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No association between Fas A/G polymorphism and therapeutic effects induced by methimazole treatment for Graves' disease in Northern Chinese

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Fas gene, which is related to apoptosis, influences the clinical remission of Graves' disease (GD). This study was performed to determine whether the Fas gene promoter polymorphism at position-670 is associated with different efficacy in GD patients treated with methimazole (MMI). Serum levels of thyroid hormone, sFas and thyrotropic-stimulating hormone receptor antibodies (TRAb) were evaluated as therapeutic efficacy parameters before and after 18-month treatment with MMI in a total of 115 subjects of North China. At the end of the follow-up study, patients were categorized into an effective group (group A) and an ineffective group (group B) according the level of thyroid hormone. Similar to the changes of thyroid hormone, the reductions of sFas and TRAb were significantly different in the two groups ($P < 0.05$). Meanwhile, we detected that Fas promoter-670 A/G polymorphism existed in the GD patients of North China. We studied the frequencies of genotypes and alleles of the A/G polymorphism in different groups. However, there was no association between different efficacy and Fas promoter polymorphism in patients with GD.

1. Introduction

Graves' disease (GD) is an autoimmune disorder with genetic predisposition. Methimazole (MMI) as a kind of antithyroid drugs is widely used in the majority of patients with GD. It is common knowledge that the helpful effect gained from MMI is due to blockage of thyroid hormone synthesis by inhibiting thyroid peroxidase. Recently, many researchers have paid close attention to another mechanism of MMI about the capacity of interfering with immunological regulation which is mediated by Fas (Tsatsoulis 2002). Fas, a member of the tumor necrosis factor receptor family, is related to the immunomodulatory remission of GD (Hara et al. 2002). The Fas A/G polymorphism in the promoter region at position-670 has an important functional significance (Kantarci et al. 2004). Mahfoudh et al. found that the polymorphism resulted in various transcriptions and expressions of Fas (Ueda et al. 2006; Mahfoudh et al. 2007). Soluble Fas (sFas) and thyrotropic-stimulating hormone receptor antibodies (TRAb) are vital immunological mediators of GD and play important roles in the development of GD. sFas and TRAb can be used as indicators of immune response progress (Wang et al. 2005; Cappelli et al. 2007). It has been well documented that MMI can significantly decrease the titers of sFas and TRAb (Hara et al. 2002; Carella et al. 2006). In clinic practice, it is common that people have different curative effects when they are treated with the same medicine. Administration of MMI is such an example. We wondered whether the different efficacy was related to the polymorphism. To our knowledge, the association between the disparity of curative effects and the polymorphism was not clear. Therefore, we analyzed the A/G polymorphism in GD patients of a Chinese population. Furthermore, the possible reason for different efficacy of MMI was investigated by assessing the correlation between the Fas polymorphism and the serum levels of related indexes.

2. Investigations and results

Distribution of the polymorphism was analyzed with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The Fas A/G polymorphism distribution was 43.48% for the AA genotype, 51.30% for the AG genotype, 5.22% for the GG genotype and was 69.13% for the A allele, 30.87% for the G allele in Group A. The distribution was in Hardy-Weinberg equilibrium.
Serum FT4, TRAb and sFas concentrations were determined by chemoluminescence method., radioreceptorassay and enzyme linked immunosorbent assay respectively. The levels of FT4, TRAb and sFas were significantly lower after treatment than their initial values ($P < 0.05$, Table 1).

Table 1: Comparison of the serum FT4, TRAb and sFas concentrations before treatment and after 18-month treatment

Time	N	FT4 (pmol/L)	TRAb (IU/I)	sFas (pg/ml)
1	115	$1.83 \pm 0.16^{a*}$	$1.03 \pm 0.26^{a*}$	$458.32 \pm 155.20^{*}$
2	115	1.21 ± 0.18^{a}	0.73 ± 0.26^{a}	235.46 ± 111.28

1: before treatment 2: after the 18 month treatment
[a] Data are converted into logarithms
* P values are considered significant if lower than 0.05

Table 2: Comparison of the serum FT4, TRAb and sFas concentrations before MMI treatment between group A and group B

Group	N	FT4 (pmol/L)	TRAb (IU/I)	sFas (pg/ml)
A	85	1.82 ± 0.18[a]	1.03 ± 0.29[a]	454.94 ± 167.07
B	30	1.86 ± 0.08[a]	1.02 ± 0.19[a]	467.90 ± 117.07

[a] Data are converted into logarithms

After 18 months treatment, patients were divided into two groups: an efficient group (group A) and an inefficient group (group B) according to their serum thyroid hormone levels. Before MMI treatment, the FT4 concentrations were not significantly different between the two groups ($P > 0.05$). The concentration of TRAb in group A was not significantly different from that in group B, neither was the sFas ($P > 0.05$). The above data indicated that these indexes had comparability between group A and group B (Table 2).

As shown in Table 3. The patients with GD in group A showed a greater reduction in serum sFas levels than those in group B ($P < 0.05$). Patients in group A also exhibited a greater TRAb concentration reduction than those in group B ($P < 0.05$).

The frequency of the genotypes and alleles in groups A and B are also presented in Table 3. There was no significant difference in the genotype and allele frequencies between group A and group B ($\chi^2 = 2.528$, $P > 0.05$; $\chi^2 = 1.311$, $P > 0.05$).

3. Discussion

Clinical trials found that serum sFas levels increased before treatment and decreased after administration with MMI in GD patients (Wu et al. 2000; Feng 2004). The same results were obtained in this study that the serum sFas concentrations declined significantly after MMI administration. Thyroid hormone was generally used to judge efficacy in clinical practice. According to the serum FT4 levels, patients were divided into an efficient group and an inefficient one. In this study we observed the serum sFas level, being an indicator, decreased differently between the two groups, and the levels in group A declined more dramatically compared with those in group B. It was indicated that, to a certain extent, the evaluation of the immunological effect of MMI was in consistence with the result of the clinical efficacy of this drug. Meanwhile, we detected that the Fas A/G polymorphism existed in the 115 GD patients of Northern China. The distribution of AA, AG, GG genotypes and A, G alleles between the two groups was not statistically different. We concluded that the inhibitory action of MMI on sFas varied in different individuals, but there was no association between the different efficacy and A/G polymorphism. An analysis of the relationship between transplantation effects and Fas A/G polymorphism was conducted in African-Americans and Caucasians patients by Girnita et al. They found that the transplantation effects were associated with the polymorphism (Girnita et al. 2006). Zhengdong Zhang et al. also conducted a study about the Fas-670 A/G polymorphism and progression of squamous cell carcinoma of the head and neck in non-Hispanic White patients (Zheng et al. 2006). The results showed that the frequencies of genotypes and alleles were AA 26.5%, AG 50.3%, GG 23.2%, A 51.70%, and G 48.30%, respectively. The frequencies of the Fas-670 gene polymorphism was 43.5% for the AA genotype, 51.3% for the AG genotype, 5.2% for the GG genotype and was 69.1% for the A allele, were AA 43.5%, AG 51.3%, GG 5.2%, A 69.1%, and G 30.9% in the patients with GD of this study. We can see that the distribution of Fas genotype and allele have great diversity in different population. It has been postulated that gene function may influence the degree to which allele frequencies differ among populations. So we cannot rule out the possibility that the association maybe exist in another population. But the speculation needs to be confirmed by further studies. Cappelli et al. proposed that the evaluation of TRAb titers or their falling rates was a better predictor of remission with higher sensitivity and specificity (Cappelli et al. 2007). MMI can reduce or eliminate antibody production mediated by Fas. We studied the relationship between TRAb titers and the Fas A/G polymorphism within the promoter region. The results showed that serum TRAb levels dropped more noticeably in group A than those in group B, and the distribution of AA, AG, GG genotypes and A, G alleles had no significant difference between the two groups. The influence of MMI on TRAb elimination mediated by the three genotypes and two alleles was similiar in the two groups.

The results of the study suggest that there is no association between the different curative effects and Fas promoter-670 A/G polymorphism. Nevertheless, the mechanism of drug action is complex and relevant mechanisms have not yet been fully clarified. Further studies are needed to define the reason for different efficacy.

4. Experimental

4.1. Study design and subjects

We studied the patients with GD who were seen in the Second Affiliated Hospital of Harbin Medical University. They had no other autoimmune disorders. The diagnosis of GD was on the basis of history and the laboratory findings, including elevated serum free triiodothyronine (FT_3), free thyroxine (FT_4) and suppressed thyrotropic-stimulating hormone (TSH) concentrations, positive TRAb and imaging diagnosis. All patients were treated with MMI at an initial dose of 30 mg/d, which was reduced gradually according to serum thyroid hormone concentration. Patients were examined every 6–8 weeks. Serum sFas, TRAb levels were measured at the onset of diagnosis and 18 months after treatment with MMI. Finally, 26 of

Table 3: Comparison of the efficacy before and after treatment and the distribution of genotype and allele frequencies between group A and group B

Group	N	$sFas^{(t0\text{-}t2)}$ (pg/ml)	$TRAb^{(t0\text{-}t2)}$ (IU/I)	Genotype and genotype frequencies (%)			Allele and allele frequencies (%)	
				AA	AG	GG	A	G
A	85	240.54 ± 101.59*	0.73 ± 0.32[a]*	35(41.18)	44(51.76)	6(7.06)	114(67.06)	56(32.94)
B	30	172.79 ± 39.55	0.60 ± 0.21[a]	15(50.00)	15(50.00)	0(0)	45(75.00)	15(25.00)

[a] Data are converted into logarithms
t0-t2: Differenece between the serum levels before treatment and after 18 months treatment with MMI
* P values are considered significant if lower than 0.05

the 141 patients had not been followed up in our study: 4 patients underwent subtotal thyroidectomy because of the side effects of MMI, 14 patients exodused for bad compliance, 8 patients needed thyroxine vicarious treatment due to hypothyroidism. The remaining 115 patients (78 females, 37 males, 31.30 ± 11.63 years of age) completed the follow-up period successfully.

At the end of the follow-up study, patients were divided into an effective group (group A, n = 85) and an ineffective group (group B, n = 30) according to the serum level of thyroid hormone. In group A, thyroid hormone decreased gradually and reached the normal level. The clinical symptoms disappeared, so they received the minimum dosages of MMI. In group B, the thyroid hormone was not in a euthyroid state; therefore they did not receive the minimum dosages. There was no significant difference of age and sex ratio in the two groups.

The study plan was reviewed and approved by the Institutional Ethics Committee of Harbin Medical University and informed consent was given by all patients. All subjects in this study were Northern Chinese.

4.2. Laboratory test and genotyping techniques

Serum concentrations of thyroid hormone and TSH were measured by chemoluminescence method. TRAb was measured by radioreceptor assay using a commercial kit (Medipan GmbH, Germany). Serum sFas was determined by enzyme linked immunosorbent assay (ELISA) with commercial kits (Diaclone, France).

Genomic DNA was extracted from peripheral white blood cells by standard procedures. We analyzed genotypes and alleles with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). PCR were performed using 400 ng genomic DNA, 2 U Taq DNA polymerase, 15 pmol of each primers (F: 3′-5′TTAGCGTATCGGGGTCGATA, R: 3′-5′TACCTGTCGGGTCAGTTTAC), 10 mmol deoxy-NTPs under the following conditions: 28 cycles of PCR consisting of 30 s at 94 °C, annealing for 30 s at 58 °C, extension for 40 s at 72 °C and a final extension for 8 min at 72 °C. The amplified products were digested with the restriction enzyme, ScrFI (New England BioLabs, USA) at 37 °C. The fragments were resolved on an 8% polyacrylamide gel and genotypes assigned accordingly. Digestion of 10 µl Fas PCR product with 2 U enzyme ScrFI at 37 °C overnight resulted in an uncut band of 193 bp when Fas only had the A allele. The G allele of the gene was dissected into two bands of 136 bp and 57 bp. When Fas had the two alleles, Fas gene was cut into three bands (193 bp, 136 bp and 57 bp) (Fig.).

4.3. Statistical analysis

All values were expressed as mean ± SD (Data were converted into logarithms to obtain a normal distribution). Comparison between groups was assessed with t-test. Genotype and allele frequencies were estimated by direct gene counting. Observed numbers of each genotype and allele were compared by using Hardy-Weinberg equilibrium for group representation. Allelic and genotypic frequency distributions were compared between groups using Chi-square test. Statistical analysis was performed by using SPSS 13.0 for Windows. P value <0.05 was considered statistically significant.

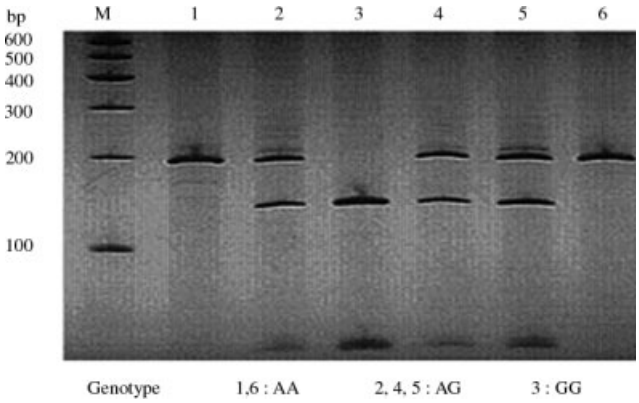

Fig.: Fas gene polymorphism: 1,6: AA genotype 2, 4, 5: AG genotype 3: GG genotype

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