APPLICATION OF Y-CHROMOSOMAL STRS TO PARENTAGE TESTING

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Abstract
Population studies were carried out on the Y-specific short-tandem-repeat (STR) polymorphisms DYS19, DYS385 I+II, DYS389 I+II, DYS390, DYS391, DYS392, and DYS393 in population samples from North India, Turkey and West Germany. In the German population, a highly unusual haplotype was observed. Sequence analysis revealed, that the variant allele DYS392*11.1 was caused by an insertion of an A (adenine). A Turkish haplotype showed 2 alleles (*15 and *16) at locus DYS19. A father and son pair shows a full haplotype with the Y-STRs tested but reveals only the X-allele when tested for the amelogenin locus.

The usefulness of Y-chromosomal markers is demonstrated in a deficient paternity case.

1. Introduction
DNA-polymorphisms on the human Y chromosome are valuable tools for evolution and migration studies as well as for identity testing. In forensics, Y chromosome markers are particulary used in rape cases and in deficient paternity cases with male offspring [1 - 6]. To date, most forensic Y-chromosome DNA work around the world has focused on a set of 7-9 short-tandem-repeat (STR) polymorphisms [7,8]. This study was performed to determine Y-STR haplotype frequencies in three population samples.

2. Materials and Methods
2.1 *Population samples*
. Jat Sikhs are endogamous but practice g*otra* (clan like organisation) exogamy. Samples from 108 unrelated individuals were collected and analysed. The samples were collected from the Punjabi University students and villagers belonging to the districts of Patiala, Fatehgarh Sahib, and Sangrur of the Panjab, India. 160 unrelated Turkish males living in Germany were tested as they were involved in legal proceedings concerning paternity.
In addition, 166 unrelated German Caucasians were analysed.

2.2 *DNA extraction*
DNA was extracted either from Iso Sticks (Schleicher and Schuell) following the company's recommendation or from EDTA blood samples by the salting out method described by Miller *et al*. [9].

2.3 *Analysis*
Primers and PCR conditions
Primers were employed as described by Kayser *et al*. [4] or Schneider *et al*. [10].
Monoplex amplification: DYS385 (HEX) [10]
Triplex amplification: DYS391 (NED), DYS392 (FAM), DYS393 (FAM)

[4]
Quadruplex amplification: DYS19 (NED), DYS389I/II (NED), DYS390 (FAM) [4].

Analysis
Capillary electrophoresis was carried out on an ABI 310 genetic analyzer (PE), using the internal standard CXR 60-400 (Promega, Madison, USA). The allele attribution was made by comparison with allelelic ladders constructed from reference samples kindly provided by Lutz Roewer, Institut f. Rechtsmedizin, Berlin. Correct allele calling was additionally assured by successful participation in the quality control tests of the Y chromosome short-tandem-repeat (STR) haplotype reference database (http://ystr.charite.de).

3. Results and Discussion

3.1 *Population studies*

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At certain loci, striking differences between the three populations can be observed. At locus DYS385, the allele pair *9 / *16 for example, seem to occur in the Sikh series only. On the other hand, at locus DYS391 the alleles *10 and *11 are common in all three populations (data not illustrated). Because most of the Y chromosome does not recombine, alleles at Y-STR loci should be combined to haplotypes. The most common haplotypes in each ethnic group are shown in table 1.

The vast majority of haplotypes was observed only once in the three populations. 68 different haplotypes were observed in 108 Jat Sikhs, 148 haplotypes were observed in 160 Turkish males, and 133 haplotypes were found in 166 German Caucasians. The most frequent haplotype in Jat Sikhs was found in 13 individuals (12 %), while in Turks and in Germans the highest frequencies were 2.5 %, and 4.8 %, respectively. These data show that a very high degree of diversity can be found in Turks, which confirms a previous study by Decorte et al. [11]. Interestingly, a German individual carried a DYS392 *11.1 allele which was caused by an insertion of A (adenine). The consensus sequence structure is mentioned elsewhere (http://ruly70.medfac. leidenuniv.nl/~fldo/) (P.de Knijff).

A Turkish father carried two alleles (*15 and *16) at locus DYS19. He inherited his haplotype to his son; duplicated alleles have been observed at various Y-STR loci [Kayser *et. al* 1997, Kayser *et. al* 2000] and are thought to reflect duplication events of larger regions including the STR locus followed by a mutation in the number of repeats. For DYS19 a frequency of 0,12% has been reported by Kayser *et al.* [12].

3.2 *Application to paternity testing*

As an example for the application of Y-STRs, a case study is provided. Because an alleged father could not be reached for testing, the court ordered his two brothers to be tested. An excerpt from the typing results is shown in table 2, which can be interpreted as follows. Strong evidence for kinship was found in the Rhesus blood group system, in DNA minisatellite polymorphisms, (despite the fact that there exist 5 RFLP alleles in the predigree at loci D12S11 and D5S110 !) and in Y-chromosomal STRs. The observed Y-chromosomal STR haplotypes revealed that the two men were half-brothers (meaning that they share the same mother but had different fathers). We have calculated that the probability for P. being the uncle of the persuing child exceeds 99.99 % (based on the analysis of 5 conventional markers, 6 DNA minisatellite polymorphisms, 8 autosomal STR polymorphisms, and 9 Y-chromosomal STRs).

3.3 A putative father did not show the Y allele with neither the PowerPlex® 16 (Promega) (Fig 1) nor the SGM Plus (Applied Biosystems). The same phenomenon was observed in his son. Both DNA samples amplified the full set of Y-STR markers studied here (Fig 2). Another example of this kind of mutation was described by Roffey et a. [13].

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Table 1: The most common haplotypes in German, Turks and Jat Sikhs

	total number of haplotypes tested	DYS19	DYS385 I-II	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	number	frequency
German	166	14	13-14	12	28	22	10	11	13	8	4.85
		14	11-14	13	29	24	11	13	13	6	3.61
		14	11-14	13	29	24	10	13	13	5	3.01
Turks	160	14	12-13	13	9	23	10	14	13	4	2.50
Jat Sikhs	108	15	9-16	13	29	22	10	14	12	13	19.12
		16	11-14	13	30	25	11	11	13	8	11.76
		14	15-19	14	30	23	10	11	13	5	7.35

Table 2
Genetic findings in a deficient paternity test (exerpt)

Serology

System	*Child*	*Mother*	*Uncle P.*	*Uncle C.*
Rhesus	(C)cD.Ee	CcD.ee	(C)cD.EE	ccD.Ee

DNA-Minisatellite-Systems (Sizes in kilobases)

Locus	*Child*	*Mother*	*Uncle P.*	*Uncle C.*
D1S7	6.1/4.7	6.1/4.8	9.4/4.3	9.4/5.6
D7S21	9.0/**6.4**	9.0/5.7	8.1/6.0	**6.4**/6.0
D12S11	10.8/7.9	7.9/6.7	10.3/4.9	9.4/9.2
D2S44	**4.3**/3.3	3.3	**4.3**/2.6	3.1/2.7
D16S309	**4.0**/2.1	2.7/2.1	2.8/2.7	**4.0**/2.4
D5S110	7.3/5.8	7.3/2.8	5.3/2.7	6.9/4.1

Autosomal-Microsatellite-Systems

Locus	*Child*	*Mother*	*Uncle P.*	*Uncle C.*
D3S1358	16/17	16	17	17
vWA	14/18	16/18	14/15	16/17
FGA	21/24	20/21	24	24
TH01	6	6/9.3	6/9	6/7
TPOX	10/11	10/11	8/9	8/11
CSF1PO	11/12	12/12	11	11/13
D5S818	11/13	10/11	13	9/13
D13S317	9/12	9	12/13	11/12

Y-chromosomal-Microsatellite-Systems

Locus	*Child*	*Uncle P.*	*Uncle C.*
DYS19	14	14	13
DYS389.I	10	10	9
DYS389.II	26	26	27
DYS390	23	23	23
DYS391	10	10	8
DYS392	11	11	11
DYS393	12	12	13

Fig. 1: Profile of son and father obtained with PowerPlex® 16 missing the Y allele of the amelogenin.

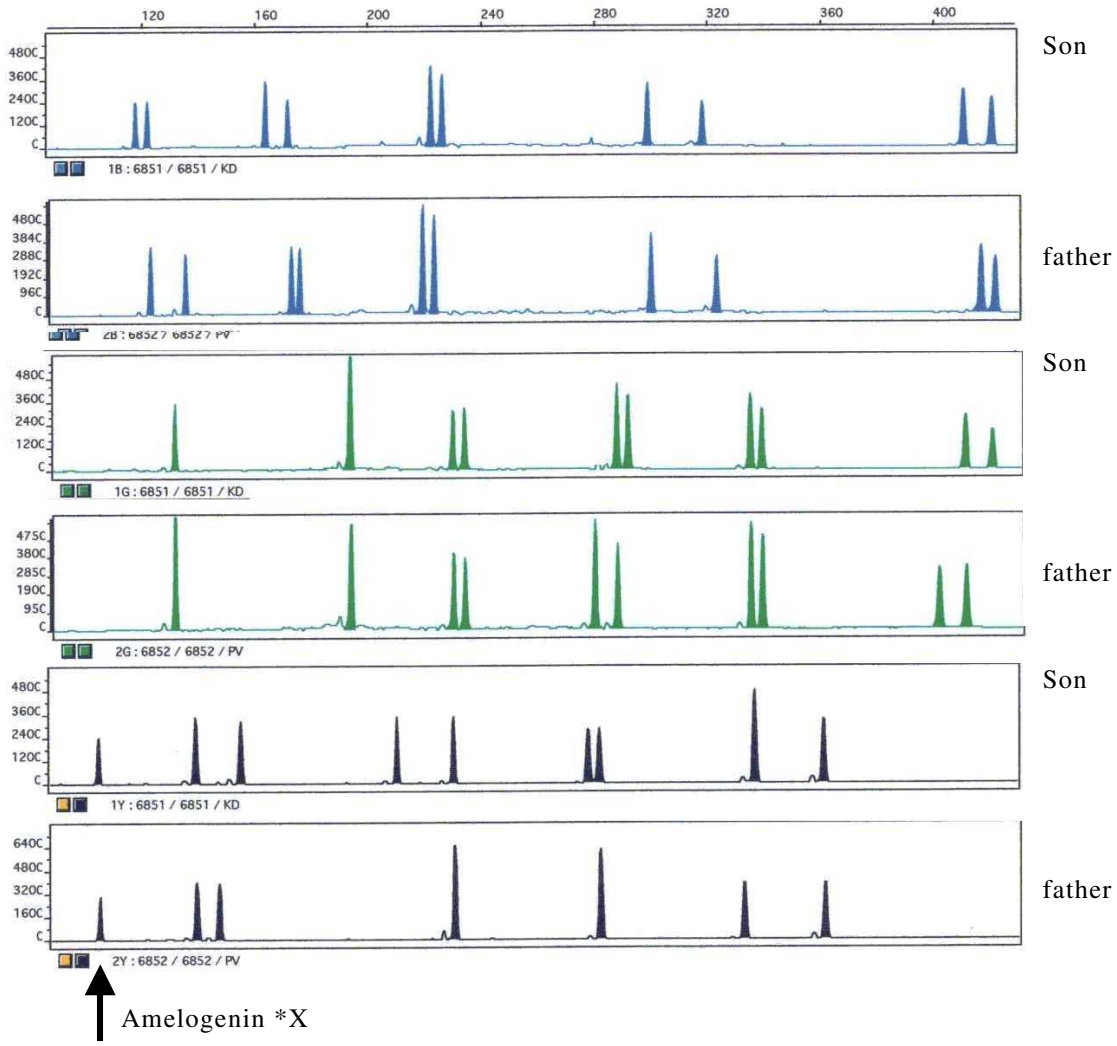

Fig. 2: Y STR profile of a son – father pair lacking the Y allele at the amelogenin locus