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Research in Genetics

Vol. 2015 (2015), Article ID 374688, 22 minipages.

DOI:10.5171/2015.374688

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Research Article

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

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Received Date: 23 June 2014;

Accepted Date: 14 August 2014;

Published Date: 13 March 2015

Academic Editor: Jian Tie

Cite this Article as: Li Li, Yan Liu and Yuan Lin (2015), " Typing of 67 SNP Loci on X Chromosome by PCR and MALDI-TOF MS," Research in Genetics, Vol. 2015 (2015), Article ID 374688, DOI: 10.5171/2015.374688

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 0.9999999999999999. 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; MALDI-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 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 primers, including a pair of PCR primers and a single base extension primer (Table1). Primers were synthesized by Sangon Biotech (Shanghai, China).

Please See Table 1 in PDF Version

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 μ L) contained 1 μ L the desired primers at their optimized concentrations, 0.625 μ L PCR buffer(10 \times) (Qiagen GmbH),

0.325 μ L 25 mmol/L MgCl₂, 1 μ L dNTP (2.5mmol/L) (Tatara Inc.), 0.1 μ L of HotStarTaq polymerase (5U/ μ L), 1 μ L of DNA , and 0.95 μ L H₂O. 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 0.17 μ L SAP buffer (10 \times), 0.3 μ L SAP (1U/ μ L), 1.53 μ L H₂O (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 9 μ L) contained 0.94 μ L extension primers at optimized concentrations, 0.1 μ L iPlex termination mix, 0.2 μ L iPlex buffer (10 \times), 0.0205 μ L iPlex enzyme and 0.7395 μ L H₂O (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/ μ L. 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: $CDP_f=0.99999999999999999999$ and $CDP_m=0.999999999999999931$. The combined values of Exclusion Probabilities in trio cases and duo case were are as follows: $CPE_{\text{trios}}=0.99999999996$ and $CPE_{\text{duos}}=0.9999995$. The results revealed that the SNP panel on X chromosome yield the equivalent power in forensic identification as 15 STR markers currently used.

Please see Table 2 in PDF version

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.

Acknowledgements

This work was supported by grants from Shanghai Natural Science Foundation and National Institute Scientific Program (Nos.08ZR1419800 and GY0805).

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