Article

Analysis of TAP1 and TAP2 Polymorphism of Mother-Infant in Chinese Patients with Pre-Eclampsia

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To analyze the polymorphism of TAP gene and the shared rates of alleles between mothers and their infants in Chinese patients with pre-eclampsia, TAP1 and TAP2 genotyping was performed by the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) in 42 patients, 106 normal pregnant women, and their neonates. The allelic frequency of TAP and the alleles shared in maternal-fetus were compared and analyzed in the two groups. Our results showed that, with totally eight alleles of TAP1 and TAP2 examined in the samples, no significant difference was found in allelic frequencies between pre-eclampsia group and control group, as well as between mothers and their neonates. Similar finding was obtained in the comparison with shared alleles. In conclusion, our results do not support a role for the polymorphisms of TAP in the etiology of pre-eclampsia. ***Cellular & Molecular Immunology*****. 2005;2(2):141-144.**

Key Words: pre-eclampsia, TAP gene, polymorphism

Introduction

Pre-eclampsia is one of the severest complications in pregnancy and represents the leading cause of both fetal and maternal morbidity and mortality. It has a series of maternal systemic syndrome, including hypertension, proteinuria and edema. A key feature of pre-eclampsia is shallow cytotrophoblast invasion into the myometrial portions of the spiral arteries and vascular endothelial cell dysfunction, leading to a poor perfusion of placenta (1, 2). A genetic component to susceptibility is supported by the presence of ethnic and racial differences in disease risk. The nature, location and relative contribution of these genetic influences are largely undefined. So far more than sixty kinds of diseases have been found the relationship with human leucocyte antigen (HLA). Carreiras et al. found several HLA genotypes abnormities were present in mothers with pre-eclampsia and their neonates, including HLA-G*0104, DRB1*07/06 (3). And the significant excess of HLA-DR homozygosity as well as the reduced antigenic variety were also found in pre-eclampsia couples (4).

The TAP1 and TAP2 genes (transporter associated with antigen processing) are localized in the class II region between HLA-DP and DQ. They are members of the ATP binding cassette (ABC) superfamily of transporter genes which play important roles in antigen processing and presentation (5). Although TAP1 and TAP2 genes are mainly involved in the HLA class I antigen-peptide binding, they also play a role in class II-restricted endogenous antigen processing (6). Both TAP1 and TAP2 are polymorphic genes. The TAP gene polymorphism can influence the specificity of peptides preferentially presented by the MHC molecules and the outcome of the immune response.

Since several HLA genotypes are associated with the susceptibility to pre-eclampsia (3), the TAP gene polymorphism may also be involved in this disease. In this study, therefore, the association of TAP gene polymorphism of mother-infant in Chinese patients with pre-eclampsia was investigated.

Materials and Methods

Subject selection

This study included a cohort of 42 patients with pre-eclampsia between Feb 2004 and Jun 2004 in Zhengzhou city from Henan province. All the pre-eclampsia patients met the criteria from the classification of pre-eclampsia described by "Obstetric and Gynecology" (9). The pregnant women in this study had an average age of 27.31 ± 8.43, average

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Table 1. Comparison of TAP allelic frequency between patients with pre-eclampsia and controls

TAP	Controls (n = 106)		Patients (n = 42)		χ^2	p
	n	Frequency (%)	n	Frequency (%)		
TAP1*0101	89	83.96	41	97.62	5.251	0.022[a]
TAP1*0201	34	32.08	10	23.81	0.984	0.321
TAP1*0301	61	57.55	27	64.29	0.567	0.452
TAP1*0401	5	4.72	3	7.14	0.346	0.556
TAP2*0101	66	62.26	28	66.67	0.252	0.616
TAP2*0102	16	15.09	6	14.29	0.016	0.901
TAP2*0103	24	22.64	3	7.14	4.845	0.028[b]
TAP2*0201	84	79.25	39	92.86	3.97	0.046[c]

[a] $p_c = 0.022 \times 4 = 0.088$; [b] $p_c = 0.028 \times 4 = 0.112$; [c] $p_c = 0.046 \times 4 = 0.184$. All $p_c > 0.05$.

Table 2. Comparison of TAP allelic frequency between neonates of patients with pre-eclampsia and those of controls

TAP	Controls (n = 106)		Patients (n = 42)		χ^2	p
	n	Frequency (%)	n	Frequency (%)		
TAP1*0101	95	89.62	38	90.48	0.024	0.877
TAP1*0201	32	30.19	11	26.19	0.233	0.629
TAP1*0301	64	60.38	28	66.67	0.506	0.477
TAP1*0401	5	4.72	3	7.14	0.346	0.556
TAP2*0101	63	59.43	24	57.14	0.065	0.799
TAP2*0102	14	13.21	8	19.05	0.811	0.368
TAP2*0103	23	21.70	4	9.52	2.989	0.084
TAP2*0201	91	85.85	38	90.48	0.576	0.448

pregnant week of 37.15 ± 3.10. The 106 normal pregnant women at term were chosen as control group. All subjects in the two groups had no consanguinity relationship. No significant difference in comparison of their ages and gestational ages.

Genomic DNA extraction

By standard phenol-chloroform extraction method genomic DNA was isolated from peripheral blood and umbilical blood (10). Adjusted concentration of DNA is 100 μg/ml.

TAP genotyping by ARMS-PCR

Tewelve pairs of special TAP primers for the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) were designed according to published protocol (10, 11). A PCR mixture is prepared containing DNA 1 μl, specific primer 0.5 μmol/L, 1 μl dNTP, 10 × buffer 1 μl and 0.3 U/well Taq DNA polymerase. PCR amplification was performed by dipol-temperature cycler: preparing denature at 96°C for 2 min and followed by 5 cycles of 96°C for 15 s, 70°C for 60 s, 72°C for 30 s, then 25 cycles of 96°C for 15 s, 65°C for 1 min, 72°C for 40 s, and finished with 72°C for 10 min. PCR products are sized-resolved by standard electrophoresis in a 2% agarose gel and detected by staining with ethidium bromide (12).

Statistical analysis

The allelic frequencies of TAP1 and TAP2 were obtained by direct counting. Chi-square and Fisher exact tests were used to compare the difference between patients and controls. Corrected p values (p_c) were obtained by multiplying the value of allele (TAP, n = 4). $p_c < 0.05$ was considered statistically significant.

Results

The allelic frequencies of TAP1 and TAP2 in patients with pre-eclampsia and normal pregnant women

The frequency of TAP1*0101 (83.96%) was highest in checked 4 kinds of TAP1 alleles in all samples. The frequency of TAP2*0201 (79.25%) was highest in checked 4 kinds of TAP2 alleles in all samples. The frequencies of TAP1*0101 and TAP2*0201 were higher in patients with pre-eclampsia than that in normal pregnant women, but the value of p_c was not significant ($p_c > 0.05$); the frequencies of TAP2*0103 was lower in patients with pre-eclampsia than

Table 3. Comparison of TAP shared allele in materno-fetus between patients with pre-eclampsia and controls

TAP	Controls (n = 106)		Patients (n = 42)		χ^2	p
	n	Frequency (%)	n	Frequency (%)		
TAP1*0101	84	79.25	38	90.48	2.620	0.106
TAP1*0201	17	16.04	7	16.67	0.009	0.925
TAP1*0301	44	41.51	21	50.00	0.880	0.348
TAP1*0401	2	1.89	1	2.38		1.000
TAP2*0101	47	44.34	18	42.86	0.027	0.870
TAP2*0102	6	5.66	1	2.38	0.175	0.676
TAP2*0103	9	8.49	2	4.76	0.187	0.666
TAP2*0201	78	73.58	31	73.81	0.001	0.978

that in normal pregnant women, but the value of p_c was also not significant (Table 1).

The allelic frequencies of TAP1 and TAP2 in neonates of patients with pre-eclampsia and normal pregnant women

The frequency of TAP1*0101 (89.62%) was highest in checked 4 kinds of TAP1 alleles in all samples. The frequency of TAP2*0201 (85.85%) was highest in checked 4 kinds of TAP2 alleles in all samples. There was no significant difference in all allelic frequencies ($p > 0.05$) (Table 2).

TAP shared gene in mother and infant of patients with pre-eclampsia and of normal pregnant women

The same genotype which mother and infant shared in the TAP locus was so-called TAP share gene. No significant difference was detected in share gene of TAP1 and TAP2 in the two groups (Table 3).

Discussion

Pre-eclampsia is one of the main reasons for the death of women in procreation period in the world. Immunological hypothesis, one of its pathogenesis, suggested that pre-eclampsia was an autoimmune disease or autoimmune response against the fetus alloantigen (13). That the clinical disorders disappeared spontaneously after delivery suggested that paternal antigen carried by fetus may contribute to the development of pre-eclampsia. Increasing evidences indicated that syncytiotrophblast lack of the expression of classical major histocompatibility complex (MHC) antigen was imperfection as materno-fetal barrier because fetal RBC, trophoblast cells, etc. could be found in maternal blood. During normal gestation about 10^5 trophoblast cells entered into the matrix and formed immune complex with maternal antibody, then were phagocytized by reticuloendothelium in matrix. The trophoblast cells in uterine veins of patients with pre-eclampsia were twenty-fold higher than those of controls. Therefore, studies on genes correlative with the processing and presentation of fetus semi-alloantigen will contribute to the etiology of pre-eclampsia.

TAP encodes a heterodimer complex, which is responsible for the transport of processed cytoplasmic peptide fragments (8~10 aa) across the endoplasmic reticulum in an ATP-dependent manner. It is required for the processing of peptides that bind to MHC class I molecules and for the stable presentation of them at the cell surface (14). It was found that in mutant B cell lines lack of TAP gene, the quantity and stability of MHC class II molecules was not altered, but proliferation of MHC class II molecules stimulated by autoreactive T cells was significantly decreased and restored after transfection by TAP cDNA (15). It suggested that TAP also participated in T cells for the recognition and activation of MHC class II molecules.

Polymorphism of TAP genes may change quaternary structure of TAP protein and affect its substrate specificity. Therefore, it is of interest to investigate whether stronger presentation of TAP to fetus antigen contributes to the pathogenesis of pre-eclampsia. TAP gene also adjusted the complete expression of HLA-G. The previous studies showed that there were defections in expression of placental HLA-G mRNA and protein in pre-eclamptic patients.

In this study, we have clearly demonstrated that no single alleles of TAP1 and TAP2 are associated with pre-eclampsia, nor are the gene shared rate of TAP. Although the frequencies of TAP1*0101 and TAP2*0201 increased in patients group, it failed to retain significance after p corrected, this may due to the small sample size, we still need a large population to reach a conclusion. Further studies, such as comparison of TAP expression in transcription and protein level, are also required to elucidate the role of TAP in the pathogenesis of pre-eclampsia.

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