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Full-length article

Effect of *MDR1* gene polymorphism on progression of end-stage renal disease[1]

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Key words

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Abstract

Aim: P-glycoprotein is localized at the apical brush-border membrane of the proximal renal tubule and functions as extruding toxins and xenobiotics out of cells. The difference of P-glycoprotein's function resulted from single nucleotide polymorphisms in *MDR1* (multidrug resistance gene encoding for P-gp) and may be the cause of interindividual differences in susceptibility to end-stage renal disease (ESRD). The purpose of this study is to compare the genotype frequency of C3435T and G1199A polymorphisms in *MDR1* between ESRD patients and healthy controls in the Chinese population to determine whether the alteration of the P-gp function is associated with ESRD. **Methods:** Two hundred and eighty-four healthy Chinese controls and 244 Chinese patients with ESRD were involved in this study. Allele specific PCR and polymerase chain reaction-restriction fragment length polymorphism assay were used to determine the genotype *MDR1* G1199A and C3435T, respectively. **Results:** The genotype distribution of 3435CC, 3435CT, and 3435TT were 0.35, 0.50, and 0.15, respectively, in the control group and 0.38, 0.47, and 0.15 in the group with the ESRD patients. No variant allele 1199G>A was found in any of the patients. The value of serum creatinine for genotypes 3435CC, 3435CT, and 3435TT in the ESRD patients were 753.8±276.0 μmol/L, 849.6±342.2 μmol/L, and 987.0±512.0 μmol/L, respectively. The difference between 3435TT and 3435CC reached statistical significance ($P<0.05$). **Conclusion:** The low expression of P-glycoprotein was not the etiological factor for the kidney disease, but it may contribute to the progression of ESRD and affect the severity. Chinese people do not carry the 1199G>A variant allele. More studies are needed to clarify the cause and interindividual differences in the susceptibility for the risk of ESRD.

Introduction

The *MDR1* (multidrug resistance gene encoding for P-gp) gene product P-glycoprotein (ABCB1) is a membrane protein, which functions as an ATP-dependent exporter of xenobiotics from cells. P-glycoprotein is expressed in normal tissues with excretory function such as the intestine, liver and kidneys, in capillary endothelial cells of brain, placenta, and testis and in peripheral blood cells[1]. In kidneys, P-glycoprotein is expressed in the brush border membrane of proximal tubular cells[1,2]. It mediates active secretion of its substrates into urine. Renal P-glycoprotein is likely to function as a protective mechanism against toxic substances in the glomerular filtrate. Both clinical and experimental studies have reported the renoprotective effects of removing uremic toxins by peritoneal dialysis and oral charcoal adsorbent in delaying the progression of chronic renal disease[3–7]. Thus, individuals with a low renal P-glycoprotein expression would potentially be exposed to higher concentrations of toxic agents and should be more susceptible to their damaging effects.

Multiple mutations were found in the human *MDR1* gene[8,9]. We selected two single nucleotide polymorphisms (SNP) that had been previously reported to be associated with the ex-

pression or activity of *MDR1*. People with mutation C3435T were associated with a lower P-glycoprotein expression in the kidneys, compared with subjects homozygous for the wild-type allele[10,11]. Another SNP G1199A has also been reported to increase the intracellular accumulation of rhodamine-123 *in vitro*[12].

As uremic toxins have been suggested to promote the progression of chronic renal failure by damaging tubular cells, based on these observations, we hypothesized that genetically predisposed subjects carrying T mutation allele at C3435T or the A mutation allele in G1199A might be at high risk for developing end-stage renal disease (ESRD). Therefore, we decided to examine whether *MDR1* is a susceptible gene for renal disease in patients.

Materials and methods

Subjects We studied 244 ESRD patients from the Division of Nephrology (Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China), and 284 healthy controls. Clinical information and biochemical parameters were retrieved retrospectively from hospital records. The subject characteristics are presented in Table 1. According to the "Practice of Internal Medicine"[13], patients with serum creatinine >442 μmol/L were allocated to the ESRD group. Two hundred and eighty-four healthy patients were randomly selected and used for comparison with the ESRD patients. The healthy patients were determined by their medical history, physical examination, routine blood tests, and electrocardiography, and had no history of hypertension, diabetes, renal failure, vascular disease, stroke and cardiomyopathy. This research was approved by the Ethics Committee of Ruijin Hospital. Informed consent was obtained from the patients and controls participating in the study, and the hospital ethical committee approved the study.

Table 1. Clinical characteristics of the study subjects. Data are mean±SD.

	ESRD	Control
Total (*n*)	244	284
Sex (M/F)	128/116	155/129
Age (years)	44±12	27±5
Serum creatinine (μmol/L)	>422	47±8
Cause of ESRD (*n*)		
Diabetic nephropathy	83	–
Nephrosclerosis	61	–
Glomerulonephritis	39	–
Other	61	–

DNA isolation and genotyping analysis Genomic DNA was obtained from peripheral blood by proteinase K digestion and phenol-chloroform extraction and ethanol precipitation. Genotyping of the C3435T polymorphism was carried out by polymerase chain reaction-restriction fragment length polymorphism assay according to the method of Hoffmeyer *et al* with minor modifications[9]. Allele specific-polymerase chain reaction (AS-PCR) was used to determine the genotype of G1199A. The AS-PCR consisted of 2 rounds of PCR. In the first round, 1 μL genomic DNA sample was added to 25 μL of reaction volume composed of PCR buffer, 1.5 mmol/L $MgCl_2$, 0.5 unit of Taq polymerase, 0.2 mmol/L dNTP, 6.25 pmol of forward primer, and 6.25 pmol of reverse primer; 35 cycles were carried out in a GeneAmp PCR system 2700 (Biocompare, CA, USA). Each cycle consisted of 30 s at 94 °C for denaturation, 30 s at 54 °C for annealing, and 30 s at 72 °C for elongation. The second round was carried out in 2 reaction tubes. Each tube contained 25 μL of reaction volume composed of PCR buffer, 1.5 mmol/L of $MgCl_2$, 0.5 unit of *Taq* polymorphism, 0.2 mmol/L dNTP, and 6.25 pmol of allele-specific primer as the reverse primer, and the same forward primer as that used in the first round of PCR. First-round products (20 times diluted) 1 μL was used as template for the second round. The second round consisted of 20 cycles performed in the same GeneAmp PCR system (30 s at 94 °C for denaturing; 30 s at 64 °C for annealing, and 30 s at 72 °C for elongation). Products (from each second-round reaction tube) in the volume of 10 μL were then analyzed directly on 1.5% agarose gel with 0.5 mg/mL of ethidium bromide. The sequence of the oligonucleotide primers employed in the AS-PCR assays are depicted in Table 2.

Statistical analyses We used the Hardy-Weinberg equilibrium for frequency deviation. The comparison of the allele and genotype frequencies between the different groups was evaluated by Chi-square test. ANOVA tests were used to compare genotype groups in terms of clinical and laboratory characteristics. The SPSS software package version 11.0 (SPSS Inc, Chicago, IL, USA) was used to perform these statistical analyses with $P<0.05$ as the minimal level of statistical significance.

Results

The different genotypic and allele frequency distributions for *MDR1* C3435T in ESRD patients and controls are shown in Table 3. The genotype distribution was consistent with the Hardy-Weinberg equilibrium. No significant differ-

Table 2. Primer sequences for determining the genotype of MDR1 C3435T and G1199A.

Name	Primer sequence
Forward primer for C3435T	5'-TGCTGGTCCTGAAGTTGATCTGTGAAC-3'
Reverse primer for C3435T	5'-ACATTAGGCAGTGACTCGATGAAGGCA-3'
Outer forward P1	5'-GCCATCTATCCACCTATCTAA-3'
Outer reverse R1	5'-TCTACCAGGACGAGTGAGAAA-3'
Inner wild reverse R2	5'-CTTCTTTTCGAGATGGGTAAC-3'
Inner mutation reverse R3	5'-CTTCTTTTCGAGATGGGTAAT-3'

Table 3. Genotype and allele frequency of MDR1 3435C>T among Chinese ESRD patients and healthy controls.

Genotype/Allele	Healthy controls *n* (%)	ESRD patients *n* (%)	Frequency in other reports of Chinese patients *n* (%)	*P* value
CC	99 (38%)	92 (35%)	85 (32%)	
CT	141 (47%)	115 (50%)	127 (48%)	
TT	44 (15%)	37 (15%)	53 (20%)	
C	59.7%	61.3%	56%	
T	40.3%	38.7%	44%	
Total	284	244	265	0.57

ence was observed in genotype frequencies between the ESRD patients and the control through the Chi-square test (P=0.573). However, as shown in Figure 1, the level of serum creatinine was significantly different between carriers with genotype CC and TT in ESRD patients, although the difference between the 3 genotypes, 3435CC, 3435CT, and 3435TT did not reach statistical significance. The value of serum creatinine for genotype 3435CC, 3435CT, and 3435TT were 753.8±276.0 μmol/L, and 849.6±342.2 μmol/L, 987.0±512.0 μmol/L, respectively. Of the 284 Chinese healthy subjects and 244 ESRD patients, no variant allele 1199G>A was found. All subjects were homozygous for 1199GG.

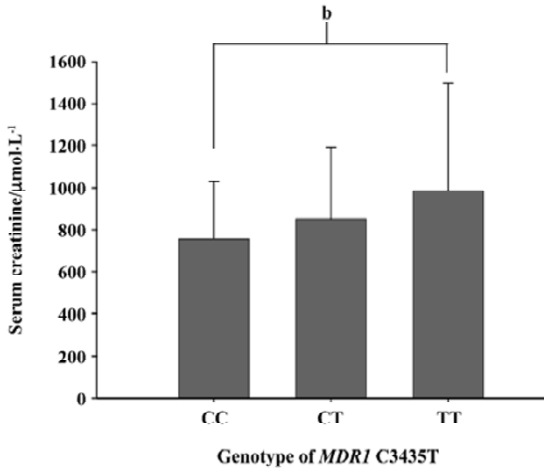

Figure 1. Effects of *MDR1* C3435T genotype on the level of serum creatinine in ESRD patients. Results are expressed as mean±SD. The columns represent the mean value and the lines represent standard deviation. The difference of mean level of Scr between CC and TT carriers is significant (^bP<0.05, ANOVA analysis).

Discussion

To date, 48 SNP have been reported in the MDR1 gene[14]. The single nucleotide polymorphisms 1236C>T, 2677G>T/A, and 3435C>T are the most common variants in the coding region of ABCB1[10]. 1236C>T and 3435C>T are synonymous SNP, while the nonsynonymous 2677G>T/A causes an amino acid substitution (899Ala>Ser/Thr). These 3 SNP are in strong linkage disequilibrium, accounting for 2 abundant haplotypes (ABCB1*1: 1236C-2677G-3435C; and ABCB1*13: 1236T-2677T-3435T)[14,16]. So we selected the polymorphism 3435C>T to represent the other 2 SNP, 1236C>T and 2677G>T/A. It was reported that individuals homozygous for 3435TT showed significantly lower P-gp expression in the intestines, liver and kidneys, with increased plasma levels of the P-gp substrate[10,16,17]. Similar to its protective role at many biological barriers, P-glycoprotein as a plasma membrane efflux pump may be involved in the clearance of

toxic compounds via the brush border of the tubular lumen and is critical in the processes of re-absorption and secretion. So we postulated that polymorphism C3435T should be associated with the severity of ESRD, or the frequency of mutation allele 3435C>T should be higher in ESRD patients compared with the controls.

In our results, we did not find any difference for the frequencies of the C3435T genotype and allele between the ESRD patients and the controls. The frequencies of C3435T in our study were consistent with other reports, as is shown in Table 3. However, the level of serum creatinine is higher in homozygote 3435TT than heterozygote 3435CT and homozygote 3435CC in ESRD patients. The difference between 3435TT and 3435CC reached statistical significance. According to the clinical characteristics in our subjects, the most common causes for ESRD were diabetes mellitus (34%), hypertension (25%), chronic glomerulonephritis (16%) and other (25%).The low expression of P-glycoprotein was not the etiological factor for the kidney disease, but it can contribute to the progression of ESRD and affect the severity.

The cause of ESRD and interindividual differences in susceptibility remain elusive. Many studies have recently focused on this aspect. Kim *et al* found that SNP and haplotypes of the *SLC12A3* [solute carrier family12 member (sodium/chloride) 3] gene, especially Arg913Gln, are significantly associated with ESRD caused by diabetic nephropathy in the Korean population[18]. It was reported that the polymorphism of promoter -511, exon -5+3953 in IL-β and a variable number of tandem repeats in the interleukin-1 receptor antagonist gene affects the risk of development of ESRD[19]. Meanwhile, Lamnissou *et al*[20] reported that patients with autosomal dominant polycystic kidney disease, who carried allele A in the nitric oxide synthase (NOS3-4) gene, progressed to ESRD more quickly. The result from Koupepidou *et al*[21] support the hypothesis that Caucasian patients with essential hypertension, homozygous for 677TT or doubly heterozygous for 677CT/1298AC genotypes in the methylenetetrahydrofolate reductase gene, are predisposed to develop hypertensive nephrosclerosis and chronic renal failure (CRF). Since more than 1 single gene was associated with the progression of ESRD, a study with the multiple linear regression method is needed in the future.

The substitution of G to A in the position 1199 of *MDR1* results in a serine-to-asparagine substitution at amino acid 400 in a cytoplasmic domain of P-gp. Alteration in the efflux transport of P-gp owing to the G1199A transition has been observed in a recombinant expression system. Mean intracellular R123 fluorescence for $MDR1_{wt}$ and $MDR1_{G1199A}$ cells were 3.91±0.11 and 18.56±0.46 (P<0.001), respectively, an approximate 4.75-fold higher accumulation of R123 in $MDR1_{G1199A}$ cells[12], which meant that G1199A mutation resulted in the decrease of the transport function of P-gp. Accordingly, the decrease efflux function of P-gp made the toxins more easily accumulated in the body, so the individual with variant 1199G>A was more likely to suffer from ESRD. It is expected that the frequency of variant allele will be higher in ESRD patients than in healthy controls. However, no variant allele was found in our study, neither in healthy controls nor in ESRD patients. Comparatively, the frequency of genotype 1199GG, 1199GA, and 1199AA is 88.9%, 11.1%, and 0 in 461 German volunteers, respectively[22]. Hoffmeyer *et al* reported that the frequency of 1199GA was 12.9% and 1199AA was zero in Caucasians[9], so there is no evidence to associate 1199G>A with the progression or severity of ESRD. As the human body is a complex organism, data obtained from *in vitro* experiments sometimes cannot be applied to the human body directly.

In conclusion, we found that SNP of the *MDR1* gene, especially C3435T, were significantly associated with the severity of ESRD in the Chinese population. Chinese people do not carry the 1199G>A variant allele. More study is needed to clarify the cause and interindividual differences in the susceptibility for the risk of ESRD.

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