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ARTICLE

Relationship between CYP2E1 Gene Polymorphism and Anti-tuberculosis Drug-induced Liver Injury

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ABSTRACT

Objective: To investigate the relationship between cytochrome P450 E1 (CYP2E1) gene polymorphisms and susceptibility to anti-tuberculosis drug-induced liver damage (ATDLI) in tuberculosis patients in the Chinese Han nationality. Methods: A retrospective analysis was performed on 360 patients with tuberculosis who had liver damage after tuberculosis treatment (case group) and 360 patients with tuberculosis who did not develop liver injury after treatment (control group). MassARRAY were used to detect CYP2E1 gene polymorphisms. Results: In a total of 8 tagged SNP loci selected, the rs8192773 locus failed to pass the test, and therefore, it is not included in subsequent analysis. At the remaining seven SNP sites, the difference in alleles was not statistically significant between the case group and the control group, suggesting that these sites may not be related to liver damage caused by anti-tuberculosis drugs. Three monomer domains were found in the seven tags SNP loci mentioned above. However, it was found that these haplotypes are not closely related to anti-tuberculosis drug-induced liver damage. Conclusion: The CYP2E1 gene polymorphism in the Chinese Han nationality is not related to the occurrence of anti-tuberculosis drug-induced liver injury.

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1. Introduction

The cytochrome P450 enzyme system is the main enzyme system that catalyzes the biotransformation of foreign compounds in the body. It was named so because of the specific absorption near the wavelength of 450 nm when it is in a reduced (ferrous) state and is bound to carbon monoxide (CO). Cytochrome P4502E1 (CYP2E1) is a member of the Phase I Metabolic Enzyme Cytochrome P450 Superfamily, which is expressed mainly in the liver and is involved in the metabolism of many drugs and carcinogens.[1] Recent studies have shown that CYP2E1 gene is polymorphic and different genotypes affect the expression of CYP2E1.[2] This difference may be related to genetic factors, and is one of the reasons for the difference in the metabolic capacity of the same substrate among different individuals and races. A number of articles have reported that the CYP2E1 gene polymorphism is associated with lung cancer, liver cancer, alcoholic cirrhosis and other diseases.[3-5] CYP2E1, as an important enzyme in the metabolic pathways of anti-tuberculosis drugs, has attracted the attention of many scholars at home and abroad due to the relationship between genetic polymorphisms and anti-tuberculosis drug-induced liver damage.[6-8] In this study, MassARRAY and case-control protocols were used to investigate the relationship between CYP2E1 gene polymorphisms and susceptibility to anti-tuberculosis drug-induced liver damage in the Chinese Han nationality.

2. Study Objects and Methods

2.1 Study Objects

Through retrospective analysis, from May 2010 to March 2016, 720 cases of primary or relapsed tuberculosis who met the inclusion criteria were selected. The patients were divided into two groups. The case group consisted of 360 patients who had liver damage after first-line anti-tuberculosis treatment (2HRZE/4HR). The liver damage induced by anti-tuberculosis drugs is defined as asymptomatic or symptoms of hepatic inflammation such as loss of appetite, nausea and vomiting, after taking anti-tuberculosis drugs, and diagnosed with the following conditions: (1) Serum AST and/or ALT is 2 times (or > 80 U/L) above the upper limit of normal (ULN), or (2) Any increase in ALT, AST, accompanied by gradual elevation of bilirubin (>2.5 mg/dl). The control group included 360 tuberculosis patients who took the same anti-TB drugs but no liver damage occurred. All patients need to meet the following conditions before they can be included in the study: Liver function is normal at the beginning of chemotherapy, and there are no factors that may cause liver damage, such as malnutrition, HIV infection, alcohol abuse, viral hepatitis, liver disease, cardiac insufficiency, and the use of other liver damage drugs, etc. Other conditions include that patients can be closely monitored for changes in liver function during treatment.

2.2 Methods

2.2.1 Extraction of Genomic DNA from Peripheral Blood Mononuclear Cells

1 ml of blood specimens of determining erythrocyte sedimentation rate (ESR) from patients with tuberculosis mentioned above were collected. DNA of the mononuclear cells in those samples was extracted using the whole genome DNA extraction kit (Tiangen Biotech, Beijing, China) according to the instructions and were immediately stored in a refrigerator at -20 °C.

2.2.2 Selection of NAT2 Gene SNPs and Detection of Their Polymorphisms

According to the gene polymorphism published in the public SNP database and related literature reports, 8 tag SNP loci from the CYP2E1 gene of the Chinese Han nationality were screened using haploview 4.2 software. See Table 1 for details. Mass spectrometry (MassARRAY, Sequenom, USA) was used to detect the genotype of each SNP locus.

2.2.3 Statistical Analysis

SPSS 13.0 and haploview 4.2 software, t-test, X2 test and other statistical methods were used to analyze the basic parameters of the case group and the control group. Pearson's chi-square test or Fisher's exact test was used to analyze the distribution of genotypes and alleles at each site in the case and control groups. The test level is α=0.05.

3. Results

3.1 Analysis of the Basic Characteristics of the Two Groups

There was no statistically significant difference in gender and age between the case group and the control group. The values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin (DBIL) and total bilirubin (TBIL) before administration were all within the normal range. Hardy−Weinberg equilibrium in control group, MAF test of all data, the percentage of non-deletional genotypes in the locus, and other gene locus related information are shown in Table 1.

3.2 Association of Individual SNP and Anti-tuberculosis Drug-induced Liver Injury

The differences in the distribution of genotypes and alleles

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Table 1. The basic of the 8 SNP loci

Name	Position	ObsHET	PredHET	HWpval	%Geno	MAF	Alleles	Rating
rs3813865	135189234	0.332	0.343	1	98.8	0.22	G:C	
rs2031920	135189835	0.31	0.326	0.4073	95.8	0.205	C:T	
rs2070673	135190557	0.491	0.484	0.4236	99.2	0.41	T:A	
rs8192773	135195964	0	0	0	0	0	T:T	BAD
rs915908	135196949	0.229	0.243	1	98.3	0.142	G:A	
rs8192775	135198016	0.361	0.349	0.8664	99.8	0.225	G:A	
rs7092584	135198247	0.496	0.491	0.5121	99.8	0.432	C:T	
rs2515641	135201352	0.298	0.318	1	98.8	0.198	C:T	

Notes:
(1) Position: Position of the locus on the chromosome.
(2) %Geno: The percentage of non-deleted genotypes on the locus for all samples (the minimum value is 75%, less than this value is considered to have failed the test).
(3) MAF: The frequency of the last allele at this site (minimum value is 0.001, less than this value is considered to have failed the test)
(4) Alleles: Major and minor alleles at the locus
(5) Rating: Genetic sites that passed all tests will be entered for follow-up analysis. Sites that did not pass one or more tests were shown as BAD and excluded.

at each site between the case group and the control group were analyzed using the Pearson chi-square test or Fisher's exact test. The results are shown in Table 2. As can be seen from the table, there was no statistically significant difference in alleles between the case group and the control group at the seven SNP loci. The rs8192773 locus failed the test, so it does not enter the subsequent analysis.

Table 2. Differences in distribution of CYP2E1 genotypes and alleles between case group and control group

Site	Alleles	Case, Control Ratio Counts	Frequencies (case, control)	χ^2	P
rs3813865	G:C	542:150, 556:158	0.783, 0.779	0.042	0.838
rs2031920	C:T	562:132, 556:162	0.810, 0.774	2.686	0.101
rs2070673	T:A	430:286, 432:282	0.601, 0.605	0.030	0.863
rs915908	G:A	602:106, 626:92	0.850, 0872	1.389	0.239
rs8192775	G:A	562:158, 552:164	0.781, 0.771	0.190	0.663
rs7092584	C:T	416:302, 402:298	0.579, 0.574	0.038	0.846
rs2515641	C:T	570:142, 572:136	0.801, 0.808	0.122	0.727

3.3 Haplotype Analysis

A set of single nucleotide polymorphisms that are related to each other in a specific region of a chromosome and tend to be inherited globally to offspring, were known as haplotypes. The linkage disequilibrium between loci was examined by methods such as D'/r^2. In the seven sites of CYP2E1, three monomer domains were found, and the linkage disequilibrium occurred in each monomer domain. For the monomer domains calculated in the linkage disequilibrium analysis, the distribution ratios of the haplotypes in the case group and the control group in each monomer domain were calculated. Overall genetic haplotypes and disease associations were examined using the Pearson chi-square test ($p<0.05$). The results are shown in Table 3. No haplotype associated with anti-tuberculosis drug-induced liver damage was found in the 3 monomer domains consisting of 7 SNP sites.

Table 3. Relationship between haplotype and liver damage

Haplotypes	Frequency	Case, Control Ratio Counts	Frequencies (case, control)	χ^2	P
Block 3					
GC	0.573	417.5 : 296.5, 403.4 : 314.6	0.585, 0.562	0.767	0.3811
CC	0.222	164.8 : 549.2, 152.4 : 565.6	0.231, 0.212	0.715	0.3978
GT	0.206	133.4 : 580.6, 161.8 : 556.2	0.187, 0.225	3.245	0.0716
Block 4					
TG	0.447	311.8 : 406.2, 331.3 : 388.7	0.434, 0.460	0.974	0.3237
AG	0.411	296.4 : 421.6, 294.0 : 426.0	0.413, 0.408	0.030	0.8629
TA	0.142	109.3 : 608.7, 94.8 : 625.2	0.152, 0.132	1.248	0.2639
Block 5					
GCC	0.562	411.2 : 308.8, 397.2 : 319.8	0.571, 0.553	0.429	0.5126
ATC	0.224	154.8 : 565.2, 166.8 : 551.2	0.215, 0.232	0.621	0.4308
GTT	0.193	136.1 : 583.9, 141.1 : 576.9	0.189, 0.197	0.130	0.7188
GTC	0.015	10.9 : 709.1, 11.0 : 707.0	0.015, 0.015	0.001	0.9776

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4. Discussion

CYP2E1 is a member of the phase I metabolic enzyme cytochrome P450 superfamily. Through the metabolism of CYP2E1 in the liver, the drug produces some toxic products, such as free radical, pro electron group, oxygen group and so on. These toxic substances could covalently bind to large molecules in the liver cells, or cause lipid peroxidation in the cell membranes and organelles, resulted in the liver damage.[9-10]

Huang YS et al.[11] conducted a retrospective study of 318 cases of pulmonary tuberculosis or extrapulmonary tuberculosis that met the inclusion criteria in the Taipei Veterans General Hospital from May 1998 to August 2001. Among them, they found 49 cases (15.4%) had drug-induced hepatitis after taking anti-TB drugs. After digesting the CYP2E1 gene-specific amplified fragments of tuberculosis patients using the restriction endonuclease RsaI, they divided the CYP2E1 allele into the wild-type c1 and the variant c2 according to the results of the fragment electrophoresis, so that the genotype could be divided into the following three types: c1/c1, c1/c2, and c2/c2. After statistical analysis, it was found that the CYP2E1 c1/c1 genotype had a higher risk of liver damage (20.0% vs 9.0%) than other genotypes containing the mutation gene c2. Using CYP2E1 c1/c2 or c2/c2 genotypes and NAT2 fast acetylated genotypes as reference, Patients with CYP2E1 c1/c1 genotype plus NAT2 slow acetylation genotype had a significantly higher risk of hepatotoxicity than CYP2E1 c1/c1 plus NAT2 fast acetylation genotypes (risk odds ratio increased from 3.94 to 7.43). In a Swiss study, Vuilleumier N et al.[12] included 89 patients with tuberculosis latent infection treated with isoniazid for prospective studies. 26 cases (29%) of their patients were found to have abnormal liver function, of which 8 cases (9%) showed isoniazid-induced hepatitis. At the same time, CYP2E1*1A/*1A genotype was found to be associated with isoniazid-induced liver dysfunction. The CYP2E1*1A/*1A genotype had a positive predictive value and a negative predictive value for isoniazid-induced liver dysfunction of 39% and 84%, respectively. Therefore, they suggested that the CYP2E1 gene polymorphism might serve as a useful predictor of anti-tuberculosis drug-induced liver damage. Wang Tao and Soukaina Guaoua et al.[13-14] supported their conclusions through research and reported that the CYP2E1 RsaI polymorphism is closely related to the occurrence of anti-tuberculosis drug-induced liver damage, and c1/c1 genotype is considered as an independent risk factor for the development of anti-tuberculosis drug-induced liver damage. However, research results were not consistent. Teixeira RL et al.[15] studied 167 patients with tuberculosis and found that there was no correlation between the CYP2E1 gene polymorphism and the occurrence of anti-tuberculosis drug-induced liver damage in Brazilian population.

In this study, we did not classify CYP2E1 into c1/c1, c1/c2, and c2/c2 genotypes through restriction endonuclease RsaI recognition sites. We screened 8 target sites according to the gene frequency in Chinese SNPs reported in the public SNP database, and used the PCR-MassARRAY method to detect the frequency of all SNP loci in the case and control groups, and then the association of these SNP loci and their haplotypes with the anti-tuberculosis drug-induced hepatic impairment was analyzed. Our results did not reveal a correlation between CYP2E1 polymorphisms and anti-tuberculosis drug-induced hepatic impairment. The difference between this study result and other reports, first of all, may be related to ethnic differences. In different countries and regions, the distribution of CYP2E1 genotypes is quite different. Secondly, in the previously reported studies, the sample size was relatively small. In particular, the number of patients with hepatic impairment in these studies was small, and it was easy to make type II errors in the statistical analysis to obtain false-negative results. Thirdly, because the use of anti-tuberculosis drugs is reported to increase the incidence of drug-induced liver damage, in the study of Vuilleumier N et al.[12], isoniazid was used as the only chemotherapy drug, while in the present study, patients were simultaneously treated with two or more chemotherapeutic agents for the treatment of tuberculosis, which may also be an important reason for the difference in results. Finally, differences in diagnostic criteria and experimental design for drug-induced liver damage can also lead to discrepancies in the results of the study.

5. Conclusion

In summary, we used the MassARRAY method to analyze 8 SNPs in CYP2E1 and found no association between CYP2E1 polymorphisms and antituberculous drug-induced hepatic impairment.

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