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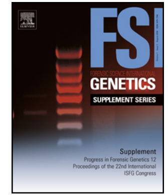

Screening of Multi-InDel markers on X-chromosome for forensic purpose

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ABSTRACT

Many studies have been proposed to identify insertion/deletion (InDel) polymorphisms in humans for forensic genetics. However, the discriminatory power of InDel was limited by the poor polymorphism of diallelic marker. To improve discriminatory power, we designed the Multi-InDel comprising more than two InDel loci that were tightly linked by their physical position as a new marker, which can be amplified by a pair of PCR primers. This strategy gave at least three haplotypes for a Multi-InDel marker. As genetic markers on X-chromosome have been recognized as useful tools to supplement traditional kinship testing, we focused on developing Multi-InDel markers on the X-chromosome (X-Multi-InDel). We explored potential X-Multi-InDel from 1000 genome database, 10 candidates for X-Multi-InDel were selected. The frequencies of the haplotypes were also investigated in Chinese population. The results showed that there were higher levels of heterozygosity in X-Multi-InDel than X-SNP or X-InDel. It implied that X-Multi-InDel markers were useful for individual identification and relationship studies.

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1. Introduction

Insertion/deletion polymorphisms (InDels) are underutilised in the forensic science, since they combine desirable characteristics of both SNPs and STRs, such as widely spread throughout the genome, low mutation rate or can be analysed as short amplicons using the established STR typing techniques [1]. InDels located on the X-chromosome (X-InDels) reflects higher efficiency than those on the autosomes in many deficiency paternity cases [2,3]. However, the use of diallelic X-InDel markers was still limited by its lower discrimination power in contrast to X-STR. Recently, potential ascendancy of haplotype block for kinship analyses has been received much attention [4]. Multi-InDel marker in autosomal has already been developed and proofed to be more effective than traditional diallelic InDel. By combined more than two InDel loci tightly linked in physical position into a specific marker and amplified by a pair primers, each multi-InDel has at least three haplotypes [5].

We present here an attempt to find such Mulit-InDel located on the X-chromosome (X-Multi-InDel). X-Multi-InDel markers that showed well proportion distribution of at least three haplotypes were chosen from the 1000 Genome database. Frequencies of haplotypes and other important biostatistic parameters were determined for each X-Multi-InDel polymorphism.

2. Materials and methods

Bloodstain obtained from 100 unrelated individuals from Chinese Han population were investigated to get haplotypes frequencies. DNA was extracted with QiaAmp Mini Kit (Qiagen).

Based on the 1000 Genome database (http://www.ncbi.nlm.nih.gov/variation/tools/), the X-Multi-InDel candidates should fulfil the following criteria: (i) minimum allele frequency (MAF) ≥0.10 in the population of Southern China (CHS); (ii) not in coding region or *cis*-acting element; (iii) in any Multi-InDel constructed, the interval between InDels <250 bp, (iv) an allele length variation of 1–20 bp of each InDel; (vi) minimum distance of 250 Kb between each candidates, (v) amplicon length <380 bp. Twenty candidates for such X-Multi-InDel loci were obtained (data available from the authors). After genotyping of 30 males and 30 females, more than two haplotypes were observed in 10 of 20 X-Multi-InDel candidates. Table 1 shows the 10 X-Multi-InDel loci selected for this study including their localization on the X-chromosome, primer sequences, InDel sizes and amplicon lengths. Primers were designed using the Primer 3 software (http://primer3.wi.mit.edu/). We inherited nomenclature of alleles that previously employed by us, named the smallest allele as 0, the other alleles as *N* if they were *n* bp greater than the smallest allele [5].

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Table 1
Ten ChrX Multi-InDel polymorphisms overview.

Marker	Locus	InDel motif	Primer sequence (5′–3′) and labeling	Haplotypes
B1	r62954660	-/A	TCAGAAATCCAGAAGTCCAGAAT(FAM)	0, 3, 4
	rs35428456	-/TTTA	gttTGGGTGACAAAGGGAGACTC	
Y1	rs34387295	-/A	GAAGCAGAGGGGAAAGCAAA	0, 4, 5
	rs199928945	-/TGAT/TGAC	TGAGGGAAGGCTTGCTACAA(TAMRA)	
R2	rs58672847	-/G	GCCATGTGTAAGGCCCTATG(ROX)	0, 3, 4
	rs35446765	-/CA	gCAGATCTCAGCTCAAGCATCA	
	rs398069136	-/CA		
Y3	rs72434864	-/A	gTGCGACAAAGACGACAAGC	0, 1, 4
	rs199900518	-/T	TTCCTCTTCGGTTTGTTGTTG(TAMRA)	
Y2	rs201888515	-/TG	CTGAGGCCAATGAAGCAGTG(TAMRA)	0, 1, 2
	rs11297248	-/T	gTATGCTGTTGCCCTCTCTTT	
B2	rs143509592	-/AA	TCAAAGGAACCTGAAGACCCT(FAM)	0, 2, 5
	rs67576675	-/ATG	gttcttTGAAGGAAAATAGTGAGAGCTGT	
G1	rs200978635	-/T	gttcttCTGCATATGAGAGCAGCTGT	0, 4, 5
	rs201816322	-/ CATAA	GTCTGTGTATATGCTGTCAACCA(HEX)	
G2	rs34524694	-/TAAA	TGAAGCCCTGAAGAATTTGCA(HEX)	0, 2, 4
	rs71948836	-/AC	gttcttTCAGGAGGGGAGGGTAGAAA	
R1	rs201836163	-/AAAC	gttcttCCTCGAAAGTGCTGGGATTAC	0, 3, 4
	rs200276682	-/A	AGTTGGTCATTGTTTCTTGTCCT(ROX)	
G3	rs200327587	-/TATG	GGGACATAGCTCTTACCACTTGA(HEX)	0, 2, 4
	rs58551815	-/TT	gttcttAATGAGCCCACCAAGAGCT	

PCR amplification was performed in one multiplex reactions with primer pairs. Thermal cycling conditions consisted of an initial step at 95 °C for 15 min; 30 cycles at 94 °C for 30 s, 62 °C for 90 s, and 72 °C for 60 s; and a final extension at 60 °C for 60 min.

All PCR products were resolved and detected by capillary electrophoresis using ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) and LIZ 500 (Golden eye 20A) as internal size standard.

Hardy–Weinberg equilibrium (HWE) was checked by exact test using Arlequin v3.5.1.2. Heterozygosity, polymorphism information content (PIC), power of exclusion (PE), mean exclusion chance (MEC) were determined using ChrX-STR.org online calculating tools (http://xdb.qualitype.de).

3. Results and discussion

Three haplotypes were observed in each of the X-Multi-InDel loci. Haplotype frequencies of the short, medium length and long alleles are in agreement with 1000 Genomes database information and are shown in Fig 1. The X-Multi-InDel markers were characterised by haplotype ratios resulting in PIC values in the range of 0.415–0.566. All loci were in HWE after Bonferroni's correction for multiple testing.

4. Conclusion

The multiplex setup allowed an accurate and efficient typing of 10 Multi-InDel markers on the X-chromosome. Such markers we selected from 1000 Genomes database were proved to have higher polymorphism than traditional X-InDels. It implied that this novel marker would have a great potential of becoming effective for individual identification and relationship studies. To enhance the ability to deal with degraded DNA samples X-Multi-InDel markers in much shorter amplicons are still warranted in the future. Samples from different ethnic groups are under investigation to obtain the haplotype distribution of these X-Multi-InDel markers.

Conflict of interest

None.

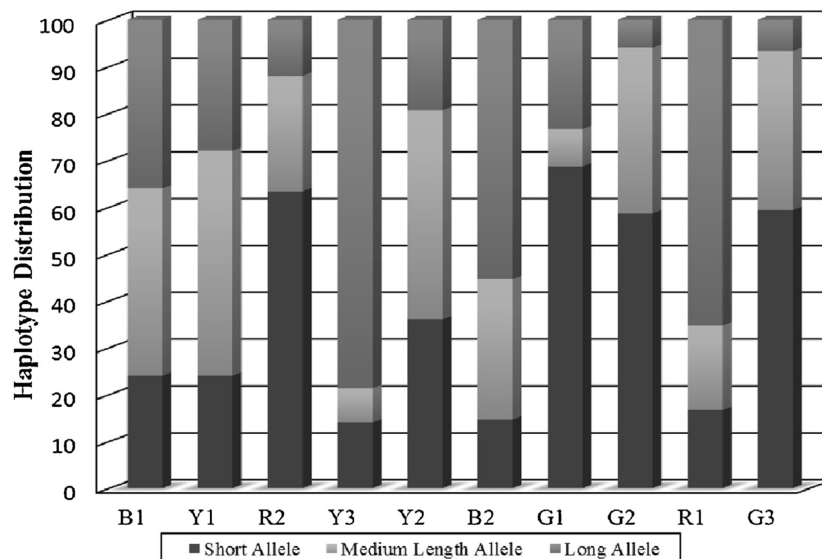

Fig. 1. Haplotype frequencies of 10 X-Multi-InDel polymorphisms. The markers are given in order of their localization from Xp-tel to Xq-tel.

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