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SHORT COMMUNICATION

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Identification of novel allele on the locus 47z (*DXYS5*) in the Korean population

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Abstract Two homogenous sequences of 47z (*DXYS5*) are located on the X (*DXYS5X*) and Y (*DXYS5Y*) chromosomes, and these are known to be useful polymorphic markers for tracing male-specific gene flow such as the migration routes of human populations. Using the *47z*/*Stu*I PCR–RFLP system, we found a novel allele which showed two bands, in contrast to the previous two allele types, one band (Y1) and three bands (Y2). This means that copies of PCR products derived from both the *DXYS5X* and *DXYS5Y* loci were clearly cut by the *Stu*I enzyme, implying that the *DXYS5X* locus of the X chromosome is polymorphic. Allelic frequencies examined in 267 male Korean individuals showed that 95.8% had Y1, 3.4% Y2, and 0.8% had the novel allele. Our findings should contribute to a better understanding of genetic polymorphism on X and Y chromosomes, the molecular evolution mechanism of sex chromosomes, and how the migration route of Koreans is related to those of other East Asian populations.

Keywords *DXYS5* · Novel allele · Allele frequency · Polymorphism · Korean population

Introduction

Polymorphisms of alleles residing in the nonrecombining portion of the human Y chromosome have been used to trace male-specific gene flow and human evolution (Spurdle et al 1994; Hammer and Horai 1995). In particular, the *47z*/*Stu*I polymorphism (Nakahori et al 1989) that is known to be specific to Asian populations is found in Japan, Korea, and Taiwan but is absent in other populations (Nakagome et al 1992; Hammer and Horai 1995; Shin et al 1998; Oh et al 2000). A simple PCR–RFLP method has been used to investigate the polymorphism, which is represented by two alleles, Y1 and Y2. Until now, the Y1 allele frequency has been shown to be higher than that of the Y2 allele in Asian populations (Shin et al 1998; Shinka et al 1999; Kim et al 2000). Chinese and Philippino populations are 100% in the Y1 allele, whereas the frequency of Y1 in Japan is relatively lower (83%) than other Asian populations.

In this study, we investigated the allelic variation in *47z*/*Stu*I polymorphism using the *47z*/*Stu*I PCR–RFLP method in 267 individual males in a Korean population. Here we report a novel allele of *47z*/*Stu*I polymorphism in addition to the two previously known alleles (Y1 and Y2), and discuss its consequences.

Materials and methods

47z/StuI PCR–RFLP

Using the standard method (Sambrook et al 1989), genomic DNAs were prepared from whole blood of 267 individual Korean males. Two copies of *DXYS5* DNA at the loci of Xq21 (*DXYS5X*) and Yp (*DXYS5Y*) were amplified by PCR using AmpliTaq DNA polymerase (Perkin-Elmer, Boston, MA, USA), subsequently cut with *Stu*I enzyme (Shin et al 1998) and separated on 3% agarose gel and 7% polyacrylamide gel. Primer

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sequences used were NS2F: 5′-TGAGTCAATGT-CAATGAATC-3′ and NS2R: 5′-TAGTTACGCCT-TGGCATAAC-3′. DNA amplification was performed. To determine the DNA sequences, the amplified DNAs were directly cloned into pGEM-T easy Vector (Promega), and sequenced with universal sequencing primers (F40 and R21) via BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Ver 3.1. Foster City, CA, USA). Individual sequences were assembled into contigs using the Sequencher 4.1.4 (Gene Codes Corp., Ann Arbor, MI, USA).

Results and discussion

The most popular method used to detect the polymorphism of the *DXYS5Y* locus on the Y chromosome is the *47z*/*Stu*I PCR–RFLP system (Shin et al 1998; Shinka et al 1999). In this study, we investigated 47z (*DXYS5*) polymorphism using 267 individual Korean males using this system. Unexpectedly, we found a new band pattern which was distinct from the two previously known types, Y1 and Y2, and clearly demonstrated it by 7% polyacrylamide gel electrophoresis (Fig. 1). The new variant showed two bands, 280 and 100 bp, which suggests that DNAs to be amplified from both *DXYS5Y* and *DXYS5X* loci were clearly cut by *Stu*I enzyme. Moreover, we analyzed the nucleotide sequence and confirmed that individuals showing the novel allele have *Stu*I restriction sites (AGGCCT) on not only the *DXYS5Y* but also on the *DXYS5X* locus (Fig. 2). Consequently, our result implies that the *DXYS5X* locus is a polymorphic site although all previous studies have only considered Y-linked polymorphism (*DXYS5Y*), disregarding X-linked polymorphism (Shin et al 1998; Oh et al 2000).

Based on these results, we reformulated allele types derived by the *47z*/*Stu*I PCR–RFLP method on the 47z (*DXYS5*) locus, as follows: X1–Y1, X1–Y2, X2–Y1, and X2–Y2. X1–Y2 and X2–Y1 correspond to the Y2 allele in previous reports, and X1–Y1 and X2–Y2 correspond to the Y1 allele of the previous and the new variant from this study, respectively (Fig. 1).

We further investigated the allele frequencies for *47z*/*Stu*I polymorphism using 267 individual Korean males, which showed 95.8% (X1–Y1), 3.4% (X1–Y2 and X2–Y1), and 0.8% (X2–Y2) or 2 out of the 267 individuals (Table 1). The frequency was similar to previous results seen in Korean populations. However, since the two bi-alleles of X1–Y2 and X2–Y1 cannot be classified by the method, previous results (Shin et al 1998; Shinka et al 1999; Oh et al 2000) may have overestimated the Y2 allele due to the misjudgment about the X and Y polymorphism. Therefore, our reformulated allele types on the 47z (*DXYS5*) locus should provide important clues for understanding not only the gene flow of the Y chromosome but also that of the X chromosome.

A recent effort highlighted that the X-transposed region (XTR) on the Y chromosome resulted from the transposition of a chromosome segment from the X chromosome to the Y chromosome, which occurred after the speciation event that lead to human and chimpanzee lineages (Ross et al 2005). Our finding suggests that the translocated fragment including *DXYS5* did not have the *Stu*I site, and after translocation the base change on the Y chromosome resulted in the *Stu*I site. This means that the same base change at the *Stu*I site happened on the X chromosome independently without changing any of the surrounding sequence, as gene conversion. Therefore, our finding supplies an important clue to not only the migration route of human lineage but also the molecular evolution mechanism.

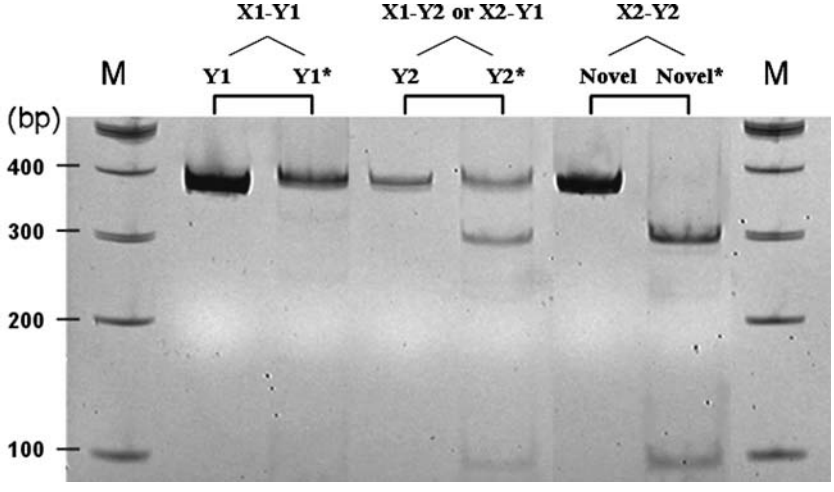

Fig. 1 Detection of the *47z*/*Stu*I polymorphism using the primers NS2-F and NS2-R. DNAs amplified from three different male individuals (*Y1, Y2, and Novel*) and subsequently digested by *Stu*I enzyme (*Y1*, Y2*, and Novel**) were separated using a 7% polyacrylamide gel electrophoresis method. All of the alleles were reformulated in this study as follows: X1–Y1, X2–Y1, X1–Y2, and X2–Y2. Expected sizes of the allelic variants are 382, 289, and 93 bp, respectively, and these depend on the sequence variation of the *Stu*I enzyme site. The numbers on the left hand side indicate a 100 bp ladder size marker (*M*)

Fig. 2 Alignment result for novel allele sequences for *47z*/*Stu*I polymorphism. Two consensus sequences derived from X and Y chromosomes were aligned by the CLUSTALW program, and a reliable region discriminated the X sequence from the Y (*gray region*). Two sequences have the sequences of the *Stu*I enzyme (*black rectangle*), and the cutting site of the *Stu*I enzyme is represented by an *arrow*

Table 1 Allele frequencies of *47z*/*StuI* in eight East Asian populations

Population (*n*)	No. (%) of		
	Y1	Y2	Novel
North Asians			
Chinese (180)	180 (100.0)	0	
Japanese (251)	208 (82.9)	43 (17.1)	
Korean (412)	386 (93.7)	26 (6.3)	
Korean (267)	*256 (95.8)*	*9 (3.4)*	*2 (0.8)*
Mongolians (116)	116 (100.0)		
South Asians			
Indonesians (42)	38 (90.5)	4 (9.5)	
Philippino (108)	108 (100)	0	
Thais (73)	71 (97.3)	2 (2.7)	
Vietnamese (78)	75 (96.2)	3 (3.8)	

This is based on Kim et al (2000), and the *italicized* values are from this study. The Y1, Y2, and Novel alleles were reformulated to X1–Y1, X1–Y2 or X2–Y1, and X2–Y2 in this study

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