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Research Article

Typing of 67 SNP Loci on X Chromosome by PCR and MALDI-TOF MS

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Abstract

A set of single nucleotide polymorphisms (SNP) on X chromosome was selected with the aim of forensic identification in Chinese Han population to complement the analysis of autosomal, Y-chromosomal or mitochondrial markers, especially in deficiency cases. 67 forensically relevant X-SNPs were screened in a Chinese Han population sample. Genomic DNA samples extracted from 295 unrelated Chinese Han individuals were typed using three 17-plex and one 16-plex amplification reactions in combination with genetic analysis assisted by mass-spectrometry based on MassARRAY MALDI-TOF MS platform (Sequenom Inc.). All the investigated loci except rs12849634 were found to be in Hardy-Weinberg equilibrium. A total of 52 of the loci showing independent inheritance and high polymorphisms with minor allele frequency (MAF) above 0.3 were finally screened out. The accumulative exclusion probabilities (CPE) in trio cases and duo cases were above 0.999999. The combined discrimination power (CDP) in female population and male populations were above 0.999999999999999. It is concluded that the panel of informative SNP markers on X chromosome may be used for forensic genetic purposes. The mass spectrometry-based method for X-SNPs profiling was suitable for high-throughput application and could have promising prospects.

Keywords: Forensic genetics; SNP; X chromosome; MALID-TOF Mass

Introduction

Single nucleotide polymorphisms (SNP) typing on X chromosome could effectively complement the analysis of autosomal, Y-chromosomal or mitochondrial markers very efficiently. The markers may be easily amplified in samples with low-quality and degraded DNA.

In some deficiency kinship cases, e.g. relationship between paternal grandmother and granddaughter or relationship between half-sibling sisters who have the same father, X-chromosome markers are valuable and necessary, as reviewed by Pereira et al (2011). Additionally, for solving special father-daughter duos or mother-daughter-father trios, in which only 1~2 loci (out of 19~39) autosomal STR （Short Tandem Repeat）loci were

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exclusionary, X-SNP loci with genetic stability may be used as supplementary markers to autosomal STR loci to further confirm or exclude paternity or maternity.

Therefore, we planned this study. 67 SNPs on X chromosome were amplified in four multiplex PCR systems followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, mentioned by Storm N et al 2003 and Tost et al 2005) in Chinese Han population samples. Hardy-Weinberg equilibrium was analyzed and forensic genetic parameters were calculated.

Materials and Methods

Sample

295 blood samples were collected from unrelated Chinese Han individuals (215 males and 80 females) under informed consent. DNA was extracted from whole blood by using BLOOD DNA EXTRACTION kit according to manufacturer's instructions (Sangon Biotech, Shanghai, China).

Primer Design

67 SNP markers (Table 1) on X chromosome with high minimal allele frequency (MAF>0.4) were selected from HapMap (*www.hapmap.org*) and NCBI SNP database (*http://www.ncbi.nlm.nih.gov/SNP*). The locus to locus space was about 3~20Mb. 67 groups of primers were designed via MassARRAY Assay Design software (Sequenom Inc.). Each group has three primes, including a pair of PCR primes and a single base extension prime (Table 1). Primers were synthesized by Sangon Biotech (Shanghai, China).

Table 1: Primers for Amplification and Extension Reactions of 67 X-SNPs

SNP_ID	Forward primer(5'→3') for PCR	Reverse primer (5'→3') for PCR	Amplicons (bp)	Primer for extension reactions	molecular weight（D）
rs203648	ACGTTGGATGTAGCTC CTCTGAGTCTTGTC	ACGTTGGATGTGAAAAG CATGCCCTCTGCG	95	CCACAGGCCCCCACAG C	5070.3
rs1166756	ACGTTGGATGGCTCA GGCAAGCACATGTTT	ACGTTGGATGGTTCTAG AGGGCAACATAGC	101	GTCATCGTCAATGCAG G	5210.4
rs611711	ACGTTGGATGGTTTT CTAGGAAAGAACAAG	ACGTTGGATGCCTCATC CTGAAAACATCGC	100	AAGAACAAGAGAACC AGT	5558.7
rs7876774	ACGTTGGATGAGAGG AGTCTGTCTGATCTG	ACGTTGGATGAGGTCAT CAGGAAAGTCATC	98	ATGGGACGAGTGTGTC TA	5594.6
rs1544545	ACGTTGGATGTTATCC CCATTCAGACACAC	ACGTTGGATGGACTCTG TTAGGGTTGATTG	102	CCAAGTCTCCCACTAT TTA	5682.7
rs5905043	ACGTTGGATGTTGAG CCTGGGATGTTAGAC	ACGTTGGATGTACTCAG CATACTTCTCCCC	96	TTCCCATTGAGAGAAA CCT	5771.8
rs1229078	ACGTTGGATGCAAAC CTGTAACACATGACC	ACGTTGGATGGTAAGAT TGTTTAAATTTAGG	97	TGACCAAAGTAACCAT TGAG	6134
rs5932595	ACGTTGGATGAAGCT TGCTTCTGCCCATTC	ACGTTGGATGTTTGCTG AGCATTATGGCCC	100	ATGATGGAACAGAAAT CAGA	6207.1
rs17264553	ACGTTGGATGTCAAA GACAGCCAGTAAGCC	ACGTTGGATGTTGAGAA TTGCAGCCATCAG	95	CAGCCAGTAAGCCTAC CCAAC	6329.1

rs2070289	ACGTTGGATGATCCTC ACGATGGCAGTAGT	ACGTTGGATGATACAGG AAGGGCGGGAGTA	98	GTAGTGCTCCAAATAT GCTAAG	6758.4
rs1874111	ACGTTGGATGCCTTTC AGATTACTGAGTAG	ACGTTGGATGTGCTCCA GATAAGCCACTTG	100	GTACAAGAAATTAAGA GTGGAA	6864.5
rs5968332	ACGTTGGATGCCACCC ACACATTATTAATGC	ACGTTGGATGGAGGCAG GTTTCCGATTTTG	100	CCACACATTATTAATG CTTATCA	6941.6
rs5931302	ACGTTGGATGTGCACA TTCCAGGCAAGGTC	ACGTTGGATGAATAGGG ACGGAAAAGTTGC	94	TCCAGGCAAGGTCCAT GCTAAAAA	7354.8
rs4442270	ACGTTGGATGAAAGA AATGACCCAGTGGAG	ACGTTGGATGGTCTGTC ATTTCGATTCAGG	82	AATGACCCAGTGGAGA ATTATGAC	7409.8
rs5923750	ACGTTGGATGGGAAG AGAGAATTTGGGAAG	ACGTTGGATGTCTTTCA GGATTCAAAAGGC	99	TTTACTCTGCCATTTT CACTGACAT	7532.9
rs5917032	ACGTTGGATGTCACCA CTGTGCTATCCTTC	ACGTTGGATGCCTCCAT TAGGGCTTTTCCA	99	TAATGCATAACTGCAA TCTAATCATG	7937.2
rs6639398	ACGTTGGATGGCTTAT ACCAGTCTCATTGC	ACGTTGGATGAATGTAT CAGGACAGAGAGAC	84	ggaTCATTGCACTAAT TGAAATATTG	8008.2
rs2209420	ACGTTGGATGCTCATT CATGTGGGAGCTTT	ACGTTGGATGACCCTTC CCAGCCTCTTGTA	99	TGATCTCGTGGAGGCA A	5250.4
rs5986750	ACGTTGGATGAGGAT TTGCAGGCCCAATAG	ACGTTGGATGGAACTCT TCAGGAGCGTATC	97	AGACACTCCACAGGTG TA	5492.6
rs5989571	ACGTTGGATGGTACA GGATGTGGGAAAGTG	ACGTTGGATGGAGAACT GATGCTCCACATA	105	TGTGGGAAAGTGCGTA TG	5634.7
rs7471388	ACGTTGGATGGTGTG TCTGCTTTTGCCTTC	ACGTTGGATGTCCTGTG GATTCTGAATGTG	89	TCATTACTGACTGAAG ACC	5771.8
rs149860	ACGTTGGATGCCAGT GCCCTATTCTGCAGT	ACGTTGGATGAAAGGAG ATGTAAGAGTGGG	97	CCTATTCTGCAGTGAC TGAG	6108
rs5954988	ACGTTGGATGTTTCA AACCTTGCCCCCATC	ACGTTGGATGGGCAACA ATAGACACTGATG	82	CCCATCCATTCTTTCC CACTC	6188
rs471205	ACGTTGGATGCTTCCA CGAAGGACAAACTG	ACGTTGGATGGACTTGG TCTCAGACATCAG	100	TCCCTCTGCTGTTATA GCCTT	6314.1
rs2214174	ACGTTGGATGTAAATC CTCTGTGTTTTCC	ACGTTGGATGTCATGTC TGAAAGCTGTTGC	99	CTCTGTGTTTTCCAAT GAAAAT	6699.4
rs2768595	ACGTTGGATGGAGGG ATTGAATCTCTAGTC	ACGTTGGATGTGGTCTA TATCCATCAAGTC	96	TTGAATCTCTAGTCTA AGATGA	6748.4
rs6639989	ACGTTGGATGGTTATG AGGCTTTGAAAAGG	ACGTTGGATGAGGACAT CGATGATAATCTG	98	TGAGGCTTTGAAAAG GAAATCG	6847.5
rs6418251	ACGTTGGATGAGCTG GAAAATCATTTGGAG	ACGTTGGATGGAATGTT ACAGTGAAAGAGC	88	TGGAGATTTACAGAGG GGATTAG	7207.7
rs9781645	ACGTTGGATGGTAAG	ACGTTGGATGGAAAGTT	93	GGGCCACAGTCCATTC	7306.8

	GCTGAGCTTTAGAGG	TGTCCTTCATCAG		CAATAGCA	
rs2694717	ACGTTGGATGTGACTA ACGAGTGCCTTAGC	ACGTTGGATGGGACACA GAACATCATTCTC	99	CTTAGCATAATGTGGA GATGATGA	7455.9
rs5912619	ACGTTGGATGTTAGTT CTGCTCTCATGTTG	ACGTTGGATGACAAGAA CAAGCCAAACCC	85	CTCTCATGTTGATTAT TACTTTTCTT	7858.1
rs1468160	ACGTTGGATGCTAAA ATCTCCATGATTCAG	ACGTTGGATGGCAATCT ACAAGTTTTGGTC	96	ATATTATTGTTATGAT CTCCTTTTTA	7906.2
rs17280621	ACGTTGGATGTGAGC AGTCAGATGGAGTTG	ACGTTGGATGAGAAGGG ACCTGCTATAGTG	89	GAGCAGTCAGATGGA GTTGAGATTCC	8075.3
rs7060326	ACGTTGGATGGACTG CCATGTCTTATTTCC	ACGTTGGATGTAAATCA GTGCCTTCCCTGC	90	TTCCTTGGCACCTCAC A	5081.3
rs2808742	ACGTTGGATGGGGAA GTAATGGGTCATAAG	ACGTTGGATGCCCTCTT ATAGTCTTAAGTC	102	AATGGAGCACTGGTAG T	5274.4
rs5920670	ACGTTGGATGTGAAG AATTCTTGGGTCTGC	ACGTTGGATGAGGAAAT AAGAGTACGCTCC	89	CTCCCAGCCAGGTCAG CC	5405.5
rs4826623	ACGTTGGATGCAGCT TCTACTTCACTAGCC	ACGTTGGATGGAAAAAC AGCTCATTTTGG	92	GAAAGTCCTGAGTGCT CTA	5827.8
rs594031	ACGTTGGATGTCTTCT CAAGGTCTCAGAGC	ACGTTGGATGACATGGG CTTAGATATGAGG	94	AGAGCTAATAAGTGAC TGC	5860.8
rs6631828	ACGTTGGATGGTTGA AATGGTAGTGTTCAG	ACGTTGGATGTTTCAGA CCATGCATCTAGG	90	AGTGTTCAGTTACAAG AAACC	6438.2
rs6646036	ACGTTGGATGCATAAA TCAGCTTCCATATC	ACGTTGGATGGATGTGA GCTAAGGGTAAGG	95	CTTCCATATCTTGATC ACTAAA	6628.3
rs3924423	ACGTTGGATGGTGAG TTTTTAGTGGCTCCC	ACGTTGGATGCCTTCCA TCTTCTCTAGGTC	87	CTCCCGACAGAGCAAT TTATGG	6719.4
rs6611148	ACGTTGGATGGGATCT ATATACTAAAAACC	ACGTTGGATGCTATCTC CTTTGATTTCTTTC	98	TATACTAAAAACCAGA AAACAGA	7050.7
rs6633608	ACGTTGGATGCAGCTA GTTCACCAAGTTGC	ACGTTGGATGAAATTTC ACATCCTGGTGAG	96	TTGCATTCTTTCTATT CATTCCTT	7200.7
rs5964206	ACGTTGGATGTGGCA ACAAGATTTCCTGTG	ACGTTGGATGAAAGAGC AGCAGTGCTGTAG	99	TGTGTAATTTTTTTTC AAATTGGA	7377.8
rs12849634	ACGTTGGATGTGAGA CCTCAGGATGACAGT	ACGTTGGATGACAGCCT CATACTGCAAGTC	98	TGACAGTGAATACAAC TGAAATGT	7408.9
rs973212	ACGTTGGATGTATTCT CTCTTAGGCACTGG	ACGTTGGATGCTTAGTC ACATCAAAGGAGC	95	TCTCTCTTAGGCACTG GTATACAAC	7591.9
rs6620798	ACGTTGGATGGCCAG CATGAGAGACAGAGA	ACGTTGGATGCATGATA GAGTTCTCATAAG	99	CCAGCATGAGAGACAG AGAGTGATG	7789.1
rs5916781	ACGTTGGATGGAAAG AAAGGCATATTTCCTG	ACGTTGGATGACTTGAA GCTATGAACTGTG	93	ttcCCTGTTTTTATTTA CACAAATGA	7885.2

rs12840669	ACGTTGGATGCTGTAC ACTTAAATTGGCAC	ACGTTGGATGCAAATGA ACTTTATTGGAG	81	gACTTAAATTGGCACA TTTTATTATA	7958.2
rs4276834	ACGTTGGATGCATTCA AAATGCTAACAGAGG	ACGTTGGATGCCACCCA AGGACAGCTATTC	97	ATGCTAACAGAGGAA AATATTTCTTA	8001.2
rs5933388	ACGTTGGATGATCACC CCAAGAAGAAACCC	ACGTTGGATGGGAGGGG TTGGAAAAATATG	83	GAAGAAACCCAGGACC C	5182.4
rs6418330	ACGTTGGATGGTTTCA TGCACTCTTTCCAC	ACGTTGGATGATGGGAT CACTGAATACTTC	95	ACTCTTTCCACTAGCA TC	5369.5
rs5927322	ACGTTGGATGTCAAA ATATATCCTGGCAGC	ACGTTGGATGCTCTGGG AATGACCTAAATC	97	CCTGGCAGCAAAGATA TA	5516.6
rs10855633	ACGTTGGATGTCTTTT AGATACATGGCCAG	ACGTTGGATGGCAATTT GGAGATTTCTCA	100	ATGGCCAGTAATAGGA TC	5547.6
rs5986751	ACGTTGGATGATCTGC CAAGACTTCACTGC	ACGTTGGATGCAGAAAG CCTGTTGTGATCC	100	TTTTGCTTCCCTCACA GTG	5720.7
rs2519557	ACGTTGGATGTGACA ACTATAAGACCCATC	ACGTTGGATGTGGCTGA TTTCGCTGACTTG	99	GACCCATCTATTAGAG AGCC	6086
rs6527549	ACGTTGGATGGAGAA ATACAGGTGTTTCGTG	ACGTTGGATGCCTGCCT AACTGTCTGTAAA	100	ggGGTGTTTCGTGTGT TGAC	6226
rs6649211	ACGTTGGATGGAGTT AAAAATTCTCCCATCC	ACGTTGGATGCTGTGAC AGTTGGTAAGGTG	84	ttgATTCTCCCATCCAC AATA	6300.1
rs5928614	ACGTTGGATGTTTGT GTCGTCTCTGTTGTG	ACGTTGGATGTGAAGGA GAAAATGTAATG	96	TTGTGTAAACTCTTTA GACAA	6419.2
rs183277	ACGTTGGATGGAAGG AACGTCAGTGGTCAG	ACGTTGGATGCTGCCAC CTATATGATCCAG	102	AACGTCAGTGGTCAGC TTTGT	6452.2
rs7880460	ACGTTGGATGCCACAT CCTTGTCAACACAC	ACGTTGGATGAGTTCAT AGCCACTTGGTTG	83	TCAACACACATTATTA TCTTTTT	6922.5
rs5955927	ACGTTGGATGGCACA GCGCCTGATATAAAG	ACGTTGGATGGCTTAAA TATGTATCTCTTCC	99	AGTAGTTTCTCAATAG CATTTTG	7043.6
rs5908324	ACGTTGGATGTTAATG GGCTGAAACAGAAC	ACGTTGGATGTTTTAAG CCTCTTTTTAAAC	98	GAAACAGAACAAATT TTATTTCAC	7327.8
rs5906919	ACGTTGGATGGCTCTA CTCACGTGCCTTTT	ACGTTGGATGCATTTCG GTTAGAGTTTAGTC	97	CCTTTTATAAAGTTTA GGTCTGAAC	7646
rs5980274	ACGTTGGATGGTGAA TCTGAATGTTCTGC	ACGTTGGATGTGGTATT GCTCGTACATGG	100	CTTTATGTATCAATGT TCCTATCATA	7885.2
rs5926442	ACGTTGGATGGACTT TCTTGCTAAAAAGTT	ACGTTGGATGCTGTAAA AACAAAATTTCTC	100	cacCTTTCTTGCTAAA AAGTTTATCAC	8168.3
rs5956616	ACGTTGGATGCTCAA ATTCTATTTGTAAGC	ACGTTGGATGTGGATTT TAGGCTTCCTGAC	82	ccCTATTTGTAAGCATA TACTCAATAC	8177.3

SNP Typing

SNP genotyping was performed using the MassARRAY MALDI-TOF MS platform (Sequenom Inc.). Briefly, three 17-plex and one 16-plex amplification reactions were processed following standard protocols for iPLEX chemistry. The reaction products were used as templates for the primer extension reactions. All reactions were performed in 384 microtiter plates (Sequenom, Inc.). PCR amplification and primer extension reaction were carried out on a GeneAmp PCR System 9700 (Applied Biosystems, Norwalk, CT), and no-template controls were carried along in every plate to exclude contaminations. A panel of genomic DNA samples genotyped by the assay was sequenced simultaneously for quality control.

Multiplex PCR

SNP genotyping was performed using the MassARRAY MALDI-TOF MS platform (Sequenom Inc.). PCRs (final volume, 5 μL) contained 1μL the desired primers at their optimized concentrations, 0.625μL PCR buffer(10×) (Qiagen GmbH), 0.325μL 25 mmol/L $MgCl_2$, 1μL dNTP (2.5mmol/L) (Tatara Inc.), 0.1μL of HotStarTaq polymerase (5U/μL), 1μL of DNA , and 0.95μL H_2O. A tag (5'-ACGTTGGATG) was included in the primer sequence. PCR conditions were 94 °C for 15 min followed by 45 cycles at 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 1 min; and finally 72 °C for 3 min.

SAP Dephosphorylation

After amplification, the products of four reactions (i.e. 17-plex, 16-plex, 17-plex and 17-plex) were treated with shrimp alkaline phosphatase (SAP) to remove excess dNTPs. This reaction contained 0.17μL SAP buffer (10×), 0.3μL SAP (1U/μL), 1.53μL H_2O (all from Sequenom Inc.). The reaction conditions were 37 °C for 40 min, followed by 85 °C for 15 min.

Primer Extension Reactions

The PCR products were then used as templates for the primer extension reactions. This reaction (final volume was 9μL) contained 0.94μL extension primers at optimized concentrations,0.1μL iPlex termination mix, 0.2μL iPlex buffer (10×), 0.0205μL iPlex enzyme and 0.7395μL H_2O (all from Sequenom Inc). Extension reactions were performed at 94 °C for 30 s followed by 40 cycles at 94 °C for 5 s and 5 cycles of 52 °C for 5 s, 80 °C for 5 s; and finally 72 °C for 3 min. The extension products were treated with a cationic exchange resin (AG® 50W-X8 Resin; Bio-Rad Laboratories, Inc.) for 30 min to remove salts.

MALDI-TOF MS

The products were spotted onto the MassARRAY SpectroCHIP with an auto-spot arm (Sequenom, Inc.) and then the target plate was inserted into the MALDI-TOF mass spectrometer of MassARRAY compact System (Sequenom, Inc.). The mass range of the MS instrument was set at 3920–12023 Da. SNP loci was genotyped by MassArray Typer Analyzer software version 4.0 (Sequenom, Inc.).

Statistical Analysis

Tests of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were performed using the Power marker v3.25 program. The formula for calculation of expected heterozygosity (H) and polymorphism information contents (PIC) were introduced by Nei and Roychoudhury **(1974)** The formula for calculation of discrimination power and exclusion probability were published by Szibor et al **(2003).**

Results and Discussion

Validation

The procedure to type SNPs used in this study was convenient with multiplexes where 67 X-SNP markers were amplified in four reactions. Validating carried out on control DNA 9947A and DNA extracts prepared from blood samples with known X-SNP profiles from MALDI-TOF MS assay by sequencing revealed fully consistent and reproducible results.

The MALDI-TOF MS technique requires the initial concentration of DNA is not less than

50ng/μL. If the amount of DNA is limited, it may be necessary to use other platform such as SNaPshot (Bouakaze et al 2007 and Wei et al 2012) or to perform a whole genome amplification of the sample previously.

Testing of Linkage Disequilibrium and Calculation for 'Forensic Statistics'

Population data of 67 X-SNPs were obtained. The exact test of 67 X-SNPs showed no deviation from Hardy-Weinberg equilibrium ($p>0.05$) except for rs12849634 ($p=0.0007$).

Two out of the other 66 X-SNP loci (i.e.rs1229078, rs1544545) were found to be low informative (MAF<0.3).Tight LD were observed at six groups （rs5986750-rs5986751, rs183277 - rs7060326, rs6611148 - rs4826623, rs5923750 - rs1166756, rs6620798 - rs5917032, rs2519557 - rs6649211）and slight LD were found at two groups (rs4276834 - rs5928614；rs5968332 - rs6418330). 52 X-SNP loci showing independent inheritance and high polymorphisms were finally selected out (Table 2). Based on the population data (Table 2), the combined values of Discrimination Power in female population and male populations are as follows: CDP_f=0.9999999999999999999 and CDP_m=0.99999999999999931. The combined values of Exclusion Probabilities in trio cases and duo case were are as follows: CPE_{trios}=0.99999999996 and CPE_{duos}=0.9999995. The results revealed that the SNP panel on X chromosome yield the equivalent power in forensic identification as 15 STR markers currently used.

Table 2: Forensic efficiency of 52 X-SNPs

No	X-SNP	allele	frequency	H	PIC	DP_{female}	DP_{male}	PE_{trios}	PE_{duos}
1	rs6639398	A/G	0.50/0.50	0.5017	0.3750	0.6250	0.5000	0.3750	0.2500
2	rs6631828	C/T	0.50/0.50	0.5017	0.3750	0.6250	0.5000	0.3750	0.2500
3	rs5917032	C/G	0.50/0.50	0.5017	0.3750	0.6250	0.5000	0.3750	0.2500
4	rs5912619	A/G	0.51/0.49	0.5016	0.3750	0.6250	0.4999	0.3750	0.2500
5	rs2070289	A/G	0.49/0.51	0.5016	0.3750	0.6250	0.4999	0.3750	0.2500
6	rs3924423	C/T	0.51/0.49	0.5015	0.3749	0.6249	0.4998	0.3749	0.2499
7	rs9781645	C/T	0.51/0.49	0.5014	0.3748	0.6248	0.4997	0.3748	0.2498
8	rs183277	G/A	0.51/0.49	0.5013	0.3748	0.6248	0.4996	0.3748	0.2498
9	rs2209420	A/C	0.51/0.49	0.5013	0.3748	0.6248	0.4996	0.3748	0.2498
10	rs5933388	C/T	0.51/0.49	0.5013	0.3748	0.6248	0.4996	0.3748	0.2498
11	rs5908324	A/G	0.48/0.52	0.5010	0.3746	0.6246	0.4993	0.3746	0.2496
12	rs5980274	C/T	0.52/0.48	0.5004	0.3744	0.6244	0.4987	0.3744	0.2494
13	rs6639989	G/T	0.53/0.47	0.4998	0.3741	0.6240	0.4981	0.3741	0.2490
14	rs5954988	C/T	0.54/0.46	0.4990	0.3737	0.6237	0.4973	0.3737	0.2487
15	rs1874111	A/T	0.46/0.54	0.4989	0.3734	0.6236	0.4972	0.3734	0.2486
16	rs2808742	A/G	0.54/0.464	0.4987	0.3735	0.6235	0.4970	0.3735	0.2485
17	rs5931302	C/G	0.54/0.46	0.4983	0.3733	0.6233	0.4966	0.3733	0.2483
18	rs6611148	G/T	0.46/0.54	0.4982	0.3731	0.6231	0.4963	0.3731	0.2481
19	rs5986750	A/G	0.55/0.45	0.4973	0.3728	0.6228	0.4956	0.3728	0.2478
20	rs5964206	C/G	0.45/0.55	0.4957	0.3718	0.6220	0.4940	0.3718	0.2470
21	rs6646036	C/T	0.45/0.55	0.4957	0.3718	0.6220	0.4940	0.3718	0.2470

No	X-SNP	allele	frequency	H	PIC	DP_{female}	DP_{male}	PE_{trios}	PE_{duos}
22	rs7876774	C/T	0.56/0.44	0.4950	0.3717	0.6216	0.4934	0.3717	0.2467
23	rs2694717	A/C	0.44/0.56	0.4945	0.3714	0.6213	0.4928	0.3714	0.2464
24	rs5920670	A/G	0.44/0.56	0.4945	0.3714	0.6213	0.4928	0.3714	0.2464
25	rs594031	C/T	0.44/0.56	0.4938	0.3710	0.6210	0.4921	0.3710	0.2461
26	rs5906919	A/G	0.57/0.43	0.4931	0.3708	0.6206	0.4915	0.3708	0.2457
27	rs471205	A/G	0.43/0.57	0.4931	0.3708	0.6206	0.4915	0.3708	0.2457
28	rs7471388	C/G	0.43/0.57	0.4931	0.3707	0.6206	0.4915	0.3707	0.2457
29	rs5928614	A/G	0.57/0.43	0.4926	0.3702	0.6203	0.4909	0.3702	0.2454
30	rs1166756	C/G	0.43/0.57	0.4924	0.3703	0.6202	0.4908	0.3703	0.2454
31	rs5926442	C/T	0.43/0.57	0.4907	0.3694	0.6193	0.4889	0.3694	0.2445
32	rs2214174	A/G	0.42/0.58	0.4902	0.3692	0.6191	0.4885	0.3692	0.2443
33	rs6418330	A/G	0.42/0.58	0.4901	0.3692	0.6190	0.4884	0.3692	0.2442
34	rs149860	C/G	0.58/0.42	0.4901	0.3692	0.6190	0.4884	0.3692	0.2442
35	rs5905043	C/T	0.42/0.58	0.4901	0.3693	0.6190	0.4884	0.3693	0.2442
36	rs6649211	A/C	0.42/0.57	0.4900	0.3691	0.6190	0.4883	0.3691	0.2442
37	rs5989571	A/T	0.58/0.42	0.4887	0.3682	0.6182	0.4870	0.3682	0.2435
38	rs17280621	A/G	0.58/0.42	0.4887	0.3684	0.6182	0.4870	0.3684	0.2435
39	rs5956616	A/G	0.42/0.58	0.4875	0.3678	0.6176	0.4859	0.3678	0.2429
40	rs203648	A/G	0.58/0.42	0.4875	0.3678	0.6176	0.4859	0.3678	0.2429
41	rs2768595	A/T	0.58/0.42	0.4874	0.3678	0.6176	0.4857	0.3678	0.2429
42	rs6633608	A/T	0.40/0.60	0.4837	0.3661	0.6156	0.4821	0.3661	0.2410
43	rs1468160	A/G	0.60/0.40	0.4829	0.3655	0.6151	0.4813	0.3655	0.2406
44	rs5955927	C/T	0.60/0.40	0.4827	0.3653	0.6150	0.4811	0.3653	0.2405
45	rs7880460	G/T	0.61/0.39	0.4792	0.3638	0.6130	0.4776	0.3638	0.2388
46	rs12840669	C/T	0.62/0.38	0.4752	0.3614	0.6107	0.4736	0.3614	0.2368
47	rs6418251	A/C	0.62/0.38	0.4734	0.3605	0.6097	0.4718	0.3605	0.2359
48	rs973212	C/T	0.62/0.38	0.4718	0.3597	0.6088	0.4702	0.3597	0.2351
49	rs5916781	A/G	0.62/0.38	0.4710	0.3592	0.6083	0.4694	0.3592	0.2347
50	rs5927322	A/G	0.64/0.36	0.4624	0.3549	0.6031	0.4608	0.3549	0.2304
51	rs5932595	G/T	0.34/0.66	0.4512	0.3486	0.5960	0.4496	0.3486	0.2248
52	rs17264553	G/T	0.34/0.66	0.4501	0.3480	0.5953	0.4486	0.3480	0.2243

Comparison of Population Genetic Data

Of the 67 X-SNP loci, 10 loci (i.e. rs1229078, rs1544545, rs4442270, rs1874111, rs5968332, rs1166756, rs12849634, rs5932595, rs203648 and rs611711) has been reported the power of discrimination and power of exclusion among Spanish population from coastal area and Pas valley by Zarrabeitia (2007). Analysis of pairwise genetic data between the Spanish population and the Chinese Han groups revealed strong

similarities at seven X-SNP loci (i.e. rs4442270, rs1874111, rs5968332, rs1166756, rs5932595, rs203648 and rs611711), but highly significant differences at three X-SNP loci (i.e. rs1229078, rs1544545 and rs12849634). For example, the discrimination power of rs12849634 in Chinese Han female population and male populations were 0.0616 and 0.0316 respectively, but the values were as high as 0.6 and 0.5 respectively in the Spanish population. As for the other two loci, rs1229078 and rs1544545, their polymorphisms in the Spanish population were also higher than that in Chinese Han population.Therefore, it is necessary to investigate the polymorphisms in particular race in order to apply the X-SNP markers to the race.

The results in this study showed that the panel consisting of 52 X-SNP markers was high informative in Chinese Han population. It gives reasonable discrimination and paternity exclusion power that is sufficient for forensic identification.

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