

FK506-binding protein 51 (FKBP5) gene polymorphism is not associated with glucocorticoid therapy outcome in patients with idiopathic thrombocytopenic purpura

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Abstract. The FKBP5 gene codes for the FK506-binding protein 51 (FKBP5), a co-chaperone of hsp90, which regulates glucocorticoid (GC) receptor sensitivity. The FKBP5 gene single nucleotide polymorphisms (SNP), rs1360780, has been found to modulate GC sensitivity in stress-related psychiatric disorders. The aim of the present study was to examine the effects of rs1360780 on the treatment outcome of patients suffering from idiopathic thrombocytopenic purpura (ITP) administered with GC. The polymorphism of FKBP5, rs1360780, was genotyped in 55 GC-resistant ITP patients, 157 GC-sensitive ITP patients and 110 unrelated healthy individuals using real-time PCR and cycling probe technology with DNA extracted from peripheral blood. No significant differences in FKBP5 rs1360780 genotypes ($P=0.51$) and alleles ($P=0.89$) were observed between the GC-resistant ITP patients and the healthy controls. There were no significant differences observed between the GC-sensitive ITP patients and the healthy controls ($P=0.40$ for genotypes and $P=0.62$ for T allele), as well as between the GC-sensitive ITP patients and the GC-resistant patients ($P=0.67$ for genotypes and for T allele). The present study demonstrates that the FKBP5 polymorphism may not affect the response of ITP patients to GC treatment.

Introduction

Idiopathic thrombocytopenic purpura (ITP) is considered to be an organ-specific autoimmune disorder characterized by a severe decrease in platelet numbers and mucocutaneous bleeding (1). ITP-associated platelets are opsonized by autoantibodies and prematurely destroyed by the reticuloendothelial

system (2-4). The incidence of ITP is estimated to be 6 cases per 100,000 individuals per year in Western developed countries (5). Glucocorticoids (GCs) in combination with romiplostim are currently the mainstay of ITP therapy (6-7). Generally, the majority of patients with ITP respond favorably to GCs and never experience serious bleeding even with severe thrombocytopenia. However, a fraction of patients with ITP develop GC resistance following 3-6 months of standard treatment with GC (8). The mechanisms by which GC resistance develops are not yet fully understood. Three possible mechanisms that could lead to the development of GC resistance have been proposed: reduced numbers of glucocorticoid receptors (GRs), altered affinity of the ligand for GRs and reduced ability of the GRs to bind the DNA (9).

The FKBP5 gene is located on the short arm in human chromosome 6 and consists of 10 exons (10). The FKBP5 gene codes for FK506-binding protein 51 (FKBP5), a co-chaperone of hsp90, which regulates GR sensitivity (11). Functionally, FKBP5 expression is induced by steroids which activate GR elements (12). In turn, *in vitro* experiments have demonstrated that FKBP5 reduces the hormone binding affinity and decreases the amount of activated GR translocation to the cell nucleus (13).

FKBP5 is recognized to be expressed as several polymorphic variants, but the FKBP5 gene single nucleotide polymorphism (SNP), rs1360780, has been shown to modulate GC sensitivity in stress-related psychiatric disorders and has been associated with altered response to antidepressant drugs (14,15). FKBP51 mRNA expression in the peripheral blood has been used to determine individual sensitivity to corticosteroids in an *in vitro* bioassay (12). In addition, Woodruff *et al* identified FKBP51 as a biomarker of GC responsiveness and a potential mediator of GC-resistant asthma (16). However, it remains unknown whether rs1360780 may influence FKBP5 protein levels to develop GC resistance in ITP. The aim of the present study was to examine the effects of rs1360780 on the treatment outcome in patients suffering from ITP administered with GC.

Subjects and methods

Subjects and study design. A total of 212 Chinese patients with ITP and 110 unrelated healthy individuals were recruited from

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Table I. Clinical characteristics of ITP subjects.

Clinical characteristics	Group		
	ITP (GC-R)	ITP (GC-S)	Control
Number of subjects	55	157	110
Gender (male:female)	23:32	53:104	43:67
Age at study entry, median (range in years)	37 (20-74)	40 (18-75)	41 (19-70)
Disease course, median (range in years)	1.2 (0.3-37)	1.0 (0.1-38)	

ITP, idiopathic thrombocytopenic purpura; GC-S, glucocorticoid-sensitive; GC-R, glucocorticoid-resistant.

Table II. Distribution and statistical analysis of rs1360780 allelic and genotypic frequencies in control and ITP patients.

FKBP5 rs1360780	Frequency			P-value	Odds ratio (95% CIs)
	ITP (GC-R) ^a	Control ^b	ITP (GC-S) ^c		
Number of subjects	55	110	157		
Allele					
C	86 (0.78)	169 (0.77)	234 (0.75)	0.89 ^a	0.92 ^a (0.53-1.60)
T	24 (0.22)	51 (0.23)	80 (0.25)	0.62 ^b	1.13 ^b (0.76-1.70)
				0.52 ^c	1.23 ^c (0.73-2.06)
Genotype					
CC	32 (0.59)	65 (0.59)	83 (0.53)	0.51 ^a	
CT	22 (0.39)	39 (0.36)	68 (0.43)	0.40 ^b	
TT	1 (0.02)	6 (0.05)	6 (0.04)	0.67 ^c	

^aITP (GC-R) vs. healthy controls; ^bITP (GC-S) vs. healthy controls; ^cITP (GC-S) vs. ITP (GC-R). CI, confidence intervals; ITP, idiopathic thrombocytopenic purpura; GC-S, glucocorticoid-sensitive; GC-R, glucocorticoid-resistant.

The First Affiliated Hospital of Dalian Medical University, Dalian, China, between January 2009 and December 2011. The patients were treated with GC and classified into 2 groups: GC-resistant and GC-sensitive (Table I). Among the patients, 55 patients were classified as GC-resistant, while 157 patients were classified as GC-sensitive. The criteria for the outcome of GC therapy (success or failure) was based on the practice guidelines of the American Society of Hematology (2) and guidelines by the General Haematology Task Force of the British Committee for Standards in Haematology (5). The Research Ethics Committee approved the study. After obtaining informed consent, all patients and controls were recruited for clinical assessment and collection of blood samples for extraction of genomic DNA.

SNP selection and genotyping. DNA was extracted from the peripheral blood using a blood extraction kit (Takara Bio, Inc., Shiga, Japan) according to the manufacturer's instructions. Real-time PCR and cycling probe technology (Takara) was used to detect the FKBP5 rs1360780 SNP. The primer design was based on published sequences for genotyping the FKBP5 polymorphism using genomic DNA. The primers and probes were as follows: 5'-AGC ACA TAA TAG CAA TTC ACA-3' (forward primer), 5'-TGG CTT TAG TCA CAT TAA ATA G-3'

(reverse primer), 5'(Eclipse)-CAA AGT TAC ACA-(FAM)3', and 5'(Eclipse)-ATA CAA AAC AAA A-(ROX)3', where underlined characters indicate the RNA (17). The PCR amplification and fluorescence detection were performed using a Roche Light Cycler 480 (Roche Bio Inc., Rotkreuz, Switzerland). Cycle conditions were as follows; holding at 95°C for 30 sec, 45 cycles of denaturation at 95°C for 5 sec, primer annealing at 55°C for 10 sec, and elongation at 72°C for 20 sec. Fluorescence was measured at 72°C.

Statistical analysis. SPSS 13.0 was used for statistical analysis. Allelic and genotypic frequencies were analyzed using the Chi-square test or Fisher's exact test in case of expected frequencies <5. Odds ratios (ORs) and 95% confidence intervals (CIs) and were also calculated. P<0.05 was considered to indicate a statistically significant difference.

Results

The distribution of CC, CT and TT genotypes was 59, 39 and 2%, respectively. The allelic frequencies of C and T in the GC-resistant ITP patients were 78 and 22%, respectively (Table II). The distribution of CC, CT and TT genotypes was 59, 36 and 5%, respectively. The allelic frequencies of C

and T in the healthy control patients were 77 and 23%, respectively. No significant differences were observed in FKBP5 rs1360780 genotypes ($P=0.51$) and alleles ($P=0.89$; OR, 0.92; CI, 0.53-1.60) between the GC-resistant ITP patients and the healthy controls. The distribution of CC, CT and TT genotypes was 53, 43 and 4%, respectively. The allelic frequencies of C and T in the GC-sensitive ITP patients were 75 and 25%, respectively. There were no significant differences observed between the GC-sensitive ITP patients and the healthy controls ($P=0.40$ for genotypes and $P=0.62$; OR, 1.13; CI, 0.76-1.70 for T allele), as well as between the GC-sensitive ITP patients and the GC-resistant patients ($P=0.67$ for genotypes and $P=0.52$, OR, 1.23; CI, 0.734-2.06 for T allele).

Discussion

GCs are currently one of the most widely used anti-inflammatory agents. SNPs in the FKBP5 gene affecting the sensitivity of GC have been investigated in stress-related psychiatric disorders, asthma, Crohn's disease and ulcerative colitis (14,16,18); however, no studies have examined the correlation between FKBP5 polymorphisms and GC efficacy in Chinese cases with ITP. In the present study, we evaluated the effects of the FKBP5 polymorphism, rs1360780, on the efficacy of GC treatment for ITP cases. We observed no significant differences in FKBP5 rs1360780 genotypes and alleles between the GC-resistant ITP patients and the healthy controls, between the GC-sensitive ITP patients and the healthy controls, as well as between the GC-sensitive ITP patients and the GC-resistant patients. Our results demonstrate that the rs1360780 SNP of FKBP5 may not be associated with GC treatment response.

Since FKBP5 protein expression may have an impact on cellular sensitivity to systemic GC treatment (19), abnormalities in FKBP5 SNPs may contribute to decreased GC responsiveness. Previous studies have suggested that FKBP5 polymorphisms are associated with GC sensitivity. Hubler and Scammell reported that polymorphisms of the FKBP5 gene (rs1360780) are linked with an increased FKBP5 protein expression; the GR regulatory region of FKBP5 mRNA is located in the region <200 bp away from the highly conserved and functional hormone response element in intron 2 of the gene (20). Ising *et al* showed that compared to the patients carrying the other alleles, the high-induction alleles of FKBP5 were associated with GR resistance in healthy controls and enhanced GR sensitivity in depressed patients (21). In addition, the rs1360780 T allele was associated with an increased cortisol response as demonstrated by investigating the cortisol reactivity of 14-month-old infants during a stress test (22). These data suggest that FKBP5 polymorphisms affect GC sensitivity. However, similar to our results, other studies have demonstrated an association between FKBP5 polymorphisms and GC sensitivity. Sarginson *et al* duplicated findings by Binder *et al* (14) in a cohort of 246 elderly patients who suffered major depression treated with either mirtazapine or paroxetine, but could not identify polymorphisms in FKBP5 gene affecting GR sensitivity (23). Furthermore, Papiol *et al* did not identify any association between FKBP5 genotypes and GC sensitivity in a Spanish study using citalopram (24). In addition, Maltese *et al* reported that the rs1360780 SNP of FKBP5 was not associated with glucocorticoid resistance in Crohn's disease and ulcerative colitis (18).

These inconsistencies may arise from the environmental and ethnic differences between the populations in the collected samples. Although the data concerning polymorphisms of FKBP5 gene influence on GC sensitivity are so far inconclusive and controversial, by comparing previous studies the present study provides further evidence supporting the close correlation between FKBP5 polymorphism and GC sensitivity.

In conclusion, this is the first attempt to correlate the response to GC with FKBP5 gene polymorphisms in ITP patients. However, several main limitations should also be considered in this study. Firstly, due to the limited availability of patient samples, our study included relatively small sample sizes. Larger case-control studies are required to further assess the role of the FKBP5 rs1360780 polymorphism and GC therapy in ITP patients. Secondly, selection of the SNP was based on FKBP5 rs1360780; thus, we cannot fully exclude the possibility that other SNPs located somewhere within the gene locus are also associated with GC therapy. Nevertheless, the present study demonstrates that the FKBP5 polymorphism does not affect the response of ITP patients to GC treatment.

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