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The association between IL-18 gene polymorphism and cerebral palsy in northern Chinese population

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ABSTRACT

Cerebral palsy (CP) in children is a seriously disabling disease which is a threat to children's health and causes enormous emotional stress and financial loss to the affected families and the community. This study aimed to explore the association of IL-18 gene polymorphism with cerebral palsy by IL-18 genotyping. This was a case-control study that used DNA from 120 children with cerebral palsy and 120 control children to test polymorphisms in exon-4 and exon-5 of IL-18 gene. A polymorphic site in exon-4 of IL-18 gene, A105C, was significantly different in genotype distribution between case group and control group. Compared with type AC and CC individuals, type AA individuals were associated with lower risk for cerebral palsy. There was no significant difference in the polymorphic site G276A in exon-5 of IL-18 gene between the two groups. Presence of polymorphism in IL-18 gene is associated with an increased risk of cerebral palsy.

Introduction

Cerebral palsy (CP) is a disorder in which children experience a non-progressive brain lesion that results in permanent and progressive secondary postural and movement disorders [1]. CP has an incidence of 2.0–2.5 occurrences per 1000 live births in developed nations, making it the most common cause of physical disability in children [2]. Risk factors for CP can be categorized as prenatally, perinatally and postnatally acquired of which about 70–80% are acquired prenatally. It is estimated that the portion of CP cases with a genetic etiology is approximately 1% to 2% in all CP cases.

The human IL-18 (hIL-18) gene is located on chromosome 11q22.2-q22.3, and is composed of six exons and five introns. The protein encoded by this gene is a proinflammatory cytokine that augments natural killer cell activity in spleen cells, and stimulates interferon gamma production in T-helper type I cells [3,4]. Recent studies have indicated that IL-18 gene polymorphism is associated with many kinds of diseases [5-8]. However, it is uncertain whether there is a relationship between IL-18 gene polymorphism and cerebral palsy [9]. In this study, two SNPs were detected in IL-18 gene, A105C and G276A, using PCR-RFLP to determine the relationship between IL-18 gene polymorphism and cerebral palsy.

Materials And Methods

Study objects

120 children with cerebral palsy and 120 control children were selected in Heilongjiang province of China from September 2007 to May 2009. The controls were matched for age, gender, health and birthplace. The parents of the children provided consent that was approved by the university human research ethics committee before data and blood collection initiation.

Experimental methods

Genomic DNA was prepared from 2ml venous blood using blood genomic DNA extraction kit (TIANGEN, China) according to the recommended procedure. Exon-4 and 5 of IL-18 gene were amplified by PCR using the following primers: exon-4, F 5'-TGT TTA TTG TAG AAA ACC TGG AAT CCT CTA-3', R 5'-CA GTC AGA ATC AGT-3'; exon-5, F 5'-AAA GTG GGA GGT GTA TTA AGG A ACA-3', R 5'- AAG GTT GGT CTGA GGA TAT TTG AGT-3'. The PCR product of exon-4 and exon-5 were digested by TaqI and Cac8I (New England Bio labs, USA) respectively, and detected by polyacrylamide gel electrophoresis.

Statistical analysis

Statistical analysis of the data was performed in statistical analysis software (SAS 9.13). The χ^2 test was used to verify whether the selected population was a genetic equilibrium group. The χ^2 test and Fisher exact test were used to compare the genotype distribution of case and control group. The calculation of odd ratios (ORS) at 95% confidence intervals (CIS) were calculated by unconditional logistic regression analysis.

Results

In this study, blood samples of 120 children with cerebral palsy and 120 control children were collected. Each group consisted of 74 males (61.7%) and 46 females (38.3%). The genotype distribution of case and control groups were corresponding with Hardy Weinberg balance law, of which the two group samples were representative.

As shown in Table I and Table II, A105C was significantly different in genotype distribution and allele frequency distribution between case group and control group ($P < 0.05$), but G276A was not ($P > 0.05$, OR = 0.8738). Compared with type AC+CC individuals, type AA individuals were associated

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with lower risk (P = 0.0283, OR = 0.496) for cerebral palsy. There was no significant difference between the two groups of the other possible polymorphic site in exon-5 of IL-18 gene, G276A.PCR results are represented in fig 1, 2, 3 and 4 below the tables.

Table I: Allele and genotype frequencies of the IL-18 A/C genetic polymorphism in Chinese CP patients and controls

Polymorphism	*Controls Number (%)*	*CP Number (%)*	*P-value*	*Odds ratio (95% CI)*
Allele frequency				
A	221(92.1)	207(86.3)		
C	19(7.9)	33(13.7)	0.040	
Genotype frequency				
AA	101(84.2)	87(80.8)		
AC+CC	19(15.8)	33(19.2)	0.028	0.496(0.263-0.934)

Table II: Allele and genotype frequencies of the IL-18 G/A genetic polymorphism in Chinese CP patients and controls

Polymorphism	*Controls Number (%)*	*CP Number (%)*	*P-value*	*Odds ratio (95% CI)*
Allele frequency				
G	207(86.3)	209(87.1)		
C	33(13.7)	31(2.9)	0.788	
Genotype frequency				
GG	89 (74.2)	92 (76.7)		
GA+AA	31 (25.8)	28 (23.3)	0.6529	0.874(0.485-1.574)

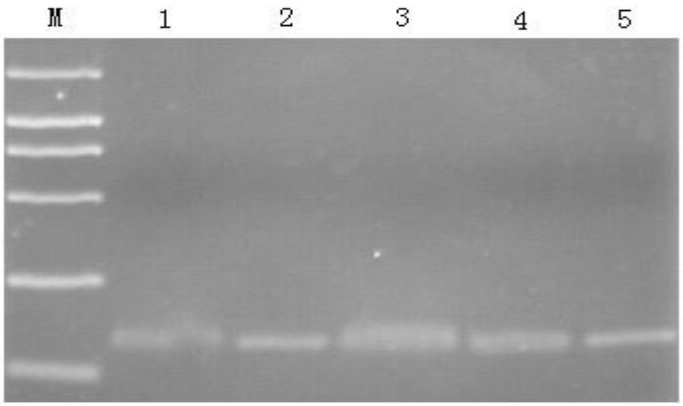

Figure 1: PCR results for exon 4 of IL-18. 1-3: *CP*; 4-5: *Controls*

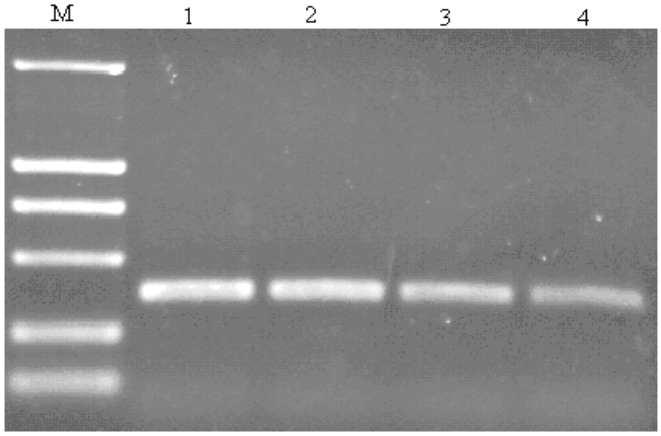

Figure 2: PCR results for exon 5 of IL-18. 1-2: *CP*; 3-4: *Controls*

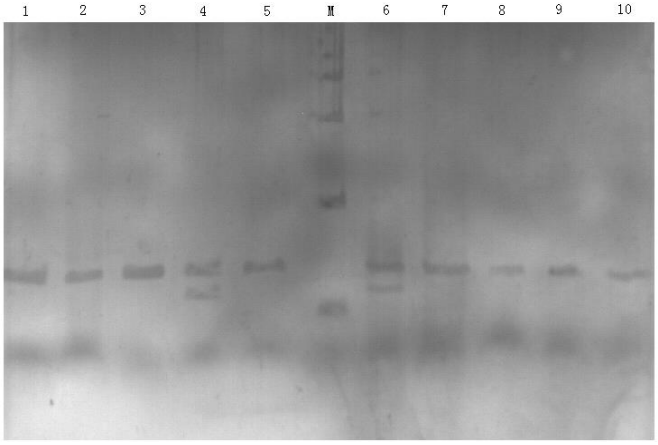

Figure 3: Results for exon 4 of IL-18 genotype. 1-3, 5, 7-10: AA homozygote; 4,6: AC heterozygote; M:DL2000 maker

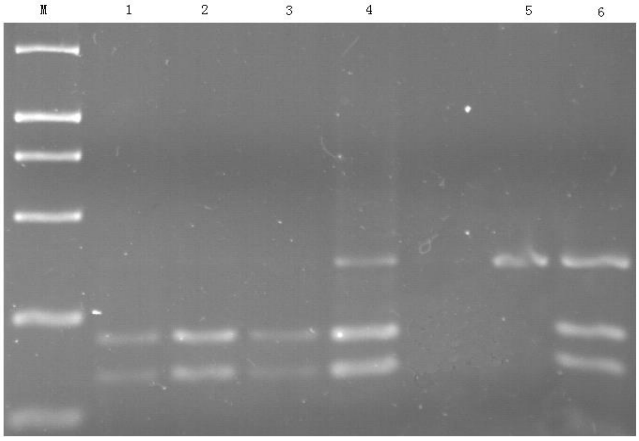

Figure 4: Results for exon 5 of IL18 genotype. 1-3: GG homozygote; 4,6: AG heterozygote; 5:AA homozygote; M:DL2000 maker

Discussions

The human IL-18 gene is located in chromosome 11q22.2-22.3, and the polymorphism of IL-18 gene increasingly caused concern because of the close relationship between IL-18 and disease occurrence. Qi [10] found that two SNPs in IL-18 were associated with cervical carcinoma. Akgun [11] found that the risk of tuberculosis in people who carried -137GG genotype of IL-18 was significantly higher than that of healthy people. In addition, it is confirmed that IL-18 is a possibly related to atopic dermatitis [12]. Novak [13] using case-control study confirmed that IL-18 is an Alzheimer's disease susceptibility gene. In another study, the relationship between SNPs in promoter (-607 to –137) of IL-18 and chronic hepatitis B was analyzed in patients of Chinese Han population [14]. The results showed that -137 allele C in IL-18 plays an important role in immune regulation and killing cells infected with virus [15]. The results from a Japanese study performed by Ide *et al.* showed that SNPs in IL-18 and 49 A/G in cytotoxic T lymphocyte- associated antigen 4 gene (CTLA-4) were related to susceptibility of diabetes type I[16]. The frequency distribution of -137G and -607C in IL-18 in the diabetic group was higher than that of the normal group. When the CTLA-4 gene was not 49GG genotype in patients, haploid frequency of IL-18 was also significantly reduced.

The relationship of IL-18 gene polymorphism and diseases of the nervous system have been rarely reported, but many studies show that in the process of a variety of brain injury (especially cerebral damage caused by infection and hypoxia ischemia which are leading causes of cerebral palsy) high IL-18 expression can be detected. Research showed that the level of IL-18 in amniotic fluid or blood of full-term and preterm

children with cerebral palsy were significantly higher than that of children without cerebral palsy. It is hence presumed a genetic relationship between IL-18 gene polymorphism and susceptibility of cerebral palsy that has not been found before may exist.

In this study, we found that the frequency distribution of IL-18 A105C genotype in the case group was 80.8% AA and 19.2% AC, CC was not detected, in the control group was 84.2% AA and 15.8% AC, and again CC was not detected. The genotype distribution of cases was significantly different from that of controls. It was valid by χ^2 test. Compared with type AC+CC individuals, type AA individuals were associated with lower risk for cerebral palsy (OR = 0.496 at 95% confidence interval (CI) = 0.263 - 0.934, P = 0.028). This means that the IL-18 A105C genotype was related to susceptibility of cerebral palsy. A105C that is located in exon-4 of IL-18 gene is a synonymous polymorphism which cannot change the amino acid encoding, so it occurs easily than that in the coding region of non-synonymous polymorphism and non-coding region polymorphisms. Although researches showed synonymous variation are neutral in function, not all synonymous polymorphisms cause no change to gene function. In a mutation experiment about human dopamine D2 receptor (DRD2) gene, researchers found that among the six synonymous mutations, C957T could affect mRNA secondary structure, change the mRNA translation level, and reduce the stability of mRNA. At the same time another synonymous mutations G1011A alone could not affect gene function, but it could eliminate the effect of C957T on DRD2 expression. Therefore, multiple synonymous mutations can change the effect of individual mutation on gene function [17]. The analysis of SNPs in exon-5 showed that frequency distribution of IL-18 G276A genotype in the case group was 76.7% GG, 20.8% GA, and 2.5% AA, while in the control group was 74.2% GG, 24.2% GA, and 1.6% AA. The genotype distribution of cases was significantly different from that of controls. It was valid by χ^2 test. Compared with type AC+CC individuals, type AA individuals were associated with lower risk for cerebral palsy(OR = 0.496 at 95% confidence interval (CI) = 0.263 - 0.934, P = 0.028). This SNP was not related to susceptibility of cerebral palsy by Fisher exact tests. However, the G276A could influence SNP sites related to cerebral palsy and indirectly play a role. Genotype frequency of G276A may be changed in certain environments and cause the occurrence of a disease, i.e. gene-environment interaction may play an important role in increasing individual susceptibility to disease mechanisms. We plan to study these issues in the subsequent work.

In this study, we found that a correlation exists between polymorphism of IL-18 gene and susceptibility of cerebral palsy. We will continue to work on other sites of IL-18, interaction of poly SNPs and their roles in pathogenesis of cerebral palsy. Because risk factors of cerebral palsy are not independent, this comprehensive study will deepen the understanding of the pathogenesis of cerebral palsy, and contribute to the establishment of effective prevention and control measures.

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Competing interest: The authors declare no conflicts of interest.

Contributors: Hong-Bin Qiu was responsible for article validation and funds. Hui Xu provided technical support and research instructions. Yun-Fei Shang and Adusei-fosu Mavis were responsible for study implementation and manuscript drafts. Qiang Zhang was responsible for data analysis and statistical processing. Ning Xie summarized and collected data.

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