International Journal of Genetics and Molecular Biology Vol. 3(10), pp. 149-154, November 2011
Available online at http://www.academicjournals.org/IJGMB
DOI: 10.5897/IJGMB11.058
ISSN2006-9863 ©2011 Academic Journals

Full length Research Paper

The Genetic variants of IL1RAPL2 gene associated with non-specific mental retardation in Chinese children

Zhang Kejin[1], He Bo[1], Gong Pingyuan[1], Gao Xiaocai[1,2], Zheng Zijian[1,2], Huang Shaoping[3] and Zhang Fuchang[1,2]*

[1]Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University) Ministry of Education, College of Life Science, Institute of Population and Health, Northwest University, Xi'an 710069, China.
[2]Institute of Applied Psychology, College of Public Management, Northwest University, Xi'an 710069, China.
[3]The 2nd Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710004, China.

Accepted 19 October, 2011

The present study investigated the association between the genetic variants of IL1RAPL2 gene and Non-syndromic mental retardation (NSMR) in the children of QinBa region of China. Five common SNPs (rs5962434, rs5916817, rs3764765, and rs5962298 and rs9887672) of IL1RAPL2 were chosen and examined their individual genotype frequencies using the conventional polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) method, and evaluated the association between these genetic polymorphisms and NSMR with the suitable bio-statistic software. Two SNPs (rs5962298 and rs9887672), whose alleles and genotypes distribution showed a significant differences between the control and NSMR groups (alleles: *p* = 0.020 and 0.017; genotypes: *p* = 0.025 and 0.053, respectively). Furthermore, the different gender effect was found out, when stratified the data set by the sex. Taken together, we provided substantial evidence that IL1RAPL2 conferred a NSMR susceptibility to children of Qinba region in China, and further work should been done.

Key words: Non-syndromic mental retardation, molecular genetics, association analysis, genetic variants.

INTRODUCTION

Mental retardation (MR) is a heterogeneous disorder, characterized by an IQ score of 70 or lower and significant limitation in the social and practical adaptive skills and leads to problems with self care, communication and school activities. MR affects 1 to 3% of children in different countries and regions (Mclaren and Bryson, 1987), and is divided into "syndromic" (SMR; the additional sdymorphic, neurological, and/or metabolic features accompany the mental deficit) and "non-syndromic" (NSMR; the cognitive impairment is the sole definable clinical feature) forms. Although the underlying causes of MR are likely to be extremely heterogeneous, a strong genetic component (even genetic etiologies) has been found in approximately two thirds of MR cases (Curry et al., 1997).

Recently, most of research works focused on the X chromosome, and try to map, identify and clone the MR candidate genes in this chromosome. So far, there were 34 candidate MR genes which were identified and cloned in X chromosome: 17 of them are NSMR-specific genes, and the other 17 genes can cause both NSMR and SMR, according to the update of Greenwood Genetic Center in July 2009 (Stevenson et al., 2000). Moreover, linkage data for 56 X-linked families have been mapped (log score >2), but based on these data, no NSMR gene has been cloned.

Carrie et al. (1999) identified a mutation encode sequence (NCBI accession number: 300206.0001) in a small family with X-linked NSMR. The sequence encodes a 696 amino acid protein with a homology to interleukin-1 (IL-1) receptor accessory protein (Carrie et al., 1999). Following in Carrie's work, several other reports also have verified the association between IL1RAPL1 and NSMR (Bahi et al., 2003; Tabolacci et al., 2006). Jin et al.

*Corresponding author. E-mail: zhfch@nwu.edu.cn. Tel: +86 29 88303328. Fax: + 86 29 88303572.

Abbreviation: NSMR, nonspecific mental retardation; **Co,** controls.

(2000) found a closely related homolog, which was designated as IL1RAPL2 gene (Jin et al., 2000). The proteins encoded by IL1RAPL1 and IL1RAPL2 share 65% sequence identity as well as a C-terminal sequence absent in other members of the IL1 receptor family. Thus, like IL1RAPL1 and IL1RAPL2 may be the mutation locus causing NSMR (Jin et al., 2000; Sana et al., 2000).

This study was aimed to study the relationship between IL1RAPL2 and NSMR by examining the genetic polymorphisms of five SNPs in IL1RAPL2 gene in a random population. We performed a case–control study to investigate the association between IL1RAPL2 and NSMR in the Han Chinese population.

MATERIALS AND METHODS

Subjects

In total, there were 118 (male/female = 56/62) MR, 116 (male/female = 52/64) borderline, and 322 (male/female = 167/155) controls in our study. All subjects were randomly collected with the requirement of case-control analysis from the Qinba region of Shaanxi province, northwest China. In addition, after the protocol had been fully explained, which was approved by the Ethical Committee of the National Human Genome Center, standard informed consents were obtained from the participants and their guardians.

Intelligence evaluation

The intelligence of each participant was screened with the Chinese Wechsler Young Children Scale of Intelligence (C-WYCSI) (Gong and Dai, 1992) for 4 to 5 year old children and the Chinese Wechsler Intelligence Scale for Children (C-WISC) (Gong and Cai, 1993) for 6 to 14 year old children. The social disability (SD) scores were assessed with the adaptive scale for infants and Children revised by Zuo et al. (1998). To further evaluate the children with an IQ < 85 and SD score no higher than 9, a subsequent clinical examination was carried out by a group of neurologists, pediatricians and gynecologists. We defined the tested IQ < 70 and SD score ≤ 8 as MR and IQ of 70 to 79 and SD score of 9 as a borderline form of MR. The definition and diagnosis criteria of MR and borderline were based on the classification of mental disorders 2nd revision (CCMD-2-R) and the classification of mental and behavioral disorders from the WHO (Cao, 1995; Who, 1992). The cases affected by trachoma, infection, trauma, dystrophy, toxicosis, cerebral palsy, birth complications or other specific clinical causes were excluded from the analysis.

All subjects were of Chinese Han population and were randomly collected. Standard informed consents were obtained from all participating subjects according to the protocol reviewed and approved by the Ethical Committee of the National Human Genome Center of China.

Variants identification and genotyping

Genomic DNAs were extracted from peripheral blood mononuclear cells using a modified phenol chloroform extraction method (Joseph and Davi, 2001) and were stored at -20°C for genotyping. Five SNPs with minor allele frequency over 5% in the IL1RAPL2 region were selected, from the dbSNP (http://www.ncbi.nlm.nih.gov/SNP), they were rs5962434, rs5916817, rs3764765, rs5962298 and rs9887672, the information of them were displayed in Table 1. The SNP rs5962434 (A/C) is in the 3'-UTR, while rs5916817 (C/T) in intron 2, and rs3764765 (C/T) in exon 9, rs5962298 (A/G), rs9887672 (C/T) are both in intron 9. The last three SNPs are in the domain which encodes the novel C-terminal sequences.

The polymerase chain reaction combined with single strand conformation polymorphism (PCR-SSCP) method and sequencing analysis were used to genotyping the five SNPs. Polymerase chain reactions were carried out in 96-well micro titer plates with each well containing a ten microliter reaction mixture of 20 mM $(NH_4)_2SO_4$, 5 mM Tris-HCl (pH 8.8 at 25), 0.01% Tween 20, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 0.3 uM of primer, 20ng DNA and 1U Taq DNA Polymerase (Fermentas International Inc. Ontario, Canada). PCR protocol included an initial 2 min at 95°C, 29 cycles at 94°C for 30 s, annealing temperature for 30 s (the annealing temperature needed for each pair of primers are listed in Table 1), 72°C for 30 s and a final extension period of 2 min at 72°C using the Mastercycler gradient 5531 PCR System (Eppendorf Inc. Hamburg, Germany). PCR products were resolved by SSCP analysis, and verified 20 to 30 samples per SNP by ABI 3700 DNA Sequencer (ABI Inc. Foster, USA) randomly.

PCR products were denatured by using the following buffer (95% formamide, 0.025% xylene-cyanole, 0.025% bromophenol blue, 10 mM EDTA, pH 8.0) at 97°C for 5 min, and snap-chilled on ice for at least 10 min. Samples were then loaded onto 11% nondenaturing polyacrylamide gels (29:1 acrylamide to bisacrylamide) containing 0.5×TBE (0.045M Tris-borate, 0.001M EDTA, pH8.0). Electrophoresis was kept at a constant temperature (4°C) with a cooling unit and was carried out in a vertical unit at 11 V/cm for 15 h. The individual's genotype was confirmed by silver-staining and gel-imaging with Bio-Rad imaging instrument. Randomly selected PCR products that exhibited different migration patterns on gels were selected for sequencing to confirm their polymorphisms and genotypes.

Statistical analysis

Demographic data (including the age and gender) and the allele and genotype frequencies for five SNPs were calculated with SPSS (SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) tests were performed for a single SNP with the method described by Guo et al. (2002). Differences in the genotype and allele distributions between MR and control groups were assessed by the Monte Carlo method with CLUMP 2.3 (10,000 simulations) (Stephens et al., 2001). The haplotypes combined by target SNPs were estimated by PHASE2.1 software package (Stephens and Donnelly, 2003; Stephens et al., 2001). The subjects' haplotypes with a confidence level of ≥ 95% were included. The pair wise linkage disequilibrium (LD), the frequencies of haplotypes and the fit of haplotypes to HWE were estimated by Cube X 2007 (Gaunt et al., 2007), a cubic exact solution online-program rather than an iterative approach (http://www.oege.org/software/cubex/). In this study, the *p*-values were two-tailed and a difference was considered statically significant when $p < 0.05$. The statistical power analysis was performed with the G*Power program (Faul et al., 2007).

RESULTS

HWE test was carried out on the genotype distribution of five SNPs. The distribution was consistent with HWE no matter in NSMR group or control group.

Table 2 shows the comparison of each SNP allele and genotype frequency distribution among NSMR, borderline and control groups. The allele and genotype frequency

distributions of rs5962298 and rs9887672 were significantly different between NSMR and control groups. The alleles frequency of X^A and X^T alleles of them were significantly higher in NSMR than that of control (p = 0.020; p = 0.025, respectively). The genotype frequency distribution also was significant different between the two groups (p = 0.017). Subsequently, the comparison were also performed for girls and boys, respectively; and the distribution differences of rs5962298 and rs9887672 were more significant in girls (allele: p = 0.004, p = 0.019; genotype: p = 0.021, p = 0.014), but vanished in boys. Moreover, no differences were detected between borderline and control groups.

The haplotypes combined by rs5962298 and rs9887672 were analyzed between NSMR and control groups in girls (Table 3). But no significant difference was found in girls. Therefore, the LD and haplotypes fit to HWE were calculated by the Cubex 2007 program. It shows that rs5962298 and rs9887672 did not have a strong LD (*D'* = -0.671, r^2 = 0.250), and their combined haplotypes did not fit to HWE, although their single SNPs were compatible with HWE.

Moreover, a power calculation was performed by the G*Power program, based on the Cohen's method (Faul et al., 2007). When an effective size index of 0.25 (corresponding to 'weak to moderate' gene effect) was used, the sample size in our study revealed > 95% power for detecting significant associations ($\alpha < 0.05$).

DISCUSSION

Qin-Ba Mountain region of Shaanxi province is a relatively isolated area in northwest China. The prevalence of MR (3.19%) in the region is much higher than that in most areas of China (1.07%); and if the number of children in the low range of normal IQ (borderline of MR) is included, the prevalence will increase to 8.23% (Zhang et al., 2004). Moreover, family clustering is obviously presented in two counties where several families have multiple affected members in one or more generations. The epidemiological survey reveals that the heritability of MR is as high as 70.23%, when the MR cases affected by trachoma, infection, trauma, toxicity, cerebral palsy and birth complications are excluded (Zhang et al., 2005, 2006). This result suggests that genetic factors play an important role in the etiology of MR in this region.

IL1RAPL1 gene is a form candidate NSMR gene, reported by Bahi et al. (2003), Carrie et al. (1999) and Tabolacci et al. (2006). Both IL1RAPL1 and IL1RAPL2 belong to the IL-1 receptor family comprised of eight members involved in the response to IL-1 and IL-18. They share the same gene structure, the same intron-exon organization and a high similarity at the protein level (Jin et al., 2000; Sana et al., 2000). IL1RAPL1 differs from IL1RAPL2 by the presence of a 150 amino acid C-terminal domain encoded by the sequence region of exon 10 and exon 11, and this domain is not present in any other members of IL-1R family. Furthermore, the novel C-terminal domain shows an important biological role, and it interacts with the neuronal calcium sensor-1 protein (NCS-1). Therefore, researchers have focused on the function of the C-terminal domain. Carrie et al. (199) and Tabolacci et al. (2006) confirmed that the polymorphism in this region (exon 10 and exon 11) affects the interaction between NCS-1 and the C-terminal domain, causing NSMR presumably through the same mechanism with IL1RAPL2 (Gambino et al., 2007).

Our study selected five SNPs of IL1RAPL2, and three of them were used to mark the polymorphism condition of the C-terminal domain. We performed a case-control study to investigate the relationship between IL1RAPL2 and NSMR within the children of Qinba region of China. The single-locus association analysis showed that the SNPs rs5962298 and rs9887672 were close to the region encoding the C-terminal domain of IL1RAPL2. We found significant differences in the allele and genotype distributions between the NSMR and control groups (rs5962298, allele: p = 0.020 and genotype: p = 0.017; rs9887672 allele: p = 0.025 and genotype: p = 0.053, respectively). Furthermore, these differences were even more significant in girls, but disappeared in boys. Surprisingly, when we examined the distribution of haplotypes combined by rs5962298 and rs9887672, no significant difference was found regardless of individual or global haplotype test. The Cubex 2007 program indicated that there was no strong LD between rs5962298 and rs9887672 (*D'* = 0.671 and r^2 = 0.250), and the combined haplotypes also did not fit to HWE (X^2=17.76, df = 5 P = 0.003) although each of them was consistent with HWE. This result implied that there were one or more recombination hotspots between these two SNPs. They just represented two different regions of IL1RAPL2, respectively. Therefore, the haplotype analysis may not be a suitable method. But our study still showed the potential association between the genetic variants of IL1RAPL2 and NSMR.

Our results can be explained at two fold: First, both SNPs (rs5962298 and rs9887672) are located in the ninth intron of IL1RAPL2, close to the region encoding the C-terminal domain. The variant of this special domain is essential for the protein's biological functions, which may cause individual NSMR. Our study provided some evidence for this assumption. Second, in additional to these two SNPs, there were other mutations related to girls' NSMR in Qinba region of China. The inconsistent results between the single-site analysis and the haplotype analysis indicated that there were more than one mutation nearby rs5962298 and rs9887672 and they may influence the function of IL1RAPL2 and the association with NSMR as well. Given properly selected genetic markers, consistent results would be expected. Meanwhile, the effect of the gender difference should be

Table 1. The primers information and PCR condition of five SNPs of *IL1RAPL2*.

SNPs	Primer sequences (5'->3')	Product size	Tm (℃)	Allele
rs5962434	Forward: CTGGGTGGAGCAGCGAGG Reversed: GTATCGGGGATTCTTGCGG	187 bp	61.5	A/C
rs5916817	Forward: CCCAAGAAGTGAAGTGTAT Reversed: TAGGGGAGAAGAAGTAATC	342 bp	58.0	C/T
rs3764765	Forward: CCCAAGTGTTGCTGATTT Reversed: TGCCATAGCCCTTTACCC	139 bp	59.0	C/T
rs5962298	Forward: ATTGCTGTCAAATCCCTCC Reversed: ATTGCTTGTTTTGTAGATGGC	125 bp	61.0	A/G
rs9887672	Forward: ACCTTTCAGTTTTTCAGTC Reversed: CAAGAAGCCATTTGTGTT	252 bp	61.0	C/T

Table 2. The alleles and genotyps frequencies of five SNPs in NSMR and control groups.

SNPs	Allele (%)		*P* value (d.f.=1)	OR (95%CI)	Genotype (%)					*P* value (d.f.=3)
rs5962434	X^A	X^C			X^AX^A	X^AX^C	X^CX^C	X^AY	X^CY	
NSMR	84 (61.3)	53 (38.7)	0.131	1.25(0.932-1.697)	18 (37.5)	20 (41.7)	10 (20.8)	28(68.3)	13 (31.7)	0.211
Co.	221(53.9)	189 (46.1)			33 (24.4)	75 (55.6)	27 (20.0)	80(57.1)	60 (42.9)	
rs5916817	X^C	X^T			X^CX^C	X^CX^T	X^TX^T	X^CY	X^TY	
NSMR	94 (58.8)	66 (41.3)	0.948	0.99(0.913-1.103)	20 (37.0)	21 (38.9)	13 (24.1)	33(63.5)	19 (36.5)	0.617
Co.	271 (59.0)	188 (41.0)			57 (37.5)	70 (46.1)	25 (16.4)	87(56.1)	68 (43.9)	
rs3764765	X^C	X^T			X^CX^C	X^CX^T	X^TX^T	X^CY	X^TY	
NSMR	47 (28.8)	116 (71.2)	0.165	1.23(0.923-1.635)	4 (6.8)	31 (53.4)	23 (39.6)	8 (17.0)	39 (83.0)	0.282
Co.	107 (23.4)	351 (76.6)			8 (5.3)	61 (40.1)	83 (54.6)	30 (19.5)	124 (80.5)	
rs5962298	X^A	X^G			X^AX^A	X^AX^G	X^GX^G	X^AY	X^GY	
NSMR	59 (33.5)	117 (66.5)	0.020[a]	1.37(1.056-1.775)	5 (8.2)	34 (55.7)	22 (36.1)	15 (27.8)	39 (72.2)	**0.017**[a]
Co.	113 (24.4)	350 (75.6)			14 (9.1)	49 (31.8)	91 (59.1)	36 (23.2)	119 (76.8)	
rs9887672	X^C	X^T			X^CX^C	X^CX^T	X^TX^T	X^CY	X^TY	

Table 2. Contd.

NSMR	135 (75.8)	43(24.2)	0.025[a]	0.72 (0.542-0.948)	33 (54.1)	25 (41.0)	3 (4.9)	44 (78.6)	12 (21.4)	0.053
Co.	386 (83.5)	76 (16.5)			109 (73.6)	33 (22.3)	6 (4.1)	135 (81.3)	31 (18.7)	

[a]Bold font indicates significant associated statistic.

Table 3. The frequencies of haplotypes combine by rs5962298 (A/G) - rs9887672 (C/T) in NSMR and controls in girls.

	The frequencies of haplotypes rs5962298- rs9887672[a]				Global *P* value
	X^{A-C}	X^{A-T}	X^{G-C}	X^{G-T}	
NSMR	0.180	0.156	0.631	0.033	0.313
Co.	0.115	0.135	0.712	0.034	
Individual *P* value	0.129	0.506	0.111	0.872	

[a]The haplotypes which frequence lower than 0.05 were excluded in global p value was calculated.

controlled.

In summary, our study has shown that the genetic variants of the region encoding the C-terminal domain of IL1RAPL2 were associated with NSMR children in Qinba region of China, but further efforts should be made to examine this locus, especially controlling the gender-difference effect and using larger sample sizes.

ACKNOWLEDGEMENTS

We sincerely thank all the participants and researchers in this study. This work was supported by 'the Tenth Five-Year Plan' National Tackle Problem Item (No.2001BA901A49), Special Prophase Project on Basic Research of The National Department of Science and Technology (2007CB516702), Shaanxi Province Science Fund (SJ08C236) and Education Foundation of Shaanxi ((No. 11JK0620).

REFERENCES

Bahi N, Friocourt G, Carrie A, Graham Me, Weiss Jl, Chafey P, Fauchereau F, Burgoyne Rdand Chelly J (2003). Il1 Receptor Accessory Protein Like, a Protein Involved in X-Linked Mental Retardation, Interacts with Neuronal Calcium Sensor-1 and Regulates Exocytosis. Hum. Mol. Genet., 12: 1415-1425.

Cao (1995). Chinese Classification of Mental Disorders Southeast University Press, Nanking.

Carrie A, Jun L, Bienvenu T, Vinet Mc, Mcdonell N, Couvert P, Zemni R, Cardona A, Van Buggenhout G, Frints S, Hamel B, Moraine C, Ropers HH, Strom T, Howell GR, Whittaker A, Ross MT, Kahn A, Fryns J, Beldjord C, Marynen P, Chelly J (1999). A New Member of the Il-1 Receptor Family Highly Expressed in Hippocampus and Involved in X-Linked Mental Retardation. Nat Genet., 2): 25-31.

Curry CJ, Stevenson RE, Aughton D, Byrne J, Carey JC, Cassidy S, Cunniff C, Graham JM, Jones MC, Kaback MM, Moeschler J, Schaefer GB, Schwartz S, Tarleton J, Opitz J (1997). Evaluation of Mental Retardation: Recommendations of a Consensus Conference: Am. Coll. Med. Genet., 72: 468-477.

Faul F, Erdfelder E, Lang AG, Buchner A (2007). G*Power 3: A Flexible Statistical Power Analysis Program for the Social, Behavioral, and Biomedical Sciences. Behav. Res. Meth., 39: 175-191.

Gambino F, Pavlowsky A, Begle A, Dupont Jl, Bahi N, Courjaret R, Gardette R, Hadjkacem H, Skala H, Poulain B, Chelly J, Vitale Nand Humeau Y (2007). Il1-Receptor Accessory Protein-Like 1 (Il1rapl1), a Protein Involved in Cognitive Functions, Regulates N-Type Ca2+-Channel and Neurite Elongation. Proc. Natl. Acad. Sci. U S A., 104: 9063-9068.

Gaunt TR, Rodriguez S, Day IN (2007). Cubic Exact Solutions for the Estimation of Pairwise Haplotype Frequencies: Implications for Linkage Disequilibrium Analyses and a Web Tool 'Cubex'. BMC Bioinformatics, 8: 428.

Gong YX, Cai TS (1993). Wechsler Intelligence Scale for Children, Chinese Revision (C-Wisc). Hunan: Map Press, Changsha.

Gong YX, Dai XY (1992). Chinses-Wechsler Yong Children Scale of Intelligence (Cwycsi). Hunan: Map Press, Changsha.

Guo Y, Guo C, Sui Q (2002). The Basis of Medical Genetics. Shandong University press, Ji Nan.

Jin H, Gardner RJ, Viswesvaraiah R, Muntoni F, Roberts RQ (2000). Two Novel Members of the Interleukin-1 Receptor Gene Family, One Deleted in Xp22.1-Xp21.3 Mental Retardation. Eur. J. Hum. Genet., 8: 87-94.

Joseph S, Davi WS (2001). Molecular Cloning: A Labortory Manual (Third Editor). Cold Spring Harbor Laboratory Press, New York.

Mclaren J, Bryson SE (1987). Review of Recent

Epidemiological Studies of Mental Retardation: Prevalence, Associated Disorders, and Etiology. Am. J. Ment. Retard., 92: 243-254.

Sana TR, Debets R, Timans JC, Bazan JF, Kastelein RA (2000). Computational Identification, Cloning, and Characterization of Il-1r9, a Novel Interleukin-1 Receptor-Like Gene Encoded over an Unusually Large Interval of Human Chromosome Xq22.2-Q22.3. Genomics, 69: 252-262.

Stephens M, Donnelly P (2003). A Comparison of Bayesian Methods for Haplotype Reconstruction from Population Genotype Data. Am. J. Hum. Genet., 73: 1162-1169.

Stephens M, Smith NJ, Donnelly P (2001). A New Statistical Method for Haplotype Reconstruction from Population Data. Am. J. Hum. Genet., 68: 978-989.

Stevenson RE, Schwartz CE, Schroer RJ (2000). X-Linked Mental Retardation, pp. Oxford University Press.

Tabolacci E, Pomponi MG, Pietrobono R, Terracciano A, Chiurazzi P, Neri G (2006). A Truncating Mutation in the Il1rapl1 Gene Is Responsible for X-Linked Mental Retardation in the Mrx21 Family. Am. J. Med. Genet. A., 140: 482-487.

WHO (1992). The Ico-10 Classification of Mental and Behavioural Disorders. WHO, Geneva.

Zhang FC, Li Rl, Gao XC, Zheng ZJ, Huang SP, Song HY, Xi H, Li F (2004). An Investigation of Mental Retarded Children Aged 0 - 14 in Qinba Mountain Areas. J. Fourth Mil. Med. Univ., 25: 511-514.

Zhang KJ, Zhang FC, Zheng ZJ, Gong PY, He G, Nan YP, Zhang R, Ms J (2005). An Analysis of Inheriting Type of Non-Causing Mental Retarded Children in Zhashui Experimental Station. J. North W. Univ. (Nat. Sci. Ed.), 35: 597-600.

Zhang SM, Zhang FC, Gong PY, Zhang KJ, Yhang XZ, He G, Li N (2006). The Discussion of Inheriting Type of Mental Retardation in Ankang Experimental Station. J. North W. Univ. (Nat. Sci. Ed.), 36: 97-100.

Zuo QH, Zhang ZX Wu LZ (1998). Adaptive Scale of Infant and Children. Med. Univ. China, Beijing, China, 36: 97-100.