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Haplotype of *BAK1* (*BCL2 antagonist killer 1*) polymorphisms associated with the risk of developing Kawasaki disease in Taiwanese children

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ABSTRACT: Kawasaki disease (KD) is a pediatric systemic vasculitis of unknown etiology for which a genetic influence has been suggested. BCL2 antagonist killer 1 (*BAK1*) has been considered to play a critical role in the development of autoimmune disease. The aim of this study was to examine the association of *BAK1* polymorphisms with KD risk in Taiwanese children. Five single nucleotide polymorphisms (SNPs)—rs210132, rs210135, rs210139, rs210145 and rs396746—in the *BAK1* gene were analysed in a case-control study comprising 93 KD patients and 680 gender- and age-matched healthy controls. The results showed that the frequencies of the SNP rs210132 TT genotypes were significantly higher in KD patients without coronary artery aneurysm than in control subjects (OR, 1.93 [95% CI: 1.08–3.46]; $p = 0.037$). The estimated frequency of the GAGC haplotype (rs210132-rs210139-rs210145-rs396746) was significantly lower in KD patients than in controls (OR, 0.60 [95% CI: 0.36–1.00]; $p = 0.047$). In addition, the frequency of the TAGC haplotype (rs210132-rs210139-rs210145-rs396746) was significantly higher in KD patients than in control subjects (OR, 9.97 [95% CI: 3.72–26.7]; $p < 0.0001$). In conclusion, the results suggest that the *BAK1* gene polymorphisms are associated with the risk of KD in the Taiwanese population.

KEYWORDS: autoimmune, coronary artery aneurysm

INTRODUCTION

Kawasaki disease (KD) is an acute self-limited immune-mediated form of vasculitis that primarily affects infants and young children. Inflammation caused by the disease can lead to coronary artery aneurysm (CAA) and heart attack, making KD the most common cause of acquired heart disease in children in developed countries[1,2]. The cause of KD remains unknown but is presumably the interaction between genetic and environmental factors, and possibly an infection[3]. Genetic factors have been suspected of contributing to KD development on the basis of the following observations. Children of Asian ethnicity are at higher risk for developing the disease; KD has been reported in most ethnic groups, but the disease is over-represented among Asian and Asian-American populations[4]. The risk of developing KD is higher in siblings of children with KD, especially twins with KD, than in other children. Moreover, children with a parent who had KD as a child also have a higher disease risk[5–7].

BAK1 (BCL2 antagonist killer 1, OMIM*600516) is a proapoptotic member of the Bcl-2 family and is located at 6p21.3[8,9]. Several studies have demonstrated that the overlapping roles of BAK1 and BAX are essential gateways for apoptosis and for maintaining B-cell homoeostasis. Deletion of *Bax* and *Bak* in adult mice results in the accumulation of immature and mature follicular B cells as well as the develop-

ment of severe autoimmune disease[10]. These findings suggest that *BAK1* might play a critical role in the development of autoimmune disease. Since KD may be an autoimmune disorder. Therefore, we examined the association of *BAK1* polymorphism with KD risk in Taiwanese children.

MATERIALS AND METHODS

Study subjects

From 1998 to 2005, we enrolled 93 individuals who fulfilled the diagnostic criteria for KD, according to the Department of Pediatrics, China Medical University Hospital, Taiwan, as previously described by Lin et al[13]. The following clinical parameters were obtained in children with KD: white blood cell (WBC) counts and levels of hemoglobin (Hb), platelet (PLT), alanine aminotransferase (AST), aspartate aminotransferase (ALT), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). The control group consisted of samples from 680 healthy children randomly selected from the Han Chinese Cell and Genome Bank, in which 3312 unrelated descendants of the Han Chinese have been recruited based on their geographic distribution across Taiwan[11,12]. Control subjects were matched for gender and age with the study patients. The study was approved by the Human Studies Committee of China Medical University Hospital, and informed consents were obtained from the participants or their parents.

SNP selection and genotyping

The 5 single nucleotide polymorphisms (SNPs), rs210132, rs210135, rs210139, rs210145, and rs396746, in the *BAK1* gene were reported from the SNP database for genotyping from the National Center for Biotechnology Information. We selected SNPs that have not been previously studied with selection criteria of minor allele frequency over 10% in HapMap-HCB population (Han Chinese in Beijing, China).

For genotyping, genomic DNA was extracted from peripheral blood leukocytes using the QIAamp Blood Kit (Qiagen). SNPs were genotyped using high-throughput matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, as described previously by Lin et al[13]. In addition, about one third of the samples were randomly selected for a predesigned TaqMan assay (Assay ID C_2502580_10, Applied Biosystems) in SNP rs210132. PCR reactions were carried out in a final reaction volume of 5 µl consisting of 2.5 µl of 2 × TaqMan Universal PCR Master Mix, 0.5 µl of 10 × Allelic Discrimination Assay Mix, and 2 µl of DNA (10 ng). PCR was done on an ABI PRISM 7900 Sequence Detection System under the following conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles at 92 °C for 15 s, and 60 °C for 60 s. The genotypes were called automatically and verified manually. The results of the selection samples on the TaqMan assay were 100% concordant with that obtained using the MALDI-TOF assay.

Statistical analysis

Pearson χ^2 or Fisher's exact tests were used to assess the difference of the genotype and allele distributions. Differences in clinical parameters of KD patients between groups were analysed using the Mann-Whitney U test. Hardy-Weinberg equilibrium (HWE) was tested for each marker using the goodness of fit χ^2 test. Haplotype analysis was performed using the HAPLOVIEW program, v4.1[14]. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for the associations between the risk alleles, genotypes, and haplotype with KD in a logistic regression model. Statistical analyses were performed using the Statistical Package for the Social Sciences software package, v15.0 (SPSS Inc.), and $p < 0.05$ was considered significant.

RESULTS

Polymorphisms in the *BAK1* gene

Allelic and genotypic frequencies of *BAK1* genetic polymorphism are shown in Table 1. Genotype frequencies of all analysed SNPs, except rs210135, were in HWE. Therefore, we excluded rs210135 from further analysis. In addition to rs210135, none of the allele or genotype frequencies of the *BAK1* gene polymorphisms showed significant differences between the KD patient group and the control group.

BAK1 polymorphisms and occurrence of CAAs

As shown in Table 2, the frequencies of the SNP rs210132 TT genotype were significantly higher in KD patients without CAA than in control subjects (OR, 1.93 [95% CI: 1.08–3.46]; $p = 0.037$). The genotype frequencies were not significantly different in KD patients with CAA as compared with those of the control subjects. Other *BAK1* genetic polymorphisms were compared between KD patients with CAA and controls or KD patients without CAA and controls, and we did not find any other significant differences in these SNPs.

Table 1 Allelic and genotypic frequency distribution in KD patients and controls.

dbSNP ID	Chromosome position	SNP allele	Subjects	Genotype			Allele 1 vs. Allele 2	Genotype 1/1 vs. 1/2 + 2/2	
		1/2		1/1	1/2	2/2	p value	p value	OR (95% CI)
rs210132	33644648	G/T	KD	31 (33.3)	41 (44.1)	21 (22.6)	0.40	0.84	0.93 (0.59–1.47)
			Control	237 (35.0)	324 (47.9)	116 (17.1)			
rs210135	33648670	A/T	KD	65 (98.5)	1 (1.5)	0	0.0001	2×10^{-7}	22.7 (3.13–165.0)
			Control	492 (74.1)	172 (25.9)	0			
rs210139	33651387	C/A	KD	59 (63.4)	31 (33.3)	3 (3.2)	0.61	0.099	1.49 (0.95–2.32)
			Control	416 (61.8)	220 (32.7)	37 (5.5)			
rs210145	33655418	C/G	KD	66 (71.0)	24 (25.8)	3 (3.2)	0.66	0.75	1.11 (0.69–1.79)
			Control	463 (68.7)	183 (27.2)	28 (4.2)			
rs396746	33665023	C/A	KD	84 (90.3)	9 (9.7)	0	0.54	0.58	1.31 (0.63–2.70)
			Control	592 (87.7)	81 (12.0)	2 (0.3)			

Table 2 Association of *BAK1* gene polymorphisms in KD patients according to the presence or absence of CAA.

dbSNP ID	Genotype	Controls	KD CAA (+)	KD CAA (-)	KD CAA (+) versus Controls		KD CAA (-) versus Controls	
		$n = 680$	$n = 30$	$n = 63$	p value	OR (95% CI)	p value	OR (95% CI)
rs210132	Containing G	561 (82.8)	27 (90.0)	45 (71.4)	0.44	Ref	0.037	Ref
	TT	116 (17.1)	3 (10.0)	18 (28.6)		0.54 (0.16–1.80)		1.93 (1.08–3.46)
rs210139	Containing C	636 (94.5)	28 (93.3)	62 (98.4)	0.68	Ref	0.24	Ref
	AA	37 (5.5)	2 (6.7)	1 (1.6)		1.23 (0.28–5.35)		0.28 (0.04–2.06)
rs210145	Containing C	646 (95.8)	28 (93.3)	62 (98.4)	0.37	Ref	0.50	Ref
	GG	28 (4.2)	2 (6.7)	1 (1.6)		1.65 (0.37–7.27)		0.37 (0.05–2.78)
rs396746	CC	592 (87.7)	28 (93.3)	56 (88.9)	0.52	Ref	0.94	Ref
	Containing A	83 (12.3)	2 (6.7)	7 (11.1)		0.51 (0.12–2.18)		0.89 (0.39–2.02)

Haplotype analysis

We analysed the 4 SNPs, rs210132, rs210139, rs210145, and rs396746, in the *BAK1* gene. Four *BAK1* gene haplotypes with frequencies of more than 5% accounted for approximately 93% of all haplotypes in both KD patients and controls. As shown in Table 3, the frequency of the estimated GAGC haplotype was significantly lower in KD patients than in controls (OR, 0.60 [95% CI: 0.36–1.00]; $p = 0.047$). In addition, the frequency of the TAGC haplotype was significantly higher in KD patients than in control subjects (OR, 9.97 [95% CI: 3.72–26.7]; $p < 0.0001$). These findings suggest that the presence of the GAGC haplotype may have a protective effect against KD, while the presence of the TAGC haplotype may increase an individual's risk of developing KD.

Table 3 Distribution of *BAK1* haplotype frequencies in KD patients and controls (C).

Haplotype	KD (%)	C (%)	OR (95% CI)	p value
GCCC	44.5	41.5	1.13 (0.83–1.54)	0.44
TCCC	35.8	36.3	0.98 (0.71–1.35)	0.91
GAGC	9.7	15.1	0.60 (0.36–1.00)	0.047
TAGC	5.1	0.5	9.97 (3.72–26.7)	2×10^{-8}

Order of SNPs comprising the *BAK1* haplotypes: rs210132, rs210139, rs210145, rs396746.

DISCUSSION

To the best of our knowledge, this is the first study evaluating the role of *BAK1* in the development of KD. Results from this case-control study showed that the frequencies of the SNP rs210132 TT genotype were associated with KD children without CAA. It indicates that rs210132 may be involved in disease susceptibility and progression. We further analysed

the clinical parameters, including WBC, Hb, PLT, AST, ALT, ESR, and CRP in KD patients with different rs210132 genotypes. There were no significant differences in the mean levels of the clinical parameters mentioned above between KD patients with the G/G or G/T genotypes and patients with the T/T genotype (data not shown).

In terms of haplotypes, there were 4 major *BAK1* haplotypes (estimated frequencies $> 5\%$) in our populations. The GAGC haplotype conferred protection against KD development, while the TAGC haplotype conferred the risk of KD development (OR, 0.60 [95% CI, 0.36–1.00]; $p = 0.047$ for haplotype GAGC; OR, 9.97 [95% CI, 3.72–26.7]; $p < 0.0001$ for haplotype TAGC). The difference between haplotypes GAGC and TAGC is due to SNP rs210132. Located in the 3′-untranslated region (3′-UTR) of the *BAK1* gene, rs210132 is suspected to have an effect on the expression level of BAK1, which confers the risk of developing KD. The biological effect of this SNP in KD pathogenesis requires further study. In addition, we also have conducted a haplotype analysis of KD cases with or without CAAs and controls. We did not observe any significant differences between the cases and the controls (data not shown). However, this result may be attributable to the relatively small sample size after stratification of the KD cases. Therefore, larger studies are required to verify our findings.

Elevated BAK expression in autoimmune diseases has been attributed to the simultaneous high local expression of interferon-γ (INF-γ), which is able to induce BAK upregulation in both epithelial and endothelial cells[15,16]. Recently, Kerekes et al and Amezcua-Guerra et al reported that serum concentrations of INF-γ were significantly higher in rheumatoid arthritis (RA) patients and INF-γ levels were associated with vascular endothelial dysfunction in patients with RA[17,18]. Although the etiology of KD remains unknown, activation of the immune system is a central feature and endothelial dysfunction is a key event in the process of atherogenesis of KD[19]. Results from a genetic study indicate that *BAK1* genetic polymorphisms influence the risk of acquiring autoimmune rheumatic diseases in Colombian women[20]. In addition, abnormal BAK1 expression can disrupt the apoptotic or survival signal, which may also result in the development of autoimmune diseases[20]. Since KD is a type of autoimmune vasculitis, this evidence suggests that *BAK1* may be involved in the pathogenesis of KD.

Several genetic association studies have reported that human major histocompatibility complex (MHC) class I and II genes contribute to the pathogenesis of KD[13,21–23]. Since *BAK1* is located in the extended MHC, an indirect association due to linkage disequilibrium with MHC loci must be considered. Another limitation of this study is that the sample size of KD patients is relatively small for a case-control association study; hence, despite the statistically significant data, the results should be interpreted with caution. Future studies with a larger number of subjects are needed to confirm these findings.

In conclusion, we have shown that *BAK1* gene polymorphism influences the risk of developing KD in Taiwanese children. According to our observations, these results may contribute to the genetic background of KD pathogenesis.

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