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REVIEW ARTICLE

R. Szibor · M. Krawczak · S. Hering · J. Edelmann
E. Kuhlisch · D. Krause

Use of X-linked markers for forensic purposes

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Abstract In forensic science, X-chromosomal short tandem repeats (ChrX STRs) bear the potential to efficiently complement the analysis of other genetic markers (autosomal, Y-chromosomal or mitochondrial). We review the population genetic properties and forensic utility of selected ChrX markers, and discuss the problems and limitations arising with their practical use. Formulae required to assess the evidential power of individual markers in different contexts are summarised and applied to ChrX STRs of interest. Since linkage and linkage disequilibrium between markers affect the inferential interpretation of genotype data, practically relevant information regarding the co-localisation and haplotypic association of ChrX STRs is provided. Finally, two examples of complex kinship testing are presented which serve to highlight the particular importance of ChrX STRs for solving deficiency cases and cases involving blood relatives.

Keywords STR typing · kinship testing · Chromosome X · Haplotype · Mapping

R. Szibor (✉) · D. Krause
Institut für Rechtsmedizin,
Otto-von-Guericke-Universität Magdeburg,
Leipziger Strasse 44, 39120 Magdeburg, Germany
Tel.: +49-391-6715812, Fax: +49-391-6715810,

M. Krawczak
Institut für Medizinische Informatik und Statistik,
Christian-Albrechts-Universität Kiel,
Brunswiker Strasse 10, 24105 Kiel, Germany

S. Hering
Institut für Rechtsmedizin, Technische Universität Dresden,
Fetscherstrasse 74, 01307 Dresden, Germany

J. Edelmann
Institut für Rechtsmedizin, Universität Leipzig,
Johannisalle 28, 04103 Leipzig, Germany

E. Kuhlisch
Institut für Medizinische Informatik und Biometrie,
Technische Universität Dresden,
Fetscherstrasse 74, 01307 Dresden, Germany

Introduction

Owing to its high individualisation power and practicality, the analysis of short tandem repeats (STR) has become wide-spread routine in forensic science. Whilst a number of articles have reviewed relevant information about STRs located on autosomes [1, 2] and the Y chromosome (ChrY) [3, 4, 5], the application of X-chromosomal (ChrX) markers has only played a minor role in forensic practice so far. However, ChrX genotyping can complement the analysis of autosomal (AS) and ChrY markers very efficiently, especially in complex cases of kinship testing.

Although, in principle, the ISFH guidelines for the forensic use of microsatellite markers [6] apply to both AS and ChrX STRs, some specific molecular and formal genetic aspects need to be taken in account when dealing with ChrX markers. The aim of this paper is to highlight the problems and potentials of ChrX marker testing, and to review some markers of particular forensic interest. Features of X-chromosomal inheritance that are relevant to forensics will be discussed on the basis of empirical data, incorporating some of our own experience in forensic ChrX marker analysis. Finally, we present two examples of the practical application of ChrX markers in complex kinship testing.

Gonosomal aberrations and ChrX marker testing

In the cells of healthy human females, ChrX is present as a homologous pair and resembles autosomes in this respect. This notwithstanding, even individuals with more than one ChrX possess only one active ChrX per cell. Additional copies are inactivated according to the Lyon hypothesis [7] which explains why ChrX monosomies, trisomies and polysomies are still compatible with life. For a given pair of parents, the presence of gonosomal irregularities can usually be excluded since these would be associated with infertility. Unexpected and undetected aberrant gonosomal karyotypes may however occur in the off-

spring, thereby affecting the accuracy of kinship testing using ChrX markers.

Genotype X0, for example, which is associated with the Ullrich-Turner syndrome, occurs at an incidence of 1 in 2,500 female live-births [8]. Both complete and partial monosomies have been observed. Another aberrant female karyotype is XY which occurs in association with androgen insensitivity and consequent testicular feminisation. The latter causes genetic males to present with an unobtrusive female phenotype [9] but the underlying condition can easily be confirmed by an amelogenin test. The posterior probability of a full or partial ChrX monosomy, or an XY female, increases when several closely linked ChrX markers appear to be homozygous. In the way they perturb kinship testing, karyotypes XO and female XY are formally equivalent to autosomal uniparental disomies [10]. Like with AS markers, paternity exclusion that relies upon ChrX marker homozygosity thus requires independent experimental verification.

When gonosomal aberrations or instances of testicular feminisation are detected, ChrX typing is no longer a valid means of kinship testing. In any case, it appears worthwhile emphasising that such findings, when inadvertently obtained during kinship testing, fall under the duty of confidentiality. Disease-relevant information should not be revealed to an affected individual unless they explicitly ask for it.

ChrX markers in forensic practice

Following the ISFH recommendations for the forensic application of microsatellite markers [6], trimeric, tetrameric and pentameric microsatellites can be used in practice if they have suitable population genetic properties (Hardy-Weinberg equilibrium, sufficiently high degree of polymorphism, known linkage disequilibrium etc.). The Genome Database (http://www.gdb.org) lists a total of 26 trinucleotide and 90 tetranucleotide repeat polymorphisms on ChrX, but only 18 tetranucleotide and 3 trinucleotide STRs, plus the VNTR locus DXS52 [11] appear to be in common forensic use. Table 1 reviews 17 of these markers. Suboptimal properties apply to DXS981 [12, 13] which combines a tetranucleotide repeat with a 3 bp insertion/deletion polymorphism, resulting in a 1 bp difference between alleles. The 17 STRs listed in Table 1 show no specific peculiarities in terms of their practical handling and are routinely used by our group.

Table 1 also contains information on the power of forensic analysis using the respective markers. Two of the parameters listed, namely the polymorphism information content (PIC) [14] and the expected heterozygosity (Het) [15], have been devised for more general purposes and are valid for both AS and ChrX markers. Formulae developed specifically in the context of kinship testing are listed in Table 2. The mean exclusion chance (MEC) was introduced by Krüger et al. [16] for AS markers typed in trios involving mother, child and putative father (formula I). This parameter is not suitable for ChrX

Table 1 Characteristics of ChrX STRs used in forensic practice

STR (synonym)	Localisation		MEC(II)	Het[d]	MEC(IV)	PD (V)	PD (VI)	Reference[e]
	Genetic (cM)[b]	RH (bin)						
DXS6807 (GATA52B03)	4.39	-	0.608	0.709	0.471	0.838	0.671	[21, 31]
DXS9895 (GATA124B04)	8.76	5	0.694	0.704	0.554	0.886	0.741	[31]
DXS8378 (GATA119E07)	-	5	0.658	0.714	0.532	0.868	0.719	[31]
DXS9902 (GATA175D03)	22.04	5	0.636	0.743	0.490	0.848	0.695	[31]
DXS7132 (GATA72E05)	83.30	16	0.687	0.883	0.557	0.883	0.731	[31]
ARA[a]	87.6–95.1[c]	16	0.893	0.857	0.814	0.982	0.901	[13,18, 20,31, 32, 33]
DXS6800 (GATA31D10)	93.17	16	0.690	0.694	0.548	0.868	0.729	[31]
DXS9898 (GATA126G01)	-	16	0.731	0.745	0.596	0.908	0.769	[22, 31]
DXS6789 (GATA31F01)	103.56	20	0.702	0.746	0.564	0.893	0.741	[24, 31]
DXS101[a]	-	19	0.78	0.885	0.794	0.978	0.889	[23, 31]
DXS7424[a] (btk5)	-	19	0.764	0.836	0.639	0.928	0.794	[25, 31]
DXS7133 (GATA81B07)	-	20	0.575	0.658	0.422	0.800	0.635	[31]
GATA172D05	116.17	20	0.775	0.804	0.654	0.935	0.808	[31]
HPRTB	150.3–183.8[c]	22–24	0.737	0.919	0.610	0.919	0.779	[20, 31, 34, 35, 36, 37]
DXS7423	-	27	0.688	0.734	0.548	0.884	0.734	[31]
DXS8377[a]	-	27	0.916	0.922	0.855	0.989	0.924	[31]
DXS11001	-	27	-	-	-	-	-	[38]

Genetic localisations were obtained from the Marshfield (or NCBI) database
RH mapping data are from own investigations using the Stanford G3 DNA panel
[a] Trinculeotide repeat
[b] Distance from the Xp telomere
[c] From NCBI database
[d] Expected heterozygosity [15]
[e] References [21, 22, 23, 24, 31, 37] contain relevant allele or haplotype frequency data
For the definition of mean exclusion chance (MEC) and power of discrimination (PD): see Table 2.

Table 2 Formulae for evaluating the forensic efficiency of genetic markers

No.	Formula	Reference
I	$\sum_i f_i^3 (1-f_i)^2 + \sum_i f_i (1-f_i)^3 + \sum_{i<j} f_i f_j (f_i+f_j)(1-f_i-f_j)^2$	[16]
II	$\sum_i f_i^3 (1-f_i) + \sum_i f_i (1-f_i)^2 + \sum_{i<j} f_i f_j (f_i+f_j)(1-f_i-f_j)$	[17]
III	$1-\sum_i f_i^2+\sum_i f_i^4-\left(\sum_{i<j} f_i^2\right)^2$	[18]
IV	$1-2\sum_i f_i^2+\sum_i f_i^3$	[18]
V	$1-2\left(\sum_i f_i^2\right)^2+\sum_i f_i^4$	[18]
VI	$1-\sum_i f_i^2$	[18]

I: MEC(mean exclusion chance) for AS markers in trios
II: MEC for ChrX markers in trios involving daughters
III: MEC for ChrX markers in trios involving daughters (Desmarais version [18])
IV: MEC for ChrX markers in father/daughter duos
V: Power of discrimination (PD) in females
VI: PD for ChrX markers in males
f_i (f_j): population frequency of the i^{th} (j^{th}) marker allele.

Table 3 Mean exclusion chance (MEC) for three unlinked ChrX STRs

Marker	Size of PCR product	PIC	MEC(I)	MEC(II)
DXS9895	139–161 bp	0.717	0.499	0.694
DXS7132	131–55 bp	0.710	0.498	0.688
GATA172D05	108–136 bp	0.790	0.612	0.775
Cumulative	-	-	0.902	0.978

PIC polymorphism information content [14]
MEC(I) calculated according to Krüger et al. [16]
MEC(II) calculated according to Kishida et. al. [17].

markers except for deficiency cases in which the paternal grandmother is investigated instead of the alleged father. Kishida et al. [17] devised an MEC for ChrX markers which covers trios including a daughter (formula II). If MEC(I) is compared to MEC(II), the latter is consistently larger (Table 3). This highlights the fact that in trios involving a daughter, ChrX markers are more efficient than AS markers. Finally, Desmarais et al. [18] introduced formulae for the mean exclusion chance of ChrX markers in trios involving daughters (formula III) and in father-daughter duos lacking maternal genotype information (formula IV). MEC(III) is equivalent to MEC(II) whilst MEC(IV) is also appropriate for maternity testing of mother/son duos.

Mutation rates of ChrX STRs

Precise mutation rate estimates are a prerequisite of reliable kinship testing using molecular genetic markers. As has been demonstrated by extensive research on human AS STRs, microsatellite mutation rates are influenced by both the structure and the length of repeat patterns involved [19]. It can be assumed that in this respect, ChrX STRs are not markedly different from their autosomal counterparts. However, owing to the uncommon application of ChrX marker in forensic practice, systematic investigations of ChrX STR mutation rates are lacking, and the number of meioses that have so far been assessed for mutations is small. Preliminary data on ChrX STR mutations, generated by our own group in the course of routine paternity testing, are presented in Table 4. These data suggest an average mutation rate of 2.09×10^{-3} per meiosis for ChrX STRs, an estimate similar to that obtained for human AS STRs. Consideration of the structure and length of the ChrX STRs involved [20, 21, 22, 23, 24, 25, 26] may help to refine these estimates even further.

Table 4 ChrX STR mutation rates as estimated from paternity tests of trios

STR	Mutations/ meioses	μ ($\times10^{-3}$)	[95%CI]
DXS6807	0/440	0.00	[0.00–8.38]
DXS9895	0/761	0.00	[0.00–4.85]
DXS8378	1/308	3.25	[0.08–18.09]
DXS9902	0/304	0.00	[0.00–12.13]
DXS7132	1/260	3.85	[0.09–21.43]
ARA	4/562	4.92	[1.01–14.37]
DXS6800	0/440	0.00	[0.00–8.38]
DXS9898	0/754	0.00	[0.00–4.89]
DXS6789	0/752	0.00	[0.00–4.91]
DXS101	0/440	0.00	[0.00–8.38]
DXS7424	0/400	0.00	[0.00–9.22]
DXS7133	0/263	0.00	[0.00–14.03]
GATA172D04	0/370	0.00	[0.00–9.97]
HPRTB	3/610	4.92	[1.01–14.37]
DXS7423	2/234	8.55	[1.03–30.87]
DXS8377	5/760	6.58	[2.13–15.35]
cumulative	16/7658	2.09	[1.25–3.32]

μ mutation rate estimate
95%CI: 95% Poisson confidence interval [CI] of μ, calculated using StatXact-4.0.1 (Cytel Software Corporation, Cambridge, MA).

ChrX marker mapping and haplotype analysis

The simultaneous analysis of STRs located on the same chromosome requires knowledge about the extent of pairwise linkage and linkage disequilibrium between them. While this is a prerequisite for STR typing in general, it is even more important for ChrX markers, and ChrX marker validation inevitably has to include precise linkage mapping. Using both physical and genetic mapping methods, we investigated the relative location of ChrX STRs of practical interest (Table 1), and combined our results with information available from www-based genetic data resources (http://www.marshfieldclinic.org/research/genetics/, http://www.ncbi.nlm.nih.gov/).

Two mapping procedures have been established in genetics. While the more classical approach to studying linkage is pedigree analysis, aiming at the estimation of recombination-related genetic distances, radiation hybrid (RH) mapping allows the physical distance between markers to be assessed directly. RH mapping uses DNA panels from somatic cell hybrids created by the fusion of a radiated human lymphoblastoid cell with non-radiated hamster recipient cells. A PCR assay is performed on these cells using the appropriate primers for the DNA of each hybrid cell line. Statistical analysis of the presence or absence of specific PCR products then allows the physical distance between new and established markers to be determined. The G3 panel (Research Genetics Invitrogen, Groningen, The Netherlands), which was used to generate the mapping data presented in Table 1, reportedly has a resolution of approximately 0.25 Mega-base pairs (Mb). As a rule of thumb, a physical distance of 1 Mb corresponds to a genetic distance of 1 centi-Morgan (cM), i.e. one expected recombination per 100 meioses. Unfortunately, genetic and physical distance are not strictly correlated [27] so that RH mapping results must be validated by additional investigations. The Stanford Human Genome Center (http://www-shgc.stanford.edu/cgi-bin/smap) calibrates the whole ChrX into 27 bins, and this definition was used here to confirm the RH map data in Table 1.

The classical approach to studying linkage between markers is via pedigree analysis. Based upon LOD (logarithm of the odds) scores calculated from family data [28], meiotic recombination fractions are estimated for pairs of markers and transformed into genetic distances (measured in cM) using appropriate mapping functions. Due to the hemizygosity of gonosomes in males, linkage analysis is particularly efficient for ChrX loci. Figure 1 illustrates the distribution of practically relevant ChrX STRs along the ChrX ideogram. Also included in Fig. 1 are the pair-wise genetic distances between STRs as estimated from 182 mother-offspring constellations with 2 or more sons involved (average number of informative meioses equal to 128). Four linkage groups could be identified when a threshold of 2.0 for the maximum LOD score was employed for significant linkage (Fig. 1). The HPRT locus was found to represent a separate linkage group on its own and was significantly linked only to GATA172D05.

Alleles of linked loci form haplotypes that recombine during meioses at a frequency corresponding to the inter-marker genetic distance. In kinship testing, haplotypes of closely linked STRs must therefore be analysed as a whole, rather than through their constituent alleles, if the meiotic stability of haplotypes is sufficiently high. Linkage disequilibrium (LD), which refers to this non-random association of alleles at different loci, measures the deviation of population-specific haplotype frequencies from the product of the corresponding allele frequencies. For markers with strong LD, haplotype frequencies cannot be inferred from allele frequencies but instead have to be estimated directly from population data. Due to their high mutation rates, STRs tend to show weaker LD than single nucleotide polymorphisms. However, LD can still occur between closely linked STRs and therefore has to be assessed prior to their practical forensic use. We analysed the inter-marker LD of the ChrX STRs in Table 1 by genotyping 210 male DNA samples. Significant LD was only observed for one pair of markers, namely DXS101 and DXS7424 (p <0.001) [25].

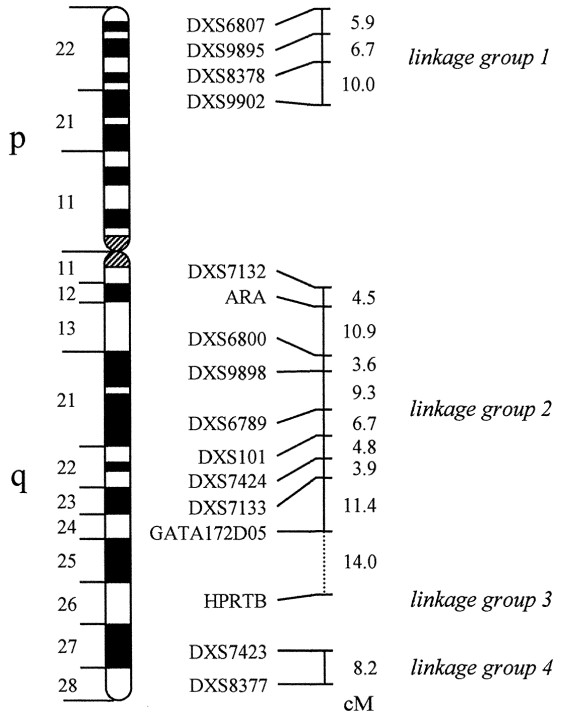

Fig. 1 Localisation of ChrX STRs used in forensic practise. The order and approximate position of STRs on the ChrX ideogram is based upon publicised map data (Marshfield, NCBI) and our own RH and genetic mapping studies. Pair-wise genetic distances (in cM) were calculated from maximum likelihood estimates of pair-wise recombination fractions using the Kosambi mapping function [28]

Use of ChrX markers in kinship testing

Paternity testing in trios and duos

Paternity cases involving the common trio constellation of mother, offspring and alleged father can usually be solved with AS STRs alone, and do not seem to require any additional or alternative markers. However, when father/daughter relationships are to be tested it may be worthwhile including ChrX markers, too. This is especially the case when difficult to analyse template materials are involved, such as DNA from exhumed skeletons or historical or prehistorical samples. In such instances sufficient statistical power has to come from a small number of low size STRs. Fortunately, ChrX STRs are usually characterised by relatively high MECs, even at a low to medium degree of polymorphism (Table 3).

Paternity cases involving blood relatives

In paternity cases involving close blood relatives as alternative putative fathers, the exclusion power of STRs is substantially decreased and ChrX STRs may be superior to AS markers. For example, if two alleged fathers are father and son, they would not share any X-chromosomal alleles identical by descent (ibd) so that ChrX markers would be more efficient than AS markers. Brothers, in contrast, share a given maternal ChrX allele with probability 0.5, which equals the probability that two alleles are shared ibd at an AS locus. For three unlinked ChrX loci, the chance of ibd sharing would be 0.5^3=0.125. However, when the markers are closely linked, they do not segregate independently. As with AS markers, they would instead represent a single haplotype that is again shared with a probability approaching 0.5. The ChrX contains three linkage groups which can provide nearly independent genotype information (Fig. 1). At present, we propose to use clusters DXS6807-DXS9895-DXS9902-DXS8378 (Xp22–21), DXS7132-ARA-DXS6800 (Xq11-Xq13), and DXS7423-DXS8377-DXS10011 (Xp27–28) to define haplotypes for forensic practice. Alternatively, two-cluster haplotypes may be chosen on the basis of Table 1 and Fig. 1.

Paternity testing in rape and incest cases

After criminal sexual assault or incest, pregnancies may be terminated by suction abortion. An aborted 6–8 week product of conception consists of small amounts of non-identifiable foetal organs as well as maternal blood and other tissues. In such cases, the microscopical dissection of chorionic villi is not generally successful, and samples most often contain a mixture of foetal and maternal DNA. Efficient paternity testing of such material is still possible for male foetuses, using ChrY markers. Paternity testing of female foetuses, in contrast, can only include AS and ChrX markers, the latter of which represent a more efficient means of paternity exclusion under all circumstances. A positive proof of paternity, however, relies mainly upon foetal alleles not shared with the respective mother. In incest cases in which a father is rightfully charged with abusing his daughter, ChrX testing of an abortus can therefore contribute only very limited information towards a positive proof of paternity. This is because all foetal alleles would necessarily coincide with alleles of the daughter.

Maternity testing

There are situations in which mother/child testing may be required. For example, due to the high rate of illegitimate paternity in modern societies, the identification of skeletons or corpses by mother/child testing is more reliable than through the assessment of father/child relationships. Although maternity can be demonstrated by sequencing mitochondrial DNA, this technique is nevertheless expensive and does not always yield the level of certainty required in forensic science. This is especially the case when individuals are involved for whom appropriate population genetic data are not available. Typing of ChrX STRs may thus represent a sensible alternative option when assessing maternity. For testing mother-daughter relationships, ChrX markers are equivalent to AS markers and do not provide any specific advantage. Testing mother-son kinship, however, is more efficiently performed using ChrX markers. The exclusion chance in such cases is identical to that of ChrX STRs in father/daughter tests (Table 2).

Deficiency paternity cases

The major advantage of ChrX markers arises in deficiency paternity cases, i.e. when a biological sample from a putative father is not available and DNA from paternal relatives has to be analysed instead. When female individuals have the same father, they also share the same paternal ChrX. An investigation of ChrX markers of two sisters or half-sisters can thus exclude paternity, namely through the presence of four different alleles or haplotypes, even when none of the parents is available for testing. AS markers cannot provide such information. A positive proof of paternity is also possible without parental genotype information, but is generally less reliable. This is due to the fact that sisters usually inherit only partially matching haplotypes from their mother. As will be shown below, the co-inheritance of two identical maternal ChrX without a recombination is not impossible, but rare.

With a total genetic length of approximately 200 cM, there are several virtually uncoupled regions on the ChrX (Fig. 1). Assuming that the number of recombination breakpoints between any two ChrX loci follows a Poisson distribution [29], the probability Φ of an individual inheriting a maternal ChrX without a single recombination event equals $e^{-L(X)}$. Here, e=2.71828... is the Euler constant, and L(X) denotes the genetic length of the ChrX. Since L(X)=200 cM=2 Morgan (i.e. the basic unit of genetic distance), we have Φ=0.135. This implies that if two maternal half-sisters share an identical haplotype A in addition to individual haplotypes B and C, then the likelihood ratio LR of shared paternity vs non-shared paternity approximately equals:

$$\frac{f(A)\cdot 1/4\cdot 2f(B)\,f(C)}{f(B)\cdot f(C)\cdot 2f(A)\cdot[1/2\phi]^2}=\frac{1}{\phi^2}=54.9. \quad (1)$$

In other words, the probability of full sisterhood, assuming equal prior odds, cannot exceed LR/(1+LR)=0.982. The explanation for the above formula is as follows:assuming shared paternity (numerator), haplotype A is of paternal origin and haplotypes B and C are of maternal origin. If B and C are sufficiently different so as to exclude their emergence from the same maternal chromosome via recombination, the likelihood of the parental

genotypes equals f(A)·2f(B)f(C). Inheritance by the two sisters of their particular maternal haplotypes occurs with probability 1/2×1/2=1/4. Assuming non-shared paternity (denominator), haplotypes B and C can be assumed to be paternal whilst haplotype A must be of maternal origin. The unlikely scenario of the two sisters inheriting two identical haplotypes from different parents can be ignored. Then, the likelihood of the parental genotypes approximately equals f(B)·f(C)·2f(A). Finally, each sister inherits a non-recombined version of maternal haplotype A with probability Φ/2.

When ChrX markers are investigated in a deficiency case, the mother of the unavailable putative father (i.e. the putative grandmother) is the key figure. Instances in which she is available for genotyping do not, strictly speaking, represent deficiency cases. All ChrX alleles of the putative father can be determined by investigating her, and the MEC can be calculated using the respective formula for AS markers (Table 2). However, ChrX marker genotypes of the putative grandmother can also be reconstructed to some extent from her children. If she has several daughters, it is possible to determine the parental origin of most of their ChrX alleles and therefore the grandmaternal genotype. If brothers of the putative father are available, the data are even more informative. Then, the grandmaternal genotype must have been heterozygous for all ChrX loci for which brothers of the putative father carry different alleles. If they carry identical alleles, the mother can be either homozygous or heterozygous at the corresponding locus. If closely linked loci have already been identified as being heterozygous, the probability of homozygosity at the original locus can be assessed by haplotyping.

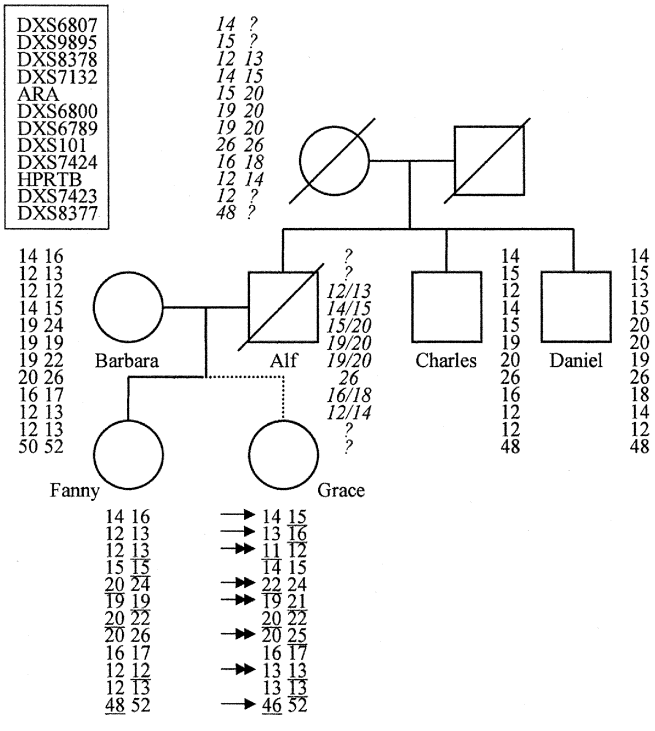

Fig. 2 Kinship testing of two putative full-sisters. STR genotypes are shown in the locus order depicted in the top left corner of the graph. Inferred genotypes are given in italics; the necessary paternal alleles of "Fanny" and "Grace" are underlined. Single-headed arrows mark STRs for which the paternal alleles of "Fanny" and "Grace" are different, i.e. which exclude full sisterhood. Double-headed arrows mark STRs at which "Alf" lacks the paternal allele of "Grace", i.e. which exclude "Alf" from being the father of "Grace"

Example 1

The considerations outlined above are exemplified in Fig. 2 for a case of complex kinship testing. Here, inspection of the genotypes of "Charles" and "Daniel" (note: all family members have been renamed for anonymity) allows the reconstruction of the grandmaternal genotype for all but four STRs, namely DXS6807, DXS9895, DXS7423 and DXS8377. Locus DXS101 would not have been informative on its own. However, since the genetic distances between DXS101 and the two flanking markers DXS6789 and DXS7424 are only 4.8 and 6.7 cM, respectively, a double recombination or the co-occurrence of two independent recombination events appears unlikely (p =4·0.048·0.067=0.013). Therefore, the deceased grandmother can be assumed to be homozygous 26-26 at DXS101. In this example, "Alf" can be excluded from being the father of "Grace" since he did not carry some of her necessarily paternal alleles.

Example 2

Since ChrX haplotyping of males is a very convenient and efficient means of kinship testing, it can compensate for informational gaps over several generations. Figure 3 illustrates a case in which a woman ("Claire") and two putative cousins ("Babsy" and "Britta") were tested for kinship. With one exception, none of the members of the parental and grandparental generation were available for testing. A total of 15 ChrX STRs were employed in this case. Inspection of the genotypes of "Babsy" and "Britta" allows the reconstruction of the genotype of their common father "Billy" for eight STRs, namely DXS6807, DXS8378, DXS7132, DXS6800, DXS6789, DXS101, DXS7424 and ARA. Comparison of the genotypes of "Carmen" and "Claire", the alleged cousin of "Babsy" and "Britta", allows the reconstruction of the genotype of her biological father at all but one locus (DXS9902). The genotypes of "Billy" and the biological father of "Claire" were found to be identical for markers DXS8378 to DXS6800 (or possibly DXS6789). The pedigree analysis program MLINK [30] was used to calculate the likelihood of the inferred genotypes assuming that the two males were either maternal brothers (hypothesis H0) or unrelated (H1). Likelihood calculations were based upon published STR allele frequencies and the genetic map depicted in Fig. 1. A likelihood ratio of 17.2 was obtained in favour of H0 which implies that, assuming equal prior odds, the probability of "Claire" being a cousin of "Babsy" and

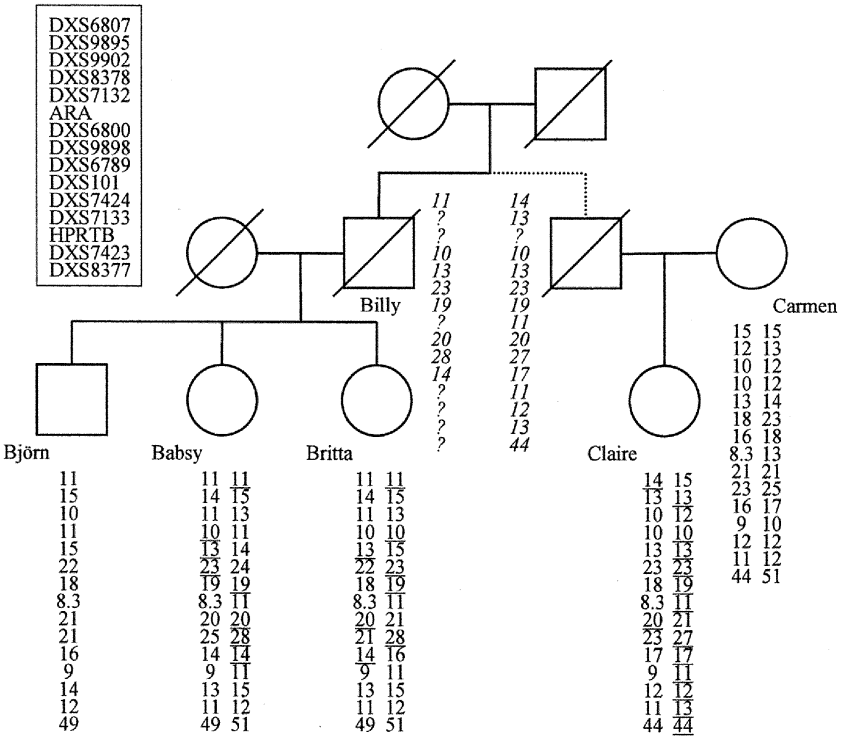

Fig. 3 Kinship testing of putative cousins. STR genotypes are shown in the locus order depicted in the top left corner of the graph. Inferred genotypes are given in italics; the necessary paternal alleles of "Babsy", "Britta" and "Claire" are underlined

"Britta" equals 17.2/(1+17.2)=0.945 (or 94.5%). Thus, ChrX haplotyping strongly supported the claim of "Claire" to be the offspring of a deceased uncle of "Babsy" and "Britta".

Power of ChrX markers in stain analysis

With a few exceptions, ChrX markers are less powerful in stain analyses than AS markers. The PD value of ChrX markers varies depending on sex, and equals that for AS (Table 2, formula V) when female traces are to be matched to female individuals. For the matching of male traces to male suspects, the PD value of ChrX markers (Table 2, formula VI) is generally smaller than that of AS markers. This is due to the fact that male ChrX analysis utilises only one allele per STR.

In a mixed female/male stain, the chance of all male alleles being included in the female component is higher for ChrX than for AS markers. Therefore, it is not advisable to use ChrX markers to test male traces where there is female contamination. In order to identify female traces in male contamination, however, ChrX markers are more efficient than AS markers since the female alleles can only be completely included in the male component if the female coincidentally happens to be homozygous at all loci. Indeed, assessment of female traces on a male rape suspect by means of ChrX typing has already been carried out successfully in practice (P. Wiegand, personal communication).

Discussion

Although their formal genetic peculiarities render them particularly suitable for kinship testing, ChrX markers have so far been employed only rarely in forensic practice. This notwithstanding, since gonosomal markers are especially efficient for solving deficiency cases, an increasing demand for their usage can be expected. The proportion of extra-marital children is constantly increasing in modern industrial societies and, for example, accounts for approximately 25% of all live births in Germany (http://www.destatis.de/basis/d/bevoe/bevoetab1.htm). In many of these instances, paternity may be disputed at some stage and, when the putative father dies early or unexpectedly, the need for a paternity test may only be recognised after the interment. A specific demand for kinship tests in which only remote relatives are available for testing can also be expected to arise, particularly from the need to rejoin families in the context of war and worldwide migration. Here, ChrX marker testing may also prove helpful since male family members are more likely to be affected by the consequences of socio-demographic unrest than females, and it may therefore be more difficult to obtain samples from them.

The present paper was intended to highlight the potential of ChrX STRs for solving some of the above problems. Technically, the implementation of ChrX marker testing in forensic practice should not pose any insurmountable problems. However, although selected haplotype data are already available, the precise location of more ChrX markers and the quantification of linkage disequilibrium between them require further intense efforts of research.

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