndrome were excluded in our study. Parents of all the children with autism were also interviewed by experienced psychiatrists. During the interview, psychiatrists inquired the developmental history of the parent subjects and briefly assessed their social ability and communicative language ability. None of the parents met the diagnostic criteria of autism according to the DSM-IV. All subjects provided written informed consent in this study. Written informed consents for children were obtained from their legal guardians. This study was approved by the Ethics Committee of the Institute of Mental Health, Peking University.

2.2. Genotyping

We selected 18 candidate genes (*RELN*, *VLDLR*, *APOER2*, *ITGA3*, *ITGB1*, *DAB1*, *SRC*, *FYN*, *CDK5*, *CDK5R1 (p35)*, *CDK5R2 (p39)*, *CUL5*, *SOCS1*, *SOCS2*, *SOCS3*, *LIS1*, *CRK*, *CRKL*) from the Reelin signaling pathway (Table 1). A list of relative nuclear and specific pathway genes reflects various aspects of Reelin signaling, including ligands, receptors, adaptors, and interactors that were reported before (Assadi et al., 2003; Bock and Herz, 2003; Dulabon et al., 2000; Fatemi et al., 2005; Feng et al., 2007; Hiesberger et al., 1999; Howell et al., 1997; Ko et al., 2001; Kuo et al., 2005; Ohshima and Mikoshiba, 2002; Park and Curran, 2008; Rice and Curran, 2001; Schmid et al., 2005; Sheldon et al., 1997; Simo et al., 2010; Trommsdorff et al., 1999). The core process and functional interactions among the genes in Reelin signaling pathway were depicted in Fig. 1.

The tagging SNPs were selected according to the dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and the HapMap phase I and phase II Chinese Han in Beijing (CHB) genotype dataset (http://hapmap.ncbi.nlm.nih.gov/). SNPs with minor allele frequency (MAF) > 0.05 were selected. Moreover, pair-wise tagging in the Tagger module implemented in Haploview version 4.1 program was used to select SNPs that could capture the common genetic variation across each gene ($r^2 > 0.8$). Meanwhile, the physical position of SNPs and the previous positive results (rs736707 and rs362691) were considered too. SNPs in *CDK5R1* (p35), *CDK5R2* (p39) and *SOCS1* failed to be selected according to this strategy.

Genomic DNA was extracted from the blood using a Qiagen QIAamp DNA Mini Kit. A total of 62 SNPs were originally selected for genotyping. Fifty-eight SNPs were successfully genotyped using the Sequenom (http://www.sequenom.com/) genotyping platform with standard protocols, which uses the MALDI-TOF primer extension assay (Jurinke et al., 2002; Storm et al., 2003). The iplex genotyping assay was employed, which has increased plexing efficiency and flexibility for the MassARRAY system through single base primer extension with mass-modified terminators. The remaining 4 SNPs, which could not be genotyped using the Sequenom system, were analyzed by direct DNA sequencing (rs4969168, rs930316, rs362691) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis (rs736707). The information of primers and PCR-RFLP analysis was given in Table S1. The PCR amplification was performed in a 25 μL volume containing GC Buffer I (TaKaRa), 200 μM of each dNTPs, 0.3 μM of each primer, 1 unit of Taq DNA polymerase, and 40 ng of the genomic DNA. The conditions used for PCR amplification were an initial denaturation phase at 94 °C for 5 min, followed by 38 cycles at 94 °C for 30 s, annealing at 53–65 °C for 30–45 s, and extension at 72 °C for 30 s, followed by a final extension phase at 72 °C for 7 min. A 15 μL aliquot of the PCR product mixtures was completely digested with 4 units of