GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

No association between *FGD1* gene polymorphisms and intellectual developmental disability in the Qinba mountain area

J.L. Li[1]*, Y.J. Li[1]*, K.J. Zhang[1], L. Lan[2], J.G. Shi[3], X. Yang[1], M.J. Zhang[1], F.C. Zhang[1] and X.C. Gao[1]

[1]Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Institute of Population and Health, College of Life Science, Northwest University, Xian, Shaanxi, China
[2]Department of Pediatrics, Tangdu Hospital, The Fourth Military Medical University, Xian, Shaanxi, China
[3]Xian Institute of Mental Health, Xian, Shaanxi, China

*These authors contributed equally to this study.
Corresponding author: X.C. Gao
E-mail: gaoxc@nwu.edu.cn

Genet. Mol. Res. 13 (1): 127-133 (2014)
Received November 21, 2012
Accepted April 15, 2013
Published January 10, 2014
DOI http://dx.doi.org/10.4238/2014.January.10.3

ABSTRACT. *FGD1* encoding a guanine nucleotide exchange factor, specifically activates Rho GTPase cell division cycle 42 (Cdc42). Dysfunction of FGD1 causes Aarskog-Scott syndrome (MIM #305400), an X-linked disorder that may affect bone and intellectual development. However, the relationship between *FGD1* and intellectual developmental disorders (IDD) remains unclear. The purpose of this study was to investigate the genetic association between the *FGD1* polymorphism and IDD. Working with families from the Qinba mountain area where the occurrence of IDD is higher than the average in China, we analyzed 456 samples from 130 nuclear families, effectively controlling for stratification and environmental factors. Five SNP loci (rs2230265, rs7881608, rs2239809, rs6614244, and

rs2284710) were selected that were well distributed within the *FGD1* gene. Genotyping was performed through single-strand conformation polymorphism and restriction fragment length polymorphism. The data were analyzed with transmission disequilibrium tests. In the Qinba mountain area, no significant association was observed between IDD and allele or genotype frequencies, or the haplotype of the 5 SNP loci of the *FGD1* gene. The results indicate that *FGD1* may not be a monogenetic X-linked factor in IDD. Further studies are required to investigate its role in intellectual development based on its specific interactions with Cdc42 or other partner proteins contributing to IDD.

Key words: *FGD1*; Restriction fragment length polymorphism; Intellectual developmental disorders; Single nucleotide polymorphism; Single-strand conformation polymorphism

INTRODUCTION

Various aspects of learning and memory are critically affected by synaptic plasticity (van Bokhoven, 2011), abnormality of which generally results in impaired intellectual development (Nimchinsky et al., 2002; Newey et al., 2005). Development of dendritic structures and dendritic spines is regulated by Rho guanine nucleotide exchange factors (RhoGEFs) and Rho proteins, like Cdc42, RhoA, and Rac. RhoGEFs and the Rho family of proteins are expressed in a wide range of tissues, including in brain. However, the roles of RhoGEFs and Rho proteins during mammalian development remain to be determined. In cellular events, RhoGEFs and Rho proteins regulate diverse cellular processes like organization of the actin cytoskeleton, polarization, proliferation, and differentiation.

In its diverse cellular roles, FGD1 may function in transcriptional and translational control, and in differentiation of neural and supporting cells of the nervous system. Alterations of several genes in the Rho signaling pathway have been shown to contribute to intellectual developmental disorders (IDD) (Crespi et al., 2010), such as *oligophrenin-1* (Nadif Kasri et al., 2009; Pirozzi et al., 2011), *ARHGEF6* (Node-Langlois et al., 2006; Ramakers et al., 2012), *PAK3* (Node-Langlois et al., 2006), *IL1RAP1* (Franek et al., 2011; Valnegri et al., 2011), *TM4SF2* (Gomot et al., 2002; Noor et al., 2009), *RSK2* (Jurkiewicz et al., 2010; Tejada et al., 2011), and *FGD1* (Lebel et al., 2002; Kleefstra and Hamel, 2005; Dierssen and Ramakers, 2006; Kaname et al., 2006). As reported previously, abnormalities in the *FGD1* gene may lead to faciogenital dysplasia and X-linked IDD (Pasteris and Gorski, 1999). *FGD1* encodes a RhoGEF that contains Dbl (DH) and pleckstrin (PH) homology domains, specifically binding to the Rho family GTPase cell division cycle 42 (Cdc42). GDP-GTP exchange of the isoprenylated form of Cdc42 can be stimulated by FGD1 (Hou et al., 2003), activating the c-Jun N-terminal kinase signaling cascade, and then regulating cell growth and differentiation. Although *FGD1* mutations are primarily associated with Aarskog-Scott syndrome (Pasteris et al., 1994), the relationship between *FGD1* and the accompanying phenotype of intelligence development is inconsistent with the mutant genotype: A missense mutation (P312L) in the *FGD1* gene is identified as being related to non-syndromic X-linked intellectual disorders (Lebel et al., 2002). Subsequently, the results from 46 independent Aarskog-Scott syndrome

male probands from different countries show that individuals with AAS are mildly affected, experiencing learning and behavioral disabilities in early childhood (Orrico et al., 2004). Another mutation (R408Q) is the cause of attention-deficit/hyperactivity disorder (Orrico et al., 2005), which is a characteristic of IDD (Melnyk and Das, 1992). However, subjects from a large Dutch family with a mutation (R402W) at position 1204 (1204C>T) exhibit a wide spectrum of intelligence levels (Verhoeven et al., 2012).

IDD is a common intellectual and neurological disorder, characterized by an intelligence quotient (IQ) significantly lower than 70, and deficiencies in cognition and social adaption. IDD affects 2-3% of the human population. Genetic defects account for about half of IDD cases. The Qinba mountain area, which is located in Shaanxi Province, northwest China, is isolated because of poor economy and lack of transportation with an altitude from 750 to 1500 m. There the prevalence of IDD (2.78%) is higher than the average in China (1.07%), and heritability is as high as 70.23% (Li et al., 1999). The present study aimed to investigate the relationship between genetic polymorphisms of *FGD1* and IDD in this area using a family-based population analysis.

MATERIAL AND METHODS

Participants

All subjects were recruited from the Qinba mountain area. Those with chromosomal and metabolic abnormality were excluded after investigation of medical history, physical examination, and the necessary clinical examination by experienced psychologists and pediatricians. Pedigree investigation and genetic sampling were carried out on IDD child and their first-degree relatives. In total, 152 children of probands (out of initially examined 7487) and their parents (304 adults) were recruited from 130 nuclear families, based upon household registration on cards. All participants gave informed consent prior to participating in this research. This project was approved by the local Ethics Committee of the National Human Genome Center.

Cognitive testing

Accurate measurements of intelligence were performed using different scales on children of different ages. Patients 4-5 years old were assessed with the Chinese Wechsler Young Children Scale of Intelligence (Gong and Dai, 1992), and children 6-14 years with the Chinese Wechsler Intelligence Scale for Children (Gong and Cai, 1993). Social adaptive behavior was evaluated with the Adaptive Scale for Infants and Children, revised by Zuo (1988). The patients were determined to have an IQ lower than 70 and social disability scores lower than 8. The adult participants were evaluated with the Chinese Classification of Mental Disorders, 2nd Revision (CCMD-2-R) (Chen, 2002).

Genotyping

Genomic DNA was extracted from whole blood (1 mL) using standard phenol/chloroform extraction methods. According to information from the National Center for Biology and

Ensemble Genome Browser, 5 loci were selected covering *FGD1* using the following criteria: The locus was near or in a functional region with a >0.1 minor allele frequency among Chinese people. Primers were designed with Primer Premier 5.0. The loci, sequences, and amplification conditions are listed in Table 1. The haplotypes of 4 loci, including rs2230265, rs7881608, rs2239809, and rs6614244 were detected by single-strand conformational polymorphism-SNP, and that of rs2284710 was analyzed using restriction fragment length polymorphism.

Table 1. Primers' sequences and PCR conditions of 5 SNP loci in *FGD1*.

SNPs	Primers	Conditions	Size (bp)	Allele
rs2230265	F: 5'-GAGGACAGGGAGATGGAGG-3' R: 5'-TGACTGAGCTGGGAGGGA-3'	94°C-30 s, 98°C-5 min, 61°C-15 s, 72°C-30 s, 30 cycles, 72°C-5 min	177	C/T
rs7881608	F: 5'-ACTTGGGCTGGGGAGAAC-3' R: 5'-AACTGAGCACCCTAGATTA-3'	94°C-30 s, 95°C-5 min, 53°C-30 s, 72°C-30 s, 30 cycles, 72°C-5 min	218	A/G
rs2239809	F: 5'-TCCACCATCACGCCCACT-3' R: 5'-TCTCCTGACTATCCCTTCCTG-3'	94°C-30 s, 95°C-5 min, 58.5°C-10 s, 72°C-30 s, 30 cycles, 72°C-5 min	183	C/T
rs6614244	F: 5'-GTATGTGACTATTTAGGAGGAG-3' R: 5'-AAGGGACTTGAGTGTTGG-3'	94°C-30 s, 95°C-5 min, 55.8°C-15 s, 72°C-30 s, 30 cycles, 72°C-5 min	195	A/G
rs2284710	F: 5'-TCTCAGGGTCTTAGTTTCC-3' R: 5'-ACTGTCTTGTTGCCTACC-3'	94°C-30 s, 95°C-5 min, 55.8°C-10 s, 72°C-30 s, 30 cycles, 72°C-5 min	207	A/G

F = forward primer; R = reverse primer; SNPs = single nucleotide polymorphisms.

Statistical analysis

Allele and genotype frequencies were calculated with the SPSS 17.0 software. The haplotype was analyzed with the Haploview 4.2 software. Transmission disequilibrium tests (TDT) were performed on the 130 families. Because the *FGD1* gene is on the X chromosome, instead of the Y chromosome, the heterozygous mothers were counted in the statistical analysis in TDT, which were completed with UNPHASED version 3.1.3.

RESULTS

The 5 SNP loci, distributing throughout the *FGD1* gene, were selected to study the association between the *FGD1* gene and IDD. There was no deviation from Hardy-Weinberg equilibrium ($P > 0.05$) in the genotyping frequencies. As for the TDT performed on the 130 nuclear families without genetic relationship, the results showed that the P values of the 5 SNP loci were greater than 0.05 with the heterozygous mothers included (Table 2). Because all 5 SNPs (rs2230265, rs7881608, rs2239809, rs6614244, and rs2284710) were in strong linkage disequilibrium ($D' > 0.80$, $r^2 > 0.45$), a haplotype block was constructed. The haplotype analysis showed no significant difference in haplotype frequency between different groups (Table 3).

Table 2. Transmission disequilibrium tests of single locus.

SNP	Allele	Transmitted	Not transmitted	Chi-square	P
rs2230265	T	36	29	0.754	0.385
rs7881608	G	40	27	2.522	0.112
rs2239809	C	37	24	2.77	0.096
rs6614244	G	35	27	1.032	0.310
rs2284710	A	31	29	0.067	0.796

Table 3. Transmission disequilibrium tests of haplotype.

	Frequency[a]	T:U	Chi-square	P
TAAAA	0.404	35:22	2.98	0.084
CATGG	0.341	27:25	0.043	0.835
CGTAG	0.055	7:8	0.084	0.773

[a]Frequecies of two alleles among all combinations. The frequencies of these haplotypes are >0.05.

DISCUSSION

The association between the *FGD1* gene and the complex disease, IDD, was investigated in a population of 456 people from 130 nuclear families in the Qinba mountain area, using a family-based method to avoid the potential confounding effects of population stratification. To the best of our knowledge, this is the first time it has been shown that there is no association between *FGD1* and IDD.

During embryogenesis, FGD1 participates in membrane transportation and actin cytoskeleton rearrangement, affects brain and skeletal formation and remodeling. When it comes to brain development, mutation in *FGD1* perturbs neuronal polarity and vesicular trafficking related to multiple aspects of neuronal development via signaling by Rho GTPases, and may lead to brain malformation (Bottani et al., 2007). Mutations in *FGD1* are related to Aarskog-Scott syndrome, which gives rise to severe speech and cognitive deficiencies, neuropsychiatric disorders, and behavioral and learning problems (Egorov et al., 2009). To date, 27 point and 2 deletion mutations have been identified in the *FGD1* gene (Orrico et al., 2007, 2010). After screening DNA from affected males, C934T, a mutation of *FGD1* was reported to be responsible for Aarskog-Scott syndrome. The mutation replaced proline with leucine, destroying secondary structure. The three affected male patients presented developmental delay, characterized by severe speech and cognitive deficiencies, while the mother showed normal developmental milestones and psychosocial adjustment, a result of random inactivation of the two X chromosomes (Lebel et al., 2002). Orrico et al. (2005) also suggested that mutation of *FGD1* might be implicated in patients with attention-deficit/hyperactivity disorder, and lower IQ due to inattentive, hyperactive, and impulsive conduct. However, the association between *FGD1* and IDD is uncertain. In this study, the association between the *FGD1* gene and IDD was studied with a family-based method based upon 130 nuclear families. The results of single-locus association were negative. Because these five loci were in strong linkage disequilibrium, a haplotype analysis was conducted. However, no association was observed in individuals or global haplotype frequencies. According to our results, the *FGD1* gene may not be significantly associated with the IDD population in the Qinba mountain area.

Comparing the data we obtained with previous reports, the following might explain our results. 1) The study recruits are from a different population to that used by other researchers. The Qinba mountain region is located at the junction of 5 provinces in central and western China, including Shaanxi, Sichuan, Gansu, Hubei, and Henan Provinces. This is one of the main poverty-stricken areas in China, and people living there are isolated for high altitude and limited access. The resources available for genetic research on family linkage/association in this closed population may eliminate differences in genetic background, and avoid false-positive results and population stratification. Thus, genes other than *FGD1* may be the causes of IDD in this isolated area. 2) As a complex disorder, occurrence of IDD may

involve a variety of abnormalities in signaling pathways or in those genes that affect neural or intellectual development. Moreover, abnormalities in dendritic structures and dendritic spine morphologies are the most consistent correlates with IDD, including genetic syndromes associated with IDD, and unclassified IDD. As reported previously, abnormalities in the *FGD1* gene may lead to faciogenital dysplasia and markedly variable levels of intelligence (Kaname et al., 2006). Nevertheless, low IQ might be due to inattentive, hyperactive, and impulsive conduct, which may be affected by more than the *FGD1* gene. *FGD1* may not be a monogenetic X-linked factor in IDD, rather it may be involved in intellectual development based on its specific interactions with Cdc42 or other partner proteins. Alternatively, *FGD1* may not be significantly involved in the genetics of IDD.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We are thankful for the efforts of the members of the Institute of Population and Health and would like to thank all the participants and researchers who took part in this study. Research supported by the National Natural Science Foundation of China (#31371327) and the Scientific Research Program Funded by Shaanxi Provincial Education Department (#JH11241).

REFERENCES

Bottani A, Orrico A, Galli L, Karam O, et al. (2007). Unilateral focal polymicrogyria in a patient with classical Aarskog-Scott syndrome due to a novel missense mutation in an evolutionary conserved RhoGEF domain of the faciogenital dysplasia gene FGD1. *Am. J. Med. Genet. A* 143 A: 2334-2338.

Chen YF (2002). Chinese classification of mental disorders (CCMD-3): towards integration in international classification. *Psychopathology* 35: 171-175.

Crespi B, Summers K and Dorus S (2010). Evolutionary genomics of human intellectual disability. *Evol. Appl.* 3: 52-63.

Dierssen M and Ramakers GJ (2006). Dendritic pathology in mental retardation: from molecular genetics to neurobiology. *Genes Brain Behav.* 5 (Suppl 2): 48-60.

Egorov MV, Capestrano M, Vorontsova OA, Di Pentima A, et al. (2009). Faciogenital dysplasia protein (FGD1) regulates export of cargo proteins from the Golgi complex via Cdc42 activation. *Mol. Biol. Cell* 20: 2413-2427.

Franek KJ, Butler J, Johnson J, Simensen R, et al. (2011). Deletion of the immunoglobulin domain of IL1RAPL1 results in nonsyndromic X-linked intellectual disability associated with behavioral problems and mild dysmorphism. *Am. J. Med. Genet. A* 155A: 1109-1114.

Gomot M, Ronce N, Dessay S, Zemni R, et al. (2002). TM4SF2 gene involvement reconsidered in an XLMR family after neuropsychological assessment. *Am. J. Med. Genet.* 112: 400-404.

Gong YX and Dai XY (1992). Chinese-Wechsler Young Children Scale of Intelligence (C-WYCSI). Hunan Map Press, Changsha, 59-128.

Gong YX and Cai TS (1993). Wechsler Intelligence Scale for Children Chinese revision (C-WISC). Hunan Map Press, Changsha.

Hou P, Estrada L, Kinley AW, Parsons JT, et al. (2003). Fgd1, the Cdc42 GEF responsible for faciogenital dysplasia, directly interacts with cortactin and mAbp1 to modulate cell shape. *Hum Mol. Genet.* 12: 1981-1993.

Jurkiewicz D, Jezela-Stanek A, Ciara E, Piekutowska-Abramczuk D, et al. (2010). Four novel RSK2 mutations in females with Coffin-Lowry syndrome. *Eur. J. Med. Genet.* 53: 268-273.

Kaname T, Yanagi K, Okamoto N and Naritomi K (2006). Neurobehavioral disorders in patients with Aarskog-Scott syndrome affected by novel FGD1 mutations. *Am. J. Med. Genet. A* 140: 1331-1332.

Kleefstra T and Hamel BC (2005). X-linked mental retardation: further lumping, splitting and emerging phenotypes. *Clin. Genet.* 67: 451-467.

Lebel RR, May M, Pouls S, Lubs HA, et al. (2002). Non-syndromic X-linked mental retardation associated with a missense mutation (P312L) in the FGD1 gene. *Clin. Genet.* 61: 139-145.

Li F, Yan FT, Han Z, Yang WF, et al. (1999). A study of the relationship between health care during labour and children's mental retardation in Qin-Ba mountain area, Shaanxi province. *J. Xi'an Med. Univ.* 20: 100-2.

Melnyk L and Das JP (1992). Measurement of attention deficit: correspondence between rating scales and tests of sustained and selective attention. *Am. J. Ment. Retard.* 96: 599-606.

Nadif Kasri N, Nakano-Kobayashi A, Malinow R, Li B, et al. (2009). The Rho-linked mental retardation protein oligophrenin-1 controls synapse maturation and plasticity by stabilizing AMPA receptors. *Genes. Dev.* 23: 1289-1302.

Newey SE, Velamoor V, Govek EE and Van Aelst L (2005). Rho GTPases, dendritic structure, and mental retardation. *J. Neurobiol.* 64: 58-74.

Nimchinsky EA, Sabatini BL and Svoboda K (2002). Structure and function of dendritic spines. *Annu. Rev. Physiol.* 64: 313-353.

Node-Langlois R, Muller D and Boda B (2006). Sequential implication of the mental retardation proteins ARHGEF6 and PAK3 in spine morphogenesis. *J. Cell Sci.* 119: 4986-4993.

Noor A, Gianakopoulos PJ, Fernandez B, Marshall CR, et al. (2009). Copy number variation analysis and sequencing of the X-linked mental retardation gene TSPAN7/TM4SF2 in patients with autism spectrum disorder. *Psychiatr. Genet.* 19: 154-155.

Orrico A, Galli L, Cavaliere ML, Garavelli L, et al. (2004). Phenotypic and molecular characterisation of the Aarskog-Scott syndrome: a survey of the clinical variability in light of FGD1 mutation analysis in 46 patients. *Eur. J. Hum Genet.* 12: 16-23.

Orrico A, Galli L, Buoni S, Hayek G, et al. (2005). Attention-deficit/hyperactivity disorder (ADHD) and variable clinical expression of Aarskog-Scott syndrome due to a novel FGD1 gene mutation (R408Q). *Am. J. Med. Genet. A* 135: 99-102.

Orrico A, Galli L, Obregon MG, de Castro Perez MF, et al. (2007). Unusually severe expression of craniofacial features in Aarskog-Scott syndrome due to a novel truncating mutation of the FGD1 gene. *Am. J. Med. Genet. A* 143: 58-63.

Orrico A, Galli L, Faivre L, Clayton-Smith J, et al. (2010). Aarskog-Scott syndrome: clinical update and report of nine novel mutations of the FGD1 gene. *Am. J. Med. Genet. A* 152A: 313-318.

Pasteris NG and Gorski JL (1999). Isolation, characterization, and mapping of the mouse and human Fgd2 genes, faciogenital dysplasia (FGD1; Aarskog syndrome) gene homologues. *Genomics* 60: 57-66.

Pasteris NG, Cadle A, Logie LJ, Porteous ME, et al. (1994). Isolation and characterization of the faciogenital dysplasia (Aarskog-Scott syndrome) gene: a putative Rho/Rac guanine nucleotide exchange factor. *Cell* 79: 669-678.

Pirozzi F, Di Raimo FR, Zanni G, Bertini E, et al. (2011). Insertion of 16 amino acids in the BAR domain of the oligophrenin 1 protein causes mental retardation and cerebellar hypoplasia in an Italian family. *Hum. Mutat.* 32: E2294-E2307.

Ramakers GJ, Wolfer D, Rosenberger G, Kuchenbecker K, et al. (2012). Dysregulation of Rho GTPases in the alphaPix/Arhgef6 mouse model of X-linked intellectual disability is paralleled by impaired structural and synaptic plasticity and cognitive deficits. *Hum. Mol. Genet.* 21: 268-286.

Tejada MI, Martinez-Bouzas C, Garcia-Ribes A, Larrucea S, et al. (2011). A child with mild X-linked intellectual disability and a microduplication at Xp22.12 including RPS6KA3. *Pediatrics* 128: e1029-e1033.

Valnegri P, Montrasio C, Brambilla D, Ko J, et al. (2011). The X-linked intellectual disability protein IL1RAPL1 regulates excitatory synapse formation by binding PTPdelta and RhoGAP2. *Hum. Mol. Genet.* 20: 4797-4809.

van Bokhoven H (2011). Genetic and epigenetic networks in intellectual disabilities. *Annu. Rev. Genet.* 45: 81-104.

Verhoeven WM, Egger JI and Hoogeboom AJ (2012). X-linked Aarskog syndrome: report on a novel FGD1 gene mutation. Executive dysfunction as part of the behavioural phenotype. *Genet. Couns.* 23: 157-167.

Zuo QH (1988). Adaptive Scale of Infant and Children. Medical University of China, Beijing.