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An association study of the *SLC26A4* gene in children with mental retardation

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ABSTRACT

It is generally considered that iodine deficiency is the single most common cause of preventable mental retardation (MR) and brain damage. The *SLC26A4* gene is expressed at the apical surface of thyrocytes and its product forms an efficient iodide-trapping mechanism. To investigate whether variability in the *SLC26A4* gene influences the risk of iodine-deficiency based MR, we undertook an association study between *SLC26A4* and MR. Participants were recruited from a relatively isolated and traditionally iodine-deficient region with a high prevalence of MR. The SNPs we selected from the dbSNP and HapMap were identified using ARMS-PCR and sequencing methods. Singular-locus and haplotype association analysis indicated no association between the *SLC26A4* gene and MR ($p > 0.05$). The negative results suggest that the *SLC26A4* gene has no measurable impact on iodine-deficiency based MR. In view of the characteristics of our samples, our study may provide a good reference for research into the transport features of pendrin in the thyrocyte apical surface.

Mental retardation (MR) is characterized by significantly sub-average cognitive functioning, commonly defined by an IQ lower than 70, and deficits in adaptive behavior, such as social and daily-living skills [14]. Around the world the overall prevalence of mental retardation is believed to be between 1% and 3%, with the rate for moderate, severe and profound retardation being 0.3% [25] defined in terms of WHO standards [24]. In China, mental retardation is perhaps the most frequent form of mental disorder – as it is in other developing countries (http://www.who.int/director-general/speeches/1999/english/19991111_beijing.html).

The causes underlying MR are extremely heterogeneous and include environmental factors, chromosomal aberrations and single gene mutations [14]. However, today it is generally considered that iodine deficiency is the single most common cause of preventable mental retardation and brain damage [26]. The iodine pathway comprises several steps in thyroid cells that involve an iodide transport mechanism via the sodium/iodide symporter (NIS) [22], iodide organification into the thyroglobulin (Tg) molecule at the apical pole that is mediated by the thyroid peroxidase (TPO), and thyroid hormone synthesis and secretion [2,3]. Any aberration occurring in the iodine pathway may influence the thyroid hormone biosynthesis. In the process of early growth and development of the brain, an insufficient supply of thyroid hormones will result in hypothyroidism and brain damage. The clinical consequence will be mental retardation [1].

The *SLC26A4* gene consists of 21 exons [9] and has been mapped to chromosome 7q31 [5,21]. Its product, commonly referred to as pendrin, is an 82 kDa protein containing 12 putative transmembrane domains. Pendrin belongs to the SLC26A family, which is referred to as a 'multifunctional anion exchanger family' because several of its members are known to transport various anions [6,10,16]. In the thyroid, pendrin is expressed at the apical surface of thyrocytes [19] and mediates Cl^-/I^- exchange at the apical membrane transporting iodide from the cell to the colloid in the follicular lumen. This forms an efficient iodide-trapping mechanism in thyroid follicles in cooperation with the sodium–iodide symporter (NIS) at the basolateral membrane that eventually leads to oxidation and organification of iodide by thyroid peroxidase [12]. In the light of this evidence and given the special location of pendrin, we hypothesized that it could play a critical role in the iodine pathway and we investigated *SLC26A4* as a candidate susceptibility gene for iodine-deficiency based mental retardation.

The Qinba mountainous area, which includes the Qinling and Daba ranges and the Hanshui Valley, is a relatively isolated and

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Table 1
Number of samples, sex ratio and mean age.

Samples	Number	Sex ratio (F/M)	Mean age ± SD
MR[a]	80	41/39	9.1 ± 2.7
Border[b]	97	51/46	9.4 ± 2.8
Controls	273	125/148	8.6 ± 2.9
Total	450	217/233	8.9 ± 2.9

[a] The definite mental retardation (MR) group.
[b] The borderline mental retardation (Border) group.

traditionally iodine-deficient region with a high prevalence of MR [15]. All the subjects in our study were children recruited from this region, and were unrelated Han Chinese from families who had lived for many generations in the Qinba mountainous area. A total of 450 participants were divided into three groups: a control group ($n = 273$), a borderline mental retardation (Border) group ($n = 97$) and a definite mental retardation (MR) group ($n = 80$) (Table 1). Informed consent was given by either participants or their guardians.

We tested the children using the Chinese-Wechsler Young Children Scale of Intelligence, C-WYCSI, and the Chinese-Wechsler Intelligence Scale for Children, C-WISC. Based on WHO standards [24], we defined IQs less than 70 accompanied by social disability (SD) scores of 8 or less as mental retardation and IQs 70–79 and SD scores of 9 as Borderline forms of MR (Border). Controls came from the same iodine-deficient areas and had IQ scores of 80 or higher with SD above 9.

The primary strategy for SNP selection in this study was to focus on tag SNPs compensated by potential functional SNPs such as nonsynonymous coding SNPs (nsSNPs) and SNPs at 5′/3′-UTR. According to HapMap data, the *SLC26A4* gene region mainly includes two LD bins in Asian populations. We selected six SNPs in the bigger LD bin: rs6970857, rs2395911, rs1858929, rs1858930, rs982663, and rs2712218 (3′-UTR). Besides the bigger LD bin, we selected a tag SNP rs2248465 in other region of the gene. Given the important functional role of 5′-UTR, we chose the sole SNP rs17154282 at 5′-UTR reported by dbSNP. In addition to tag SNPs, we compensated two nsSNPs: rs17154335 at exon 17 and rs17154353 at exon 19. We were interested in looking at the frequencies of rs17154335 and rs17154353 in our samples, regardless of their low minor allele frequencies (MAF) in Asian populations (reported by HapMap). The two SNPs frequencies in our isolated population were speculated possibly different from those of dbSNP and HapMap.

Genomic DNA was extracted from peripheral leukocytes using standard phenol–chloroform procedures and was stored at −20 °C. Rs1858929 and rs1858930 were genotyped by sequencing since they are adjacent. The remaining candidate SNPs were detected on an ABI PRISMs 7900HT Sequence Detection System using ARMS-PCR technology as described by Germer et al. [11]. After genotyping by the ARMS-PCR method, we randomly sequenced three SNPs to check their genotypes according to the standard procedure of the BigDye Terminator cycle sequencing kit (Applied Biosystems) and either the forward or reverse primers were used as sequencing primers. Electrophoresis was conducted on ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystem). The details of primers used in ARMS-PCR and sequencing are provided in Table 2.

Microsoft Visual FoxPro (version 6.0) software was used for the preliminary preparation of data for analysis. Hardy–Weinberg expectation tests were performed for each polymorphism on an online calculator (http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm). Statistical analyses were conducted on the software of SAS 9.1 (SAS Institute Inc., Cary, US). The significance of the differences in observed frequencies of polymorphisms and haplotypes in the three groups was assessed using the χ^2 test. The software program UNPHASED (3.0.13) was used to

Table 2
Details of primers used in ARMS-PCR and sequencing.

Methods	Primer name	Sequence	Melting temperature
ARMS-PCR	rs17154282F	5′-GAAAGACCGCAGCCTGTGTGT-3′	59 °C/45 s
	rs17154282RG	5′-CCTTCCGCTGCCTTTATACCC-3′	
	rs17154282RC	5′-CCTTCCGCTGCCTTTATACCG-3′	
ARMS-PCR	rs2248465FT	5′-AGGGTTATTATTTTCCAGGAAATACTTCTT-3′	57 °C/30 s
	rs2248465FC	5′-AGGGTTATTATTTTCCAGGAAATACTTCTC-3′	
	rs2248465R	5′-CAATTTACAAATCACAAAGTTATGAAC-3′	
ARMS-PCR	rs2395911FT	5′-CCTTCAATAGTCCTATTTGTGTGTGATTTT-3′	61 °C/30 s
	rs2395911FG	5′-CCTTCAATAGTCCTATTTGTGTGTGATTTG-3′	
	rs2395911R	5′-GGCTTGACGTTTATCTACACACATACTG-3′	
ARMS-PCR	rs17154335F	5′-TTGACAATTAAGTTGACAGTGTTTTCTTCG-3′	55 °C/30 s
	rs17154335RG	5′-AGGCTCAAAAGCATTATTTGTTGGAC-3′	
	rs17154335RT	5′-AGGCTCAAAAGCATTATTTGTTGGAA-3′	
ARMS-PCR	rs982663FA	5′-TGTAGACTATATTGGCCAGAGAGTTAAA-3′	57 °C/30 s
	rs982663FG	5′-TGTAGACTATATTGGCCAGAGAGTTAAG-3′	
	rs982663R	5′-ATTCTGGCATTAAATATGTTAAGACTGT-3′	
ARMS-PCR	rs17154353FG	5′-AGTGAAATCTCAAGAGGGTCCAG-3′	58 °C/45 s
	rs17154353FA	5′-AGTGAAATCTCAAGAGGGTCCAA-3′	
	rs17154353R	5′-CATCTGTAGAAAGGTTGAATATTTACCG-3′	
ARMS-PCR	rs2712218F	5′-AACTCATTTTAATCACCCTGGTTATG-3′	58 °C/45 s
	rs2712218RC	5′-GACATAAAAACAGTGCTATTCTGATTG-3′	
	rs2712218RT	5′-GACATAAAAACAGTGCTATTCTGATTA-3′	
Sequencing	rs2248465F	5′-GCGCCTGGATTAGGAGTCTGA-3′	61 °C/30 s
	rs2248465R	5′-CCACTCCAAGATGGGCACAAG-3′	
Sequencing	rs982663F	5′-TGTGCTTGGCAAAAGAGTATGAG-3′	60 °C/30 s
	rs982663R	5′-TGGGAGAAACTGGGTAAGGGGTA-3′	
Sequencing	rs2712218F	5′-GCAGACAAATGAAATAATAAAGAGA-3′	50 °C/40 s
	rs2712218R	5′-GGAAAAAAATAAATAAAAAGAAAAG-3′	

F: forward primer; R: reverse primer.