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Variants in the *RAB3A* gene are not associated with mental retardation in the Chinese population

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Abstract

Mental retardation is a common form of cognitive impairment among children. The underlying causes of mental retardation are extremely heterogeneous, and include significant genetic factors. The coexistence of neuropathology and cognitive deficits supports the view that mental retardation is a disorder of brain development and plasticity. Rab3A, a member of the Rab small G protein family, is a key molecule in modulating basal neurotransmission and contributes to synaptic plasticity. The *RAB3A* gene is located on chromosome 19p13.11, near a region shown by a linkage study to be involved in the etiology of mental retardation. Because of both its function and chromosomal location, *RAB3A* is a potential candidate susceptibility gene for mental retardation. To investigate the possible genetic contribution of the *RAB3A* gene, we performed a case-control association study focused on the Han population of northwestern China using four common SNPs in the gene (rs7259012, rs17683539, rs2271882, and rs874628). Pairwise linkage disequilibrium analysis showed that the four SNPs were in linkage disequilibrium. However, there were no significant differences of either allele or genotype frequencies at any of the SNPs nor any significant differences in haplotype distributions between cases and controls. In conclusion, we have found no evidence for *RAB3A* conferring susceptibility on mental retardation in the Han Chinese population.

Keywords: Mental retardation; *RAB3A*; Genetic polymorphisms; Association study; Chinese population

Mental retardation (MR) is a common form of non-progressive cognitive impairment which initially occurs during childhood and which is characterized by an inability to cope with everyday life. MR affects 0.28–1.2% of children in different countries and regions [17]. The underlying causes of MR are established in less than half of all cases, but appear, to a significant extent, to be genetic in nature. The coexistence of neuropathology and cognitive deficits supports the view that MR is a disorder of brain development and plasticity [21].

The synaptic plasticity in the brain is believed to underlie learning, memory, cognition and behavior. Neurotransmitter release plays critical roles in synaptic plasticity [24]. Rab3A is a key molecule in modulating the levels of neurotransmitter release in neurons and is the most abundant member among more than 60 different Rab small G proteins in mammals in the brain [27]. It is required for brain-derived neurotrophic factor-induced synaptic plasticity [29]. Studies on Rab3A-deficient mice have revealed an important insight into the Rab3A function. Rab3A is required to maintain a normal reserve of synaptic vesicles and to regulate synaptic vesicle fusion at a late stage [12,13]. Long-term potentiation (LTP) cannot be induced at the mossy fiber synapses of Rab3A-knockout mice, which provides the first link between a protein of the secretory machinery at the synapse and a form of long-term synaptic plasticity [6]. Under the high frequency stimulation, Rab3A-knockout mice exhibit a decrease in the recruitment of synaptic vesicles at the presynaptic membrane. Concomitantly, the secretion response and the

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replenishment of docked vesicles in the brain nerve terminals are also impaired after exhaustive stimulation [16].

The *RAB3A* gene is located on chromosome 19p13.11, near a region which suggested by a linkage study is involved in the etiology of MR [3]. In the linkage study the authors evaluated clinically and genetically four related consanguineous Israeli Arab families with autosomal recessive non-syndromic MR. All affected participants were mild psychomotor developmental delay during early childhood, had speech difficulties and were mentally retarded. There were no autistic features or seizures. These phenotypes are consistent with what our samples exhibit except that there are few patients with hearing impairment in our samples. On the 5′ end of *RAB3A*, only about 3.7 kb upstream, is the 'dunce-like' cAMP-specific nucleotide phosphodiesterase gene *PDE4C* which shares sequence homology with the Drosophila dunce gene [28]. The 'dunce' gene is one of several genes critical to normal learning and memory in Drosophila [19].

Because of both its function and map location, *RAB3A* is a potential candidate susceptibility gene for MR. In this work, we performed a case-control association study of MR, using four individual markers and their constituted haplotypes covering the whole *RAB3A* gene and using samples from northwestern China.

The study included three groups, 338 controls (average age 10.1 ± 2.9 years; 162 females, 176 males), 93 subjects with MR (average age 10.6 ± 2.7 years; 45 females, 48 males), and 121 subjects with a borderline form of MR (Border; average age 10.8 ± 2.9 years; 64 females, 57 males). All subjects were identified and recruited from Zha Shui and An Kang counties in the Qin-Ba mountain region of Shaanxi province, northwestern China, where the prevalence of MR (2.78%) is higher than in most other areas of China (1.07%) [18]. Moreover, familial clustering was found in the two counties with several families having multiple affected members in one or more generations. It is possible that genetic factors may interact with a mountainous environment to determine the overall risk of MR.

Participants were screened to obtain social disability (SD) scores using the Adaptive Scale for Infants and Children [37]. Children from 3 to 5 years old were tested using the Chinese-Wechsler Young Children Scale of Intelligence (C-WYCSI) [15]. Those from 6 to 16 years old were tested using the Chinese-Wechsler Intelligence Scale for Children (C-WISC) [14]. We defined IQs of less than 70 accompanied by social disability scores of 8 or less as MR, IQs 70–79 and SD scores of 9 as a Borderline form of MR (Border). Among the patients with IQ < 70, we classified them into four subtypes: Mild MR, IQ scores from 69 to 50; Moderate MR, IQ scores from 49 to 35; Severe MR, IQ scores from 34 to 20 and Profound MR, IQ scores below 20, according to Chinese Classification of Mental Disorders 2nd revision (CCMD-2-R) and the classification of mental and behavioral disorders from the World Health Organization [22,30]. A neurological examination, conducted by physicians, included tests of hearing, vision, voice and speech, reflexes, posture and gait. Cases of MR affected by trachoma, infection, trauma, toxicity, cerebral palsy, or birth complications were excluded from further study. Controls came from the same areas and were selected from families with no history of MR. Blood samples were taken for routine hematology, serology, and DNA analysis.

All subjects gave standard informed consent to the protocol which was reviewed and approved by the Ethical Committee of the National Human Genome Center. All subjects were Han Chinese in origin.

RAB3A contains 5 exons and spans 7.9 kb in the human genome. Four SNPs (rs7259012, rs17683539, rs2271882, and rs874628) with minor allele frequency over 5% were selected from the dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). rs17683539 is in intron 1 of *RAB3A*, and rs2271882 is in intron 3. These two SNPs are separated by 3.2 kb. rs7259012 is located in the 3′UTR of *PDE4C* and is 7.7 kb upstream of the initiation codon of *RAB3A*. rs874628 is 2.9 kb downstream of the 3′UTR of *RAB3A* and is in the exon 2 of *Homo sapiens hypothetical protein MGC12972 (FKSG24)* responsible for the nonsynonymous change of amino acid between valine and methionine.

Genomic DNA was extracted from peripheral blood using a modified phenol/chloroform method. Genotyping was conducted according to our standard protocol [8] apart from the annealing temperature (57 °C) of individual polymerase chain reaction (PCR) condition. The primers were 5′-AGCCTCTTACGATGCCCTGGT-3′ (forward) and 5′-GCTCCTCTTCCTGCCCCCTA-3′ (reverse) for rs7259012; 5′-TAGGACCACGGGCTTCTACCA-3′ (forward) and 5′-TGAACTGGACACTGGGGACACA-3′ (reverse) for rs17683539; 5′-GTCCACCCAGATCAAGACCT ACT-3′ (forward) and 5′-CCCTTCTTCACTACCCACCACT-3′ (reverse) for rs2271882; 5′-CCCGACCTATGAGAGGCAAAG-3′ (forward) and 5′-GGGGCTTGTAGTGGAAGAATG-3′ (reverse) for rs874628.

Hardy–Weinberg equilibrium (HWE) tests were performed for each polymorphism on an online calculator (http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm). The mean age of each group, allele and genotype frequencies of each polymorphism were calculated using SPSS (version 13.0). CLUMP program (version 2.3) [25] was used to compare the discrepancies of allele, genotype and haplotype frequencies between cases and controls. Pairwise linkage disequilibrium (LD) of all possible pairs of the four polymorphisms was estimated using 2LD software [34]. The haplotype frequencies were estimated using EHPLUS software [33,36]. After the estimated frequencies of each haplotype were calculated, CLUMP was used again to compare the difference in haplotype frequencies between cases and controls. Those haplotypes with a frequency under 5% were excluded from the analysis. In this study, the *P*-values were two tailed and significance was accepted when $P < 0.05$. Odds ratios with 95% confidence intervals (CI) were estimated for the effects of high-risk haplotypes and calculated using an internet-based facility (http://www.pedro.fhs.usyd.edu.au/Utilities/CIcalculator.xls). The statistical power of our sample size were estimated using the G*Power program [11].

We analyzed the rs7259012(C/T), rs17683539(A/G), rs2271882(T/C), and rs874628(T/C) spanning *RAB3A* in 338 controls, 93 MR and 121 Borderline MR children. Among the 93 MR children, there were 75 (81%), 11 (12%), 4 (4%) and 3 (3%) mild, moderate, severe and profound MR subjects,