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SHORT COMMUNICATION

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A novel dimorphism in the human SRY gene: usefulness in human migration studies

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Abstract A nucleotide polymorphism of C or T was detected at position 465 in the sex-determining region Y (SRY) gene. To evaluate the utility of this dimorphism in human population studies, the frequency and the frequency of the haplotype combined with the two polymorphic loci YAP and M9 were examined in a total of 130 unrelated Japanese and 130 unrelated German males. The T nucleotide was found in 24.6% (32/130) of the Japanese but not in any of the 130 German males. Accordingly, four of the eight possible combination haplotypes of SRY/YAP/M9 were identified in the Japanese population, but one of the four haplotypes comprising SRY(T) was absent in the German samples. This suggests that the C to T transition may be more recent than the YAP insertion or the M9 transversion and the change might have occurred in an ancestral Asian population. These results imply that the dimorphism at the SRY gene is one of the Y-linked markers useful for human population studies and also for ethnic identification of forensic samples.

Keywords SRY gene · Single nucleotide polymorphism · YAP element · M9 mutation · Japanese · German

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Introduction

The Y chromosome is paternally inherited and insulated from meiotic recombination except for the pseudoautosomal (PA) region. Accordingly, polymorphic sites of DNA sequence on the non-PA region of the Y chromosome are useful for human population studies based on male lineages. There are many reports on Y-specific polymorphic markers (Nakahori et al. 1989; Mathias et al. 1994; Spurdle et al. 1994; Jobling and Tyler-Smith 1995; Roewer et al. 1996; Knijff et al. 1997), most of which consist of microsatellites, including CA-repeats. Certain microsatellites have been successfully used for comparing closely related populations (Knijff et al. 1997; Jobling et al. 1997; Forster et al. 1998), but some may not be suitable for population studies because tandem repeats are more prone to mutation due to replication slippage. On the other hand, markers detecting single nucleotide polymorphisms (SNPs) are relatively stable and several SNP probes on the Y chromosome have also been reported. These include A to G transitions (DYS271) (Seielstad et al. 1994), C to T transitions (DYS199) (Underhill et al. 1996), 3 point mutations at the DYS278 locus (Hammer et al.1997) and 19 biallelic markers (Underhill et al.1997). A combined use of Y-polymorphisms is required to infer an evolutionary context (Knijff et al. 1997).

The sex-determining region Y (SRY) gene on the Y chromosome (Su and Lau 1993) is Y-specific and highly conserved. We have identified a novel single-base pair substitution (C to T) at nucleotide position 465 in the SRY gene. In this paper we have studied the feasibility of the novel dimorphism by examining the frequency of the combined haplotypes at the SRY, YAP and M9 loci in 130 Japanese and 130 German males. The YAP locus is an Alu element on the Y chromosome (DYS278) and shows a presence or absence polymorphism while the M9 comprises a polymorphism resulting from a C to G transversion (Underhill et al.1997). Both polymorphisms have proved to be powerful tools for population studies (Hammer 1994; Hammer and Horai 1995; Underhill et al. 1997;

Hurles et al. 1998; Karafet et al. 1999). Here we show that there are significant differences in the haplotype frequencies between the two populations, indicating the usefulness of this marker in human population studies.

Materials and methods

Samples

Genomic DNA was isolated from blood samples of 130 unrelated Japanese males (from Niigata) and 130 unrelated German males (from Munich) by proteinase K digestion and phenol/chloroform extraction.

Direct DNA sequencing

Direct DNA sequencing of the SRY gene (nucleotide positions 118–512) in eight Japanese males was performed with the ABI PRISM 310 Genetic Analysis System (Perkin-Elmer, Foster City, Calif.). The sequencing primers used were as follows:

– HSRY-ORF-F:5'-CTTCCTTTGCACTGAAAGC-3'
– HSRY-ORF-R:5'-CTCCATTCTTGAGTGTGTG-3'

PCR and electrophoresis

The SRY dimorphism was analysed by the PCR-APLP (amplified product length polymorphism) method (Watanabe et al. 1997) using three primers as follows:

– HSRY-F1: 5'-TATCGACCTCGTCGGAAGG-3' (forward, common)
– HSRY-R2: 5'-GTTGTCCAGTTGCACTTCG-3' (reverse, for C type)
– HSRY-R3: 5'-AAGGAGTTGTCCAGTTGCACATCA-3' (reverse, for T type)

These yielded a 99-bp product for the C type and a 104-bp product for the T type. The reaction was performed in 10 µl of PCR buffer containing 30 ng template DNA, 0.21 µM HSRY-F1 primer, 0.08 µM HSRY-R2 primer, 0.16 µM HSRY-R3 primer and 0.5 U of AmpliTaq Gold (Perkin-Elmer). The cycling conditions were 95°C for 9 min and 40 cycles of 95°C for 15 s, 60°C for 10 s, and 72°C for 10 s. Amplified products were separated by electrophoresis in 13% polyacrylamide gels and visualised by ethidium bromide staining or silver staining.

The YAP element was detected by PCR amplification using the flanking primers as described by Hammer and Horai (1995). The dimorphism of M9 was determined by *Hin*fI digestion of DNA fragments that were amplified using the primers described by Underhill et al. (1997).

Results

A 395-bp-fragment in the coding region of the SRY gene (nucleotide position 118–512 from the translation initiation site) was amplified by PCR for eight Japanese males and the products were sequenced. Figure 1a shows the sequences of two samples which demonstrate a nucleotide polymorphism of T or C at position 465. To detect this dimorphism without the use of a sequencer, we designed a set of three primers for PCR amplification (see Materials and Methods). With this PCR assay, the one nucleotide difference was detected simply by running PCR products on an acrylamide gel. Figure 1b shows the gel patterns of eight samples where a T or C at the position 465 is clearly identified by the migration difference in gel electrophoresis. Among the eight samples, three males DNA were T (SRY-T type) and the other five were C (SRY-C type). The typing results were consistent with those of the direct DNA sequencing (data not shown), indicating the accuracy of this method of PCR typing. This SRY typing was

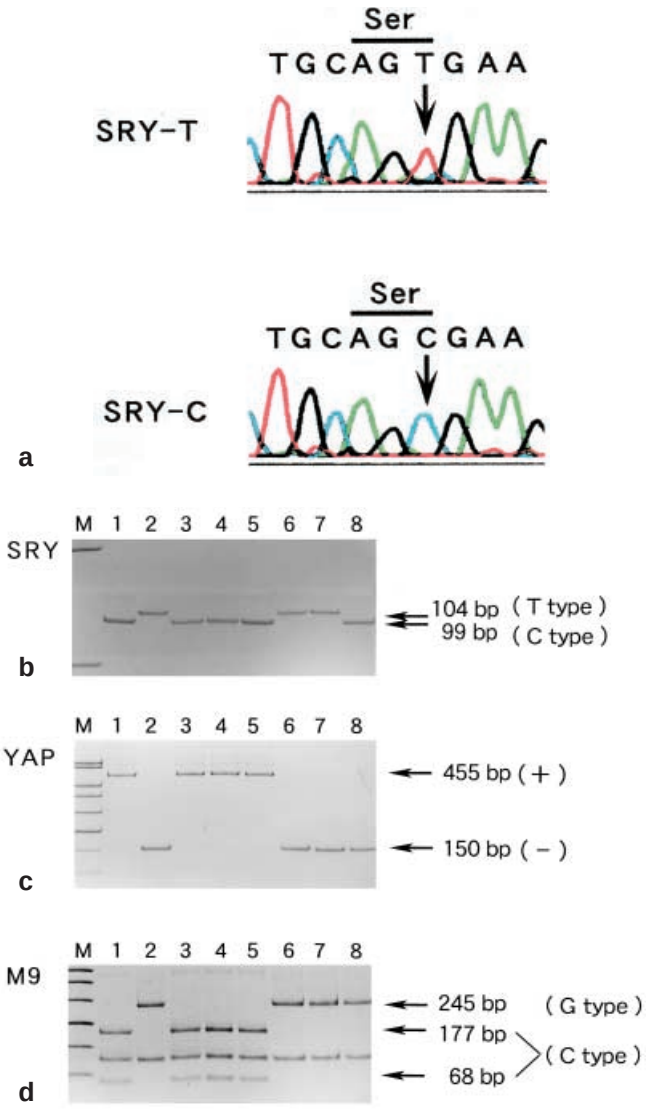

Fig. 1a–d Detection of the SRY polymorphism, the YAP element and the M9 mutation in eight unrelated Japanese males (*lanes 1–8*). **a** Sequencing patterns of two samples showing dimorphism. The arrows indicate a T peak for SRY-T type and a C peak for SRY-C type at nucleotide 465 of the SRY gene. **b** PCR-APLP analysis of the SRY polymorphism. A 104-bp DNA product was amplified for the T-type and a 99-bp fragment for the C-type. **c** PCR assay of the YAP locus. The amplification was performed in 10 µl of PCR buffer containing 30 ng template DNA, 0.1 µM each primer, and 0.5 U of AmpliTaq Gold. The thermal cycle profile was 38 cycles of 94°C for 45 s, 52°C for 45 s, and 72°C for 1 min. Electrophoresis was carried out on 12% polyacrylamide gels in a 0.5×TBE buffer containing 5% glycerol (*lane M*: size marker pBR322/*Hin*fI). **d** PCR-RFLP analysis of M9. The amplified 340-bp product including the M9 polymorphic site was digested with *Hin*fI. The M9 C-type produced a 68-bp fragment. The conditions of PCR and electrophoresis were the same as for **c**

Table 1 The frequencies of SRY/YAP/M9 combination haplotypes in Japanese and German populations

Haplotype				Japanese *n* (frequency)	German *n* (frequency)
No.	SRY nt 465	YAP element	M9 (C/G)		
1	C	–	C	6 (0.046)	37 (0.285)
2	C	+	C	62 (0.477)	11 (0.085)
3	C	–	G	30 (0.231)	82 (0.631)
4	T	–	G	32 (0.246)	0
Total				130	130

then applied to a total of 130 unrelated Japanese males and 130 unrelated German males. The observed allele frequencies are summarised in Table 1. The SRY-T type was found in 24.6% of the Japanese but not in any of the German males. In parallel, typing of the YAP present/absent polymorphism and the M9 G or C dimorphism was performed for the same DNA samples. Figure 1c,d shows gel electrophoresis patterns of the same eight males and Table 1 summarises the results of the 260 Japanese and German males. Insertion of the YAP element was detected in 47.7% of the Japanese and in 8.5% of the German males, whereas the M9 G-type was found in 47.7% in Japanese and in 63.1% of the German males. Of the eight possible SRY/YAP/M9 combination haplotypes, four were observed in the Japanese population, while only three were detected in the German population. In the Japanese samples, the frequencies of SRY(C)/YAP(–)/M9(C), SRY(C)/YAP(+)/M9(C), SRY(C)/YAP(–)/M9(G) and SRY(T)/YAP(–)/M9(G) haplotypes were 0.046, 0.477, 0.231 and 0.246, respectively, whereas in the German samples those of SRY(C)/YAP(–)/M9(C), SRY(C)/YAP(+)/M9(C), and SRY(C)/YAP(–)/M9(G) were 0.285, 0.085 and 0.631, respectively (Table 1). It should be noted that the SRY(T)/YAP(–)/M9(G) haplotype was observed in the Japanese males but not in the German males.

Discussion

In this study, a dimorphism (C/T) resulting in a silent mutation (Ser to Ser) was found at position 465 in the SRY gene. The nucleotide positions 465 and 466 in the SRY gene consist of the CpG dinucleotide which is a hot-spot for the mutation of C to T transition in vertebrate genomes (Strachan and Read 1996). We therefore interpreted this to show that the SRY dimorphism occurred by the C to T transition but not the T to C transition. This interpretation is consistent with the observation that the SRY-T allele was detected in 24.6% of the Japanese males but not in any of the 130 German males.

An unrooted tree shown in Fig. 2 was constructed with the frequencies of the SRY/YAP/M9 haplotypes in the Japanese and German samples conforming to the mutation tree by Jobling et al. (1997). This suggests that insertion of the YAP element and the C to G transversion of M9 occurred independently in a SRY(C)/YAP(–)/M9(C) chromosome (haplotype-1 changed to haplotype-2 and haplotype-3, respectively) and that the SRY transition took place in the haplotype-3 chromosome that had received the M9 mutation. A significant difference ($p < 0.001$) in the SRY/YAP/M9 frequency was detected between the geographically isolated Japanese and German populations.

The frequency of the YAP(+) was 47.7% in the Japanese and 8.5% in the German population (Table 1). The result at the YAP locus is consistent with the results of previous investigations (Hammer 1994; Hammer et al. 1997). In those studies the YAP element was frequently observed in Japanese but interestingly it was absent or at a very low frequency in some other Asian populations as well as in European populations. Our preliminary studies of Korean males (Yuasa et al. unpublished results) revealed that the YAP(+) frequency is low(1/74), which is consistent with previous data (Kim et al. 1998). According to Hammer and Horai (1995), YAP(+) chromosomes migrated to Japan with the Jomon people more than 10,000 years ago

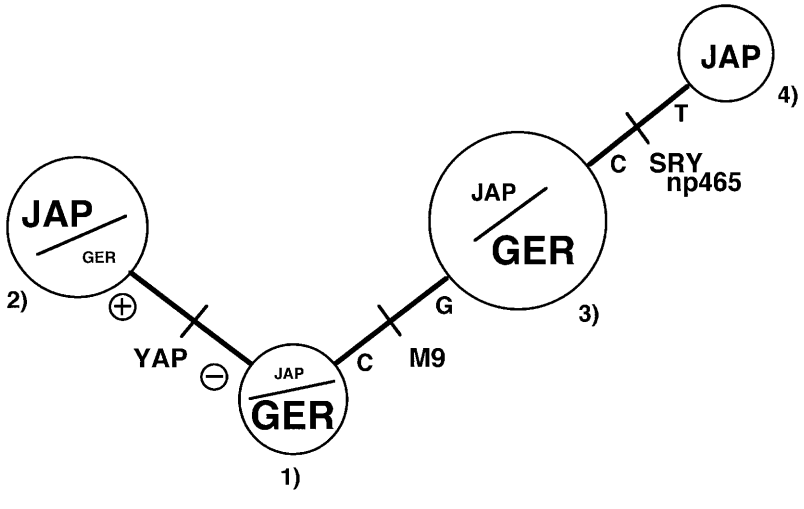

Fig. 2 The unrooted tree of the combination haplotypes of SRYnp465, YAP insertion and M9 mutation for Japanese and German populations. The four haplotypes (*1–4*) are shown in Table 1 (The size of the circles and the three-letter population codes *JAP* Japanese, *GER* Germans reflects the frequency of the haplotype)

and then there was a large infusion of YAP(–) chromosomes with the Yayoi migration from the Korean peninsula to Japan 2,300 years ago. As a result, modern Japanese have genes deriving from both the Jomon and Yayoi people. It is interesting that the SRY(T)/YAP(–) haplotype was detected in a Korean population at a frequency similar to that of Japanese (Yuasa et al. unpublished results). This suggests that the Japanese entered from the Korean peninsula, which is consistent with previous studies (Hammer and Horai 1995; Kim et al. 1998).

In contrast with the YAP insertion, which is common in Africans and Japanese, the M9 C to G mutation has been found in all geographic regions except Africa (Underhill et al. 1997). This study shows that the frequencies of YAP(–)/M9(C), YAP(+)/M9(C) and YAP(–)/M9(G) were 0.046, 0.477 and 0.477 in Japanese and 0.258, 0.085 and 0.631 German samples, respectively. The results of the YAP/M9 haplotypes coincide with the frequencies reported by Underhill et al. (1997) and Karafet et al. (1999).

In conclusion, the SNP in the coding DNA region of the SRY gene was examined in this study and the geographic distribution of the SNP was skewed. Therefore, the novel dimorphism should be one of the useful Y-linked markers for human population studies. In addition, it may be applicable to ethnic identification of forensic samples. Further investigations of the distribution and frequency of the SRY polymorphism in a variety of populations, especially in Asia, may give more information about human migration.

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