The high incidence of STR D21S1446 homozygosity in Han and She populations living in South Eastern China

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Abstract

Purpose This study investigated the allelic frequency of 4 short terminal repeats (STRs) in Han and She populations from eastern China.

Methods DNA samples from Han (N=110) and She (N=110) healthy subjects were amplified using PCR and primers specific for the STRs. D21S11 and D21S1446 (chromosome 21) and D18S865 and D18S535 (chromosome 18). The frequency and homozygosity of different STRs were evaluated. **Results** For both populations, D21S11 and D18S535 were more polymorphic than D21S1446 and D18S865, and the D21S1446 1 allele was very common (48.6% and 55.0% for the Han and She groups, respectively). The Han and She groups significantly differed from one another in respect to the distribution of D21S11, D18S865, and D18S535 allelic

frequency (all P -values ≤ 0.48) but had a similar allelic distribution for D21S1446 ($P=0.106$). D21S1446 was found to be homozygous about 50% of the time which was significantly greater compared to the other STRs analyzed.

Conclusion This study found that the Han and She differ genetically at 3 autosomal STRs located on chromosome 18 and 21. The STR D21S1446 was often homozygous within these groups, indicating it would be poor marker for analyzing trisomy within these populations.

Keywords Short tandem repeat · Trisomy detection · Allele homozygosity · She group · Genetic variant · Polymerase chain reaction

Capsule The allelic frequency of 4 short terminal repeats (STRs) in Han and She populations from Eastern China was investigated. We found that the Han and She differ genetically at 3 autosomal STRs located on chromosome 18 and 21. The STR D21S1446 was often homozygous within these groups

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Introduction

Aneuploidy is the most common chromosome abnormality reported in abortions and newborn surveys. The majority of aneuploidy involves chromosomes 13, 16, 18, 21, X and Y. Aneuploids of chromosome 13, 18, 21, X, and Y represent the majority (95%) of live born abnormalities [1]. In 2006, the Centers for Disease Control and Prevention estimated the rate of trisomy occurs at one per 733 live births in the United States (5,429 new cases per year) and approximately 95% of these are trisomy of chromosome 21, the genetic bases for Down syndrome [2].

Down syndrome occurs in all ethnic groups and among all economic classes. The karyotype of patients with Down syndrome can be divided into three main types. The standard type which accounts for about 95% of all Down syndrome cases is caused by a meiotic nondisjunction event and has the karyotype of (47,XX,+21). The extra chromosome 21 material that causes Down syndrome can also result from a Robertsonian translocation in the karyotype of one or the parents. In this case, the long arm of chromosome 21 joins to another chromosome (most commonly chromosome

14) (45,XX,der[14;21][q10;q10]). A person with such a translocation is phenotypically normal however during meiotic disjunction there is a significant chance of creating a gamete with an extra chromosome 21 producing a child with Down syndrome. Mosaic Down syndrome occurs when some of the cells in the body are normal and other cells have trisomy 21 (46,XX/47,XX,+21). Mosaicism can occur due to a nondisjunction event during a cell division early in embryogenesis of a normal embryo. Alternatively, a Down syndrome embryo undergoes nondisjunction reverting some of the cells to the normal chromosomal arrangement [3].

Members of the She nationality live scattered in vast mountainous territories in the southeastern part of China including Fujian Province, Zhejiang Province, Guangdong Province, Jiangxi Province, and Anhui Province. The population of the She nationality was 709,592 in 2000 (statistics of the fifth Chinese national census in 2000).

Marriage customs within the She society has influenced the genetic diversity within this population. The marriage between Han and She were prohibited before the establishment of People Republic of China. In She society, monogamous marriage is common. The person chooses a partner within the 4 surnames of the She nationality: Pan, Lan, Lei, and Zhong. It is uncommon to marry someone with the same surnames. The limited surnames and the fact that villages often have many relatives result in the levels of consanguinity within a village being high, although not all families with common surnames are relatives [4, 5].

Highly polymorphic short tandem repeat PCR (STR-PCR) is an efficient and cost effective method for identifying trisomy in prenatal screening [6, 7]. STR-PCR is a powerful technique for diagnosing Down syndrome and can detect about 90% of the chromosomal abnormalities and when combined with conventional karyotyping can detect 97.8% of them [7]. However, PCR and conventional karyotype analysis is not always optimal due to factors such as cost etc.

This study used PCR to investigate the distribution of 4 STR loci located on chromosome 18 and 21 (D21S11 [21q21.1], D21S1446 [21q22.3], D18S865 [18q12.3], D18S535 [18q12.3]) in the She and Han Chinese populations [6–9]. We chose these loci as they are commonly used for diagnosing trisomy [7, 8]. In addition, the D21S11 locus is used by the US Federal Bureau of Investigation in the Combined DNA Index System (CODIS) and its distribution in various populations has been well studied [6, 9]. There is currently no data on the allelic distribution of these loci in the She population.

Methods

Data collection

All subjects gave their informed consent.

Han samples

Blood ($n=65$) and saliva ($n=35$) samples of healthy Han individuals was obtained from Wenzhou Medical College. Tissue ($n=5$) and amniotic fluid ($n=5$) samples were obtained from the Forensic Centre of Wenzhou Medical and the Reproductive Center, the First Affiliated Hospital of Wenzhou Medical College, respectively.

She samples

Blood samples ($n=80$) from healthy individuals of She nationality from Southern Zhejiang were obtained from the blood bank in the Jingning area, Zhejiang Province. Saliva samples ($n=29$) were obtained from volunteers in the She community in Binhai, Cangnan County and 1 amniotic fluid sample was provided by the laboratory of the People's Hospital of Cangnan County.

DNA extraction and STR-PCR

Chromosomal DNA was extracted by Chelex 100 resin (Sigma-Aldrich, St.Louis, MO, USA) using standard procedures. Reagents for PCR reactions were purchased from MBI, Taiwan. DNA primers were purchased from Sangon (Shanghai, P. R. China).

Primer sequences

D21S11:	F: 5'-TATGTGAGTCAATCCCCCAAG -3 ' R: 5'-GTTGTATTAGTCAATGTTCTCC -3 '
D21S1446:	F: 5'-ACCATCTTACTGGTTTATGTAT -3 ' R: 5'-GTCCCAAAGGACCTGCTC -3 '
D18S865:	F: 5'-GGGTGATTTCTCTTTGGT-3 ' R: 5'-TGATTCCAATCATAGCCACA-3 '
D18S535:	F: 5'-TGTGTCTATCCCACTGTATTA -3 ' R: 5'-CACTCTCTCTCTCTC TTCAT-3 '

PCR amplification and analysis

DS21S11 amplification

Reactions were heated once to 95°C, then 30 cycles of 95°C for 15 s, 58°C for 60 s, and 60°C for 60 s, followed by 60°C for 20 min.

D21S1446 amplification

Reactions were heated at 94°C for 4 min; then 35 cycles of 94°C for 40 s, 54°C for 50 s, and 72°C for 50 s; followed by 70°C for 10 min.

D18S865 and D18S535 amplification

Reactions were heated at 94°C for 3 min, then 35 cycles of 94°C for 35 s, 58°C for 35 s, and 72°C for 55 s, followed by 72°C for 10 min.

All PCR reactions were stored at 4°C.

PCR reaction products were separated using 6% denaturing PAGE (29:1) on DY III 28AH sandwich-type vertical electrophoresis tank which was run for 2 h at 500 V constant voltage. The sizes of the fragments were estimated using a standard DNA sizing ladder (purchased from Forensic Laboratory, Huaxi Institute of Basic and forensic Medicine, Sichuan University, Peoples Republic of China). The gel was fixed with 10% ethanol for 10 min then soaked in 1% nitric acid, and washed with dH₂O twice (20 s each). The gels were stained with 0.2% silver nitrate for 20 min and washed 3 times (20 s each) with dH₂O. The staining was developed using 3% sodium carbonate (plus formaldehyde) and the reaction was terminated with dH₂O. Images of the gels were captured with a digital camera and stored on a computer.

Statistical analysis

Allele frequency distributions of the 4 autosomal STRs in Han and She population were presented by percentages. Each STR loci allele frequency distribution was

determined using Fisher's exact test. Data were analyzed using SAS 9.0 (SAS Institute Inc., Cary, NC, USA.). All *P*-values were two-sided and were considered significant if *P* was less than 0.05.

Results

The allelic frequency of 4 autosomal STRs located on chromosomes 21 (D21S11 and D21S1446) and 18 (D18S865 and D18S535) were evaluated from a total of 220 samples obtained from healthy Han (N=110) and She (N=110) individuals. For both the Han and She groups, D21S11 and D18S535 were more polymorphic than D21S1446 and D18S865 (Table 1). The most common alleles were 30 and 31 (D21S11) and 13 and 14 (D18S535) ranging from 20.9% to 33.6%. D18S865 identified 3 alleles (8, 10, and 11) that had similar frequencies (range 22.5% to 30.5%; Table 1). In contrast, D21S1446 identified 1 allele (#10) that was more common than the others (48.6% and 55.0% for the Han and She groups, respectively). The frequencies of the different genotypes were in Hardy-Weinberg equilibrium. In addition, the heterozygosity, polymorphism information content, power of exclusion, and average power of discrimination of the different STRs was similar between populations (Table 2).

The Han and She groups significantly differed from one another in respect to the distribution of alleles identified by

Table 1 Allele frequency distribution of 4 autosomal STR loci in Han and She population (*n*=110)

Allele	D21S11		D21S1446		D18S865		D18S535	
	Han	She	Han	She	Han	She	Han	She
7	—	—	—	—	1.80%	—	—	—
8	—	—	—	0.50%	22.70%	25.90%	0.50%	0.50%
9	—	—	—	0.50%	18.60%	11.40%	10.90%	18.60%
10	—	—	48.60%	55.00%	30.00%	27.70%	5.50%	6.80%
11	—	—	20.00%	22.30%	24.10%	30.50%	4.10%	1.40%
12	—	—	5.50%	5.90%	2.70%	4.50%	9.10%	10.50%
13	—	—	24.50%	14.10%	—	—	20.00%	21.80%
14	—	—	1.40%	1.80%	—	—	33.60%	20.90%
15	—	—	—	—	—	—	15.90%	17.70%
16	—	—	—	—	—	—	0.50%	1.80%
28	—	3.20%	—	—	—	—	—	—
29	6.80%	8.60%	—	—	—	—	—	—
30	27.30%	23.60%	—	—	—	—	—	—
31	25.50%	33.20%	—	—	—	—	—	—
32	19.10%	12.70%	—	—	—	—	—	—
33	12.70%	8.60%	—	—	—	—	—	—
33.2	5.00%	8.20%	—	—	—	—	—	—
34	0.90%	0.90%	—	—	—	—	—	—
34.2	2.70%	0.90%	—	—	—	—	—	—
<i>P</i> -value*	0.012		0.106		0.048		0.026	

**P*<0.05 represent a significant difference between allele frequency distribution between Han and She groups

Table 2 Summary of heterozygosity (H), polymorphism information content (PIC), average power of discrimination (PD) and power of exclusion (PE)

Polymorphism parameters	H	PIC	PD	PE
D21S11				
Han	0.782	0.770	0.919	0.566
She	0.745	0.770	0.905	0.502
D21S1446				
Han	0.645	0.640	0.813	0.349
She	0.595	0.580	0.810	0.280
D18S865				
Han	0.836	0.720	0.886	0.668
She	0.773	0.700	0.876	0.549
D18S535				
Han	0.773	0.770	0.929	0.644
She	0.773	0.800	0.943	0.549

D21S11, D18S865, and D18S535 within the population (all P -values ≤ 0.48 ; Table 1) but had a similar allelic distribution for the D21S1446 loci ($P=0.106$).

Analysis of the homozygosity of the 4 STRs indicated that in both the Han and She populations, the genotype of D21S1446 was homozygous about 50% of the time which was significantly greater than the other STRs analyzed (Fig. 1).

Discussion

This study investigated the allelic frequency of 4 STRs in Han and She populations of southeastern China. It found

that for 3 STRs, the allelic frequency significantly differed between the populations. This finding is consistent with little genetic flow (or exchange of alleles) between the 2 populations. The lack of genetic flow may result from cultural and/or geographical isolation. It is not clear if the 2 populations arose from a similar genetic origin and then subsequently diverged or were derived from different founder groups. Further analysis using STRs from Y chromosomes is needed to elucidate the phylogenetic relationship between these two nationalities.

The D21S1446 STR was found to have a high frequency of homozygosity in both the Han and She groups. One allele (#10) for this STR was also highly common (about 50%) in both populations. The data extracted from The Human Genome Diversity Project by Foundation Jean Dausset [10] found that the percentage of the D21S1446 #10 allele in Han Chinese living in San Francisco, CA, USA was 60%. These results indicate that the high frequency of this allele is at least common to the Han population, and suggest that there may be selective pressure on its sequence [6, 11]. For example, it is possible that the sequence of D21S1446 STR plays a role in gene function or regulation or chromosome stability [6].

Prenatal chromosomal content analysis has been used extensively to identify fetuses with potential chromosome abnormalities, such as trisomy. Multiple approaches are available to determine if a fetus has abnormal chromosomes including karyotyping [12–14], genomic sequencing [15–17]; micro-array analysis [18] and PCR based methods [8]. The non-microscope techniques have the advantage of being rapid and cost effective.

Identifying trisomy by using the highly polymorphic STRs and PCR requires measuring the ratio of a minimum

Fig. 1 Homozygote rate of 4 autosomal STR loci in the Han and She populations. The frequency of the homozygous STRs (from left to right) D21S11, D21S1446, D18S865, and D18S535 in the Han and She populations are indicated (bars). * indicates $P<0.05$ compared to other homozygote rates

of 2 informative STR markers [7]. The presence of disomic and diallelic STRs is important for accurately determining STR copy numbers. A normal chromosomal number would result in a PCR pattern in which the ratio of the alleles is approximately 1:1 [7, 19]. Trisomy would be indicated by the presence of a tri-allelic pattern with a ratio of 1:1:1 or trisomic diallelic pattern of 2:1 [7, 19, 20]. A high degree of homozygosity at a given STR, for example D21S1446, would result in an increased chance of a 2:1 diallelic pattern, possibly resulting in false positives for trisomy. Both the Han and She populations are highly homozygous for D21S1446 indicating it is not an optimal marker for measuring trisomy in these populations. Care should be taken in these (and other) populations to use multiple STRs and potentially a second aneuploidy detecting approach when diagnosing trisomy.

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Conflict of interest The authors declare no conflict of interest

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