

## Research Article

# Comparison of SNP Polymorphism of Alcohol Metabolizing Related Enzyme Genes in Intoxicated Individuals and Nonalcoholic Population

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## Abstract

**Objective:** To compare the distributions of SNP allele and genotype about alcohol dehydrogenase 2 (ADH2), aldehyde dehydrogenase 2 (ALDH2) and CYP2E1 in intoxicated individuals and nonalcoholic population.

**Methods:** 100 individuals who were penalized due to intoxicated driving were genotyped for 40 SNPs of ADH2, ALDH2 and CYP2E1 using multiplex PCR and iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer and compared with a reference group of 99 blood donors. Allele frequencies and genotype frequencies of 40 SNP loci were calculated and compared in the two groups.

**Results:** Among the 40 SNP loci, seven SNPs (e.g. rs698, rs2241894, rs1789915, rs13306164, rs671, rs28371746 and rs2515641) were polymorphic in the intoxicated population, but only six SNPs (e.g. rs698, rs2241894, rs13306164, rs671, rs28371746 and rs2515641) were polymorphic in the control individuals. Two SNP loci (rs671 in and rs2515641) were found to have a significant difference in frequency distribution between the two populations ( $p < 0.01$ ).

**Conclusion:** Among the above 40 SNP loci of ADH2, ALDH2 and CYP2E1 genes, rs671 and rs2515641 in the intoxicated population were found to differ in allele and genotype frequency distribution from the nonalcoholic population and may be related to alcohol intoxication.

**Keywords:** ADH; ALDH; CYP2E1; Single nucleotide polymorphism; Genetic polymorphism

## Introduction

The metabolism of drug shows inter-individual variation and inter-ethnic variation. Because of the difference of Drug-Metabolizing Enzyme (DMEs) activity, the individuals can be divided into Poor Metabolizers (PMs), Extensive Metabolizers (EMs) and Ultra-Rapid Metabolizers (UMs) [1,2]. Therefore, it is usually a hard work to interpret drug related poisoning or death in forensic science [3]. Now punitive measures against people who drink and drive have been strictly enforced. In China, current standard for judging drunk drinking and drunken drinking is mainly based on the concentration of ethanol in blood. The driver whose alcohol concentration above 0.2mg/mL in blood is convicted of drunk drinking and the driver whose alcohol concentration above 0.8mg/mL in blood is convicted of drunken drinking, but, except for alcohol dehydrogenase (ADH), aldehyde dehydrogenase 2 (ALDH2) and cytochrome P450 2E1 enzyme (CYP2E1) has also been found to be involved in alcohol metabolism [4-7], which means that ALDH2 and CYP2E1 with gene polymorphism may also play dominant role in alcohol metabolism. Individuals with distinct genotypes are different in alcohol tolerance and show unequal behavioral responses after drinking [8]. Because of high concentration of acetaldehyde in blood transformed from ethanol, poor metabolism of acetaldehyde may lead to traffic accident

even if the individual's alcohol concentration below 0.2mg/mL in blood. The individual differences are mainly caused by the gene polymorphisms of ADH, ALDH and CYP2E1. It was confirmed that the presence of the less-active form of alcohol dehydrogenase-1B encoded by ADH1B\*1/\*1 and active form of ALDH2 encoded by ALDH2\*1/\*1 increases the risk of alcoholism in East Asians [9]. SNPs were found to be related to the polymorphism of enzyme activity [8], base deletions, insertions, substitutions or transversions in genes of ADH, ALDH2 and CYP2E1 may lead to changes of amino acid sequences and result in enzyme activities lost or decreased or increased. In order to investigate SNP polymorphism of alcohol metabolizing-related enzyme genes in Chinese Han population, we genotyped 40 SNPs of ADH2, ALDH2 and CYP2E1 based on multiplex amplification and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [10,11] and compared the SNP polymorphism in intoxicated population and nonalcoholic population.

## Materials and Methods

### Selection of SNP loci and design of primers

Corresponding to sequences of genes coding for ADH2, ALDH2 and CYP2E1, 40 SNP loci (Table 1) were selected via NCBI website: <http://www.ncbi.nlm.nih.gov/>. Forty groups of primers were designed

**Table 1:** 40 SNPs in Alcohol Metabolizing Related Enzyme Genes.

Gene	SNP Loci	Variation	Gene	SNP Loci	Variation
ADH2	rs2066702	C/T	CYP2E1	rs35844228	C/G
	rs55882921	C/T		rs72559710	A/C/G
	rs41275697	C/T		rs28371740	A/G
	rs1126440	C/T		rs60719153	C/T
	rs41275699	A/G		rs56864127	A/G
ADH3	rs67420531	A/G	rs60452492	A/G	
	rs56247447	C/T	rs56040284	A/G	
	rs698	A/G	rs28371743	C/T	
	rs1042756	C/G	rs41299426	A/G	
	rs55717907	A/G	rs61710826	A/G	
	rs2241894	A/G	rs28371746	A/C	
	rs1789915	A/G	rs61644766	C/G	
ALDH2	rs6490301	C/T	rs41299434	C/G	
	rs1064903	C/G	rs59981143	A/G	
	rs13306164	C/T	rs55897648	A/G	
	rs58280059	A/G	rs60207639	A/G	
	rs1062136	A/T	rs59656378	C/G	
	rs1064933	A/G	rs57702102	A/G	
	rs671	A/G	rs2515641	C/T	
	rs59868347	A/G	rs55982231	A/G	

**Table 2:** 40 groups of primes used for SNP genotyping.

SNP_ID	Forward primer (5'→3') for PCR	Reverse primer (5'→3') for PCR	Primer for extension reactions
rs2066702	ACGTTGGATGGCATGTGGTTGTCTAAATG	ACGTTGGATGCTCTATTGCCTCAAAACGTC	gCTTCTTTCTATTGCAGTATC
rs55882921	ACGTTGGATGAAGAGTAAAGAAGGTATCCC	ACGTTGGATGCATGGGTTATTAACGCATCC	ccccAACTTGTGGCTGATTTTAT
rs41275697	ACGTTGGATGCAGTGGCCAAAATTGATGC	ACGTTGGATGGACCCATAACCAGTCGAGAA	AAAATTGATGCAGCCTC
rs1126440	ACGTTGGATGTGAACCTCTGGTGCCATC	ACGTTGGATGAACCCGGAGAGCAACTACTG	TCCTGCAGGGTCCCCCG
rs41275699	ACGTTGGATGAACACTCTCCACGATGCCG	ACGTTGGATGATGACCACGTGGTTAGTGGC	CTGCCTCATGGCCTAAA
rs67420531	ACGTTGGATGTGTCTCTTTCTTCTATTGC	ACGTTGGATGCTACAAGGGAAGGCATCTGT	TTCTTTCTATTGCAGTATC
rs56247447	ACGTTGGATGATAAATGAAGGATTGACC	ACGTTGGATGCGCTACTGTAGAATACAAAG	AGGATTTGACCTGCTTC
rs698	ACGTTGGATGAAGAAGTTTTCACTGGATGC	ACGTTGGATGAGAGCGAAGCAGGTCAAATC	CACTGGATGCATTAATAACAAT
rs1042756	ACGTTGGATGCCAGTGAAGAACTTCTTAGCC	ACGTTGGATGCTTCTTTTCAGGCTTTAAGAG	AAAGTCAGCCACAAGTTT
rs55717907	ACGTTGGATGCAGACCCATAACCAGTCGAA	ACGTTGGATGCCAAAATTGATGCAGCCTCG	CCATAACCAGTCGAAAATCCACA
rs2241894	ACGTTGGATGTCGGCGTCAGCACCTTCTC	ACGTTGGATGCGAGGCTGCATCAATTTTGG	CGGCGTCAGCACCTTCTCCCAGTACAC
rs1789915	ACGTTGGATGGTGATAAAGTCATCCCCTC	ACGTTGGATGAAGCAGTAGTTGCTTTCTGG	TGTGGAAAATGCAGAATTTG
rs6490301	ACGTTGGATGTGACAAGAGCGCGGCCCA	ACGTTGGATGTCCGCTAGCCCGCTGCGAT	acggtGCCGAAGCGGGCGGCAGC
rs1064903	ACGTTGGATGCTAGCCCGCTGCGATGTTG	ACGTTGGATGTGGCGCGGCTGACAAGAG	TGCCGCGCTTCGGGCCCGCCTGG
rs13306164	ACGTTGGATGCATGGACGCATCACACAGG	ACGTTGGATGTAGTCCGCTCCGCTCGAT	ACGCATCACACAGGGGCCGGCTG
rs58280059	ACGTTGGATGCAGTTCCAGTTGCCAAGG	ACGTTGGATGGCTGTTTGTTCAGTGG	CCATGCTTGCATCCAGGAG
rs1062136	ACGTTGGATGAGGATGACATCTATGATGAG	ACGTTGGATGTATCAAAGGGTTCCCGAC	ggCATCTATGATGAGTTTGTGG
rs1064933	ACGTTGGATGTGTATGCCTGCAGCCCGTA	ACGTTGGATGACCTTTGGTGGCTACAAGA	CCGTA CTGCCCCA ACTCCCGGCCACT
rs671	ACGTTGGATGCAGGTCCACACTCACAGTT	ACGTTGGATGAGTTGGCGAGTACGGGCTG	AGGTCCACACTCACAGTTTCACTT
rs59868347	ACGTTGGATGTTCTCTGCTGGTGTCCAT	ACGTTGGATGATGATGGGAAGCGGAAAGG	gtTGTCCATGTGGAGGCAG
rs35844228	ACGTTGGATGCTTCCCTTACCAGCCTTGTAG	ACGTTGGATGTTCCGGCCGGTGTTCACG	ATCACCACCATGCGCTGCGAGCCCA
rs72559710	ACGTTGGATGCTTCCCTTACCAGCCTTGTAG	ACGTTGGATGGCCGGTGTTCACGCTGTA	GTAGCCGTGCATCACCACCATG
rs28371740	ACGTTGGATGAAGGAAGCGCTGCTGACTA	ACGTTGGATGTCCCTGTGCGCATGGAAC	GGACTACAAGGACGAGTTCTC
rs60719153	ACGTTGGATGATAATGGACCTACCTGGAAG	ACGTTGGATGTTCCCATCCCATAGTTCC	CCTACCTGGAAGGACATC
rs56864127	ACGTTGGATGTTCCCATCCCATAGTTCC	ACGTTGGATGATAATGGACCTACCTGGAAG	GTGGTCAGGGAAAACCGC
rs60452492	ACGTTGGATGTTTTGTAGGCCAGCCTTTC	ACGTTGGATGGAAGAGGATGTCGGCTATGA	ACCCACCTTCTCATC
rs56040284	ACGTTGGATGTTTTGTAGGCCAGCCTTTC	ACGTTGGATGGAAGAGGATGTCGGCTATGA	GACCCACCTTCTCATCGCTGC
rs28371743	ACGTTGGATGTGATGAGAAGTTTCTAAGGC	ACGTTGGATGAGGGAGTGTGAGTAGGTG	TAAGGCTGATGATTTGTTTAA
rs41299426	ACGTTGGATGTTGCATCCAGAAAAAGTAG	ACGTTGGATGAAGTAGTGTAGAAAGCTGGG	ggTTCCCTCTAGCTTTAC
rs61710826	ACGTTGGATGAAAGAACAGGTGCGCCACAG	ACGTTGGATGCGGTATCACAGGAAAAGCAC	CACAGTCACGGTGATAC
rs28371746	ACGTTGGATGGAGAATCAGGAGCCCATATC	ACGTTGGATGTGTGGCCGACCTGTTCTTTG	AGCCCATATCTCAGAGTTGTGCTGGT
rs61644766	ACGTTGGATGAATTGACAGGGTGATTGGGC	ACGTTGGATGACCACAGCATCCATGATGGG	AGCCGAATCCCTGCCAT
rs41299434	ACGTTGGATGTCATGAGATTCAGCGGTTCC	ACGTTGGATGCTCTGAAAAATGGTGTCTCGG	cGTTATCACCCCTCGTCCCT

SNP_ID	Forward primer (5'→3') for PCR	Reverse primer (5'→3') for PCR	Primer for extension reactions
rs59981143	ACGTTGGATGGGTATCCTCTGAAAATGGTG	ACGTTGGATGGAGATTGAGCGGTTTCATCAC	GAAAATGGTGTCTCGGG
rs55897648	ACGTTGGATGTCAGGAAATTCTGGTTGTC	ACGTTGGATGGTCTTTGTTTCTCCTAGGGC	tagcGTCCAGAGTTGGCACTA
rs60207639	ACGTTGGATGCTGGATCAGGAAATTCTTGG	ACGTTGGATGCTTTGTTTCTCCTAGGGCAC	GGTTGTCATACAAAACAGAG
rs59656378	ACGTTGGATGCTGGATCAGGAAATTCTTGG	ACGTTGGATGCTTTGTTTCTCCTAGGGCAC	agaacTTCTTGGTTGTCATACAAAA
rs57702102	ACGTTGGATGGTTTAAGCCAGAACACTTCC	ACGTTGGATGTCTCACCTGTGAAAATGGC	cGAACACTTCTGAATGAAA
rs2515641	ACGTTGGATGGCCAGAACACTTCTGAATG	ACGTTGGATGTCTCACCTGTGAAAATGGC	CCTGAATGAAAATGGAAAGTT
rs55982231	ACGTTGGATGTCATGAGCGGGGAATGACAC	ACGTTGGATGTATCGACCTCAGCCCTATAC	gcggtGAATGACACAGATTTGTAA

**Table 3:** Genotype frequencies of 40 SNPs in intoxicated population (n=100) and control population (n=99).

Gene	SNP Loci	Variation	Genotype frequencies in intoxicated population	Genotype frequencies in control population
ADH2	rs2066702	C/T	CC:1.0000	CC:1.0000
			CT: 0.0000	CT: 0.0000 TT:0.0000
			TT:0.0000	
	rs55882921	C/T	CC:1.0000	CC:1.0000
			CT: 0.0000	CT: 0.0000 TT:0.0000
			TT:0.0000	
	rs41275697	C/T	CC:1.0000	CC:1.0000
			CT: 0.0000	CT: 0.0000 TT:0.0000
			TT:0.0000	
	rs1126440	C/T	CC:0.0000	CC:0.0000
			CT: 0.0000	CT: 0.0000 TT:1.0000
			TT:1.0000	
	rs41275699	A/G	AA:1.0000	AA:1.0000 AG:0.0000
			AG:0.0000	GG:0.0000
			GG:0.0000	
	rs67420531	A/G	AA:0.0000	AA:0.0000 AG:0.0000
			AG:0.0000	GG:1.0000
			GG:1.0000	
ADH3	rs56247447	C/T	CC:0.9900	CC:1.0000 CT:0.0000
			CT:0.0100	TT:0.0000
			TT:0.0000	
	rs698	A/G	AA:0.8400	AA:0.8889 AG:0.1111
			AG:0.1500	GG:0.0000
			GG:0.0100	
	rs1042756	C/G	CC:1.0000	CC:1.0000 CG:0.0000
			CG:0.0000	GG:0.0000
			GG:0.0000	
	rs55717907	A/G	AA:0.0000	AA:0.0000 AG:0.0000
			AG:0.0000	GG:1.0000
			GG:1.0000	
	rs2241894	A/G	AA:0.0400	AA:0.0505 AG:0.9495
			AG:0.9600	GG:0.0000
			GG:0.0000	
	rs1789915	A/G	AA:0.9800	AA:0.9899 AG:0.0101
			AG:0.0200	GG:0.0000
			GG:0.0000	
	rs6490301	C/T	CC:1.0000	CC:1.0000 CT:0.0000
			CT:0.0000	TT:0.0000
			TT:0.0000	
ALDH2	rs1064903	C/G	CC:0.0000	CC:0.0000 CG:0.0000
			CG:0.0000	GG:1.0000
			GG:1.0000	
	rs13306164	C/T	CC:0.9200	CC:0.8788 CT:0.1212
			CT:0.0800	TT:0.0000
			TT:0.0000	
	rs58280059	A/G	AA:0.0000	AA:0.0000 AG:0.0000
			AG:0.0000	GG:1.0000
			GG:1.0000	
	rs1062136	A/T	AA:1.0000	AA:1.0000 AT:0.0000
			AT:0.0000	TT:0.0000
			TT:0.0000	
	rs1064933	A/G	AA:0.0000	AA:0.0000 AG:0.0000
			AG:0.0000	GG:1.0000
			GG:1.0000	
	rs671	A/G	AA:0.0000	AA:0.0404 AG:0.4141
			AG:0.1000	GG:0.5455
			GG:0.9000	
	rs59868347	A/G	AA:0.0000	AA:0.0000 AG:0.0000
			AG:0.0000	GG:1.0000
			GG:1.0000	

CYP2E1	rs35844228	C/G	CC:0.0000 CG:0.0000 GG:1.0000	CC:0.0000 CG:0.0000 GG:1.0000
	rs72559710	A/C/G	AG:0.0100 CG:0.0000 GG:0.9900	AG:0.0101 CG:0.0101 GG:0.9798
	rs28371740	A/G	AA:0.0000 AG:0.0000 GG:1.0000	AA:0.0000 AG:0.0000 GG:1.0000
	rs60719153	C/T	CC:1.0000 CT:0.0000 TT:0.0000	CC:1.0000 CT:0.0000 TT:0.0000
	rs56864127	A/G	AA:0.0000 AG:0.0000 GG:1.0000	AA:0.0000 AG:0.0000 GG:1.0000
	rs60452492	A/G	AA:0.0000 AG:0.0000 GG:1.0000	AA:0.0000 AG:0.0000 GG:1.0000
	rs56040284	A/G	AA:0.0000 AG:0.0000 GG:1.0000	AA:0.0000 AG:0.0000 GG:1.0000
	rs28371743	C/T	CC:0.0000 CT:0.0000 TT:1.0000	CC:0.0000 CT:0.0000 TT:1.0000
	rs41299426	A/G	AA:1.0000 AG:0.0000 GG:0.0000	AA:1.0000 AG:0.0000 GG:0.0000
	rs61710826	A/G	AA:0.0000 AG:0.0000 GG:1.0000	AA:0.0000 AG:0.0000 GG:1.0000
	rs28371746	A/C	AA:0.0000 AC:0.0200 CC:0.9800	AA:0.0000 AC:0.0606 CC:0.9394
	rs61644766	C/G	CC:1.0000 CG:0.0000 GG:0.0000	CC:1.0000 CG:0.0000 GG:0.0000
	rs41299434	C/G	CC:1.0000 CG:0.0000 GG:0.0000	CC:1.0000 CG:0.0000 GG:0.0000
	rs59981143	A/G	AA:0.9900 AG:0.0100 GG:0.0000	AA:1.0000 AG:0.0000 GG:0.0000
	rs55897648	A/G	AA:0.0000 AG:0.0000 GG:1.0000	AA:0.0000 AG:0.0000 GG:1.0000
	rs60207639	A/G	AA:1.0000 AG:0.0000 GG:0.0000	AA:1.0000 AG:0.0000 GG:0.0000
	rs59656378	C/G	CC:0.0000 CG:0.0000 GG:1.0000	CC:0.0000 CG:0.0000 GG:1.0000
	rs57702102	A/G	AA:1.0000 AG:0.0000 GG:0.0000	AA:1.0000 AG:0.0000 GG:0.0000
	rs2515641	C/T	CC:0.6200 CT:0.3600 TT:0.0200	CC:0.6566 CT:0.3333 TT:0.0101
	rs55982231	A/G	AA:0.0000 AG:0.0000 GG:1.0000	AA:0.0000 AG:0.0000 GG:1.0000

via Mass ARRAY Assay Design software (Sequenom, Inc.). Each group has three primers, including a pair of PCR primers and a single base extension primer (Table 2). Primers were synthesized by Shanghai Biological Engineering Technology Corporation.

#### DNA extraction

Blood samples were collected from 100 unrelated Han individuals who were penalized duo to intoxicated driving. Another 99 blood samples for control were collected from nonalcoholic males. DNA was extracted according to the instructions of the blood genomic DNA Mini Kit (Sangon Biotech, Shanghai, China).

#### Multiplex PCR amplification

Aliquots of 1  $\mu$ L DNA were amplified in a total volume of 5  $\mu$ L. Each reaction contained 0.625  $\mu$ L PCR buffer (10 $\times$ ) (Qiagen GmbH), 0.325  $\mu$ L 25 mmol/L MgCl<sub>2</sub>, 1  $\mu$ L dNTP (2.5mmol/L) (Tatara Inc.), 0.1  $\mu$ L of HotStarTaq polymerase (5U/ $\mu$ L), 0.95  $\mu$ L H<sub>2</sub>O, and 1  $\mu$ L the designed primers at their optimized concentrations. Using a Gene Amp PCR System 9700 (Applied Biosystems, Norwalk, CT), the reaction mixtures were incubated at 94°C for 15 min and then cycled 45 times through desaturation at 94°C for 20 s, annealing at 56°C for 30 s and extension at 72°C for 60 s and finally incubated at 72°C

**Table 4:** Allele frequencies of seven polymorphic SNPs in intoxicated population (n=100) and control population (n=99).

Gene	SNP Loci	Variation	Allele frequencies in intoxicated population	Allele frequencies in control population
ADH3	rs698	A/G	0.9150/0.0850	0.9444/0.0556
	rs2241894	A/G	0.5200/0.4800	0.5253/0.4747
	rs1789915	A/G	0.9900/0.0100	0.9949/0.0051
ALDH2	rs13306164	C/T	0.9600/0.0400	0.9394/0.0606
	rs671	A/G	0.0500/0.9500	0.2475/0.7525
CYP2E1	rs28371746	A/C	0.0100/0.9900	0.0303/0.9697
	rs2515641	C/T	0.8000/0.2000	0.8232/0.1768

**Table 5:** Comparison of allele frequency of seven polymorphic SNPs in intoxicated population (n=100) and control population (n=99).

Population	Numbers of allele 1	Numbers of allele 2	Total of alleles	X <sup>2</sup>	P value
rs698 (A/G)					
intoxicated population	183	17	200	1.3189	0.2508
control population	187	11	198		
rs2241894(A/G)					
intoxicated population	104	96	200	0.0110	0.9165
control population	104	94	198		
rs1789915(A/G)					
intoxicated population	198	2	200	0.0000	1.0000
control population	197	1	198		
rs13306164(C/T)					
intoxicated population	192	8	200	0.8852	0.3468
control population	186	12	198		
rs671(A/G)					
intoxicated population	10	190	200	30.7291	<0.0001
control population	49	149	198		
rs28371746(A/C)					
intoxicated population	2	198	200	1.1791	0.2775
control population	6	192	198		
rs2515641(C/T)					
intoxicated population	160	40	200	4.6574	0.0309
control population	163	35	198		

for 3 min. No-template controls were carried along in every plate to exclude contaminations.

### SAP process

After PCR, the products were treated with hrmp alkaline phosphatase (SAP) to remove excess dNTPs. This dephosphorylation reaction contained 0.3μL SAP (1U/μL), 0.17μL SAP buffer (10×), 1.53μL H<sub>2</sub>O (Sequenom, Inc.) was carried out at 37°C for 40 min, and 85°C for 15 min.

### Primer extension reactions

The PCR products were used as templates for the primer extension reactions. Extension reactions (final volume, 9μL) contained 0.2μL iPlex buffer (10×), 0.1μL iPlex termination mix, 0.0205μL iPlex enzyme, 0.7395μL H<sub>2</sub>O (all from Sequenom, Inc.), and 0.94μL extension primers at optimized concentrations. On a Gene Amp PCR System 9700 (Applied Biosystems, Norwalk, CT), extension reactions were performed at 94°C for 30 s followed by 40 cycles (94°C for 5 s, followed by 5 cycles of 52°C for 5 s, 80°C for 5 s); and finally 72°C for 3 min. The final nucleotide extension products were treated with a cationic exchange resin (AG<sup>+</sup> 50W-X8 Resin; Bio-Rad Inc.) for 30 min to remove salts. All reactions, including PCR amplification, shrimp alkaline phosphatase treatment, and base extension, were performed in 384 microtiter plates (Sequenom Inc.).

### MALDI-TOF-MS

The reaction products were spotted onto the Mass ARRAY SpectroCHIP with an auto-spot arm (Sequenom, Inc.). The target plate was then inserted into the MALDI-TOF mass spectrometer of Mass ARRAY compact System (Sequenom, Inc.), and analysis was performed with 180 nitrogen laser shots for each sample. The mass range of the MS instrument was set at 3920–12023 Da. SNP loci was genotyped by Mass ARRAY Type Analyzer software version 4.0 (Sequenom, Inc.).

### Statistical analysis

The data were analyzed with SPSS 13.0. The statistical information included genotype frequency, allele frequency and *p* values.

## Results and Discussion

### Evaluation of the MALDI-TOF MS genotyping assay

To evaluate the established SNP genotyping assay, we analyzed 100 genomic DNA samples from individuals of Chinese origin who had previously been genotyped for some of the SNPs by TaqMan assay and got consistent results.

### Polymorphisms of SNPs

Among the 40 SNP loci in intoxicated population, three SNPs (rs698, rs2241894, rs1789915) in ADH3 gene, two SNPs (rs13306164,

rs671) in ALDH2 gene, two SNPs (rs28371746, rs2515641) in CYP2E1 gene were found to be polymorphic, i.e. the minor allele frequency(MAF) of each SNP locus was above 1%, while others were not ( the MAF of them was below 1%). Among the 40 SNP loci in control population, the seven SNPs except rs1789915 were found to be polymorphic. The observed genotype and allele frequencies in intoxicated population and control population were shown in table 3 and 4 respectively.

### Population comparison

The allele frequencies of seven polymorphic SNPs (rs698, rs2241894, rs1789915, rs13306164, rs671, rs28371746, rs2515641) in intoxicated population were compared to the data about the control population. It's found that two SNPs (rs671 in and rs2515641) with a significant difference in frequency distribution between intoxicated population and control population ( $p < 0.01$ , (Table 5) might be related to alcohol intoxication.

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