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TECHNICAL NOTE

X chromosomal recombination—a family study analysing 39 STR markers in German three-generation pedigrees

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Abstract Typing of polymorphisms on the human chromosome X (ChrX) has become a standard technique in forensic genetics, and a growing number of short tandem repeats (STRs) has been established. Knowledge of marker recombination is of great significance especially when ChrX typing is used in forensic kinship testing. It is known that meiotic recombination is not a simple function of physical distance but crossing over events tend to be clustered. Information on genetic distances between markers can be gathered by family studies and by interpolation of gene bank data such as the Rutgers map. We typed DNA samples of pedigrees consisting of mothers with several sons and grandfather–mother–son constellations and report here the recombination characteristics of 39 ChrX STRs in up to 135 meioses.

Keywords X-chromosome · STR · Kinship testing · Haplotyping · Recombination

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Introduction

Over the past few years, numerous short tandem repeats (STRs) distributed over the whole X chromosome (ChrX) have been detected and established for usage in forensic kinship testing, especially in deficiency cases [1]. The simultaneous analysis of markers located on the same chromosome requires knowledge about linkage and linkage disequilibrium. A first recombination study was performed analysing 16 ChrX STRs in mothers with several sons [2]. The counting of visible recombination events following LOD score analysis (logarithm of the odds that the loci are linked rather than unlinked) resulted in the formation of four marker groups with significant linkage of the markers within each group. A set of eight STRs (two markers chosen from each linkage group) could be typed using the commercially available forensic ChrX typing kit Mentype® Argus X-8 [3]. When linked markers are used in complex pedigree analysis, special linkage programmes are necessary for likelihood calculations considering genetic distance between markers. Approximate genetic distance can be created from physical maps assuming that 1 Mb corresponds to 1 cM [4]. ChrX markers which are separated by short physical

distances show low recombination frequencies in female meiosis. It can be assumed that such marker clusters segregate as stable haplotypes because of linkage disequilibrium. Several studies exist for closely linked markers [5–9]. Information about recombination patterns of the female X chromosome is very important for the application of ChrX haplotyping in kinship analysis. It is known that meiotic recombination is not a simple function of physical distance [10]. The locations of crossover events tend to be highly clustered. The increasing availability of large-scale population genetic data on tightly linked markers identified a large number of hotspots where recombination appears to take place considerably more frequently than in the surrounding sequence [11]. A review of direct (cytological examination) and indirect (genotype data in families) methods studying human meiotic recombination is given by Lynn et al. [12]. Several markers such as RFLPs, STRs and single nucleotide polymorphisms (SNPs) were used to construct genetic maps with different densities, analysing Centre d'Études du Polymorphisme Humaine (CEPH) family panels or deCODE families (Icelandic pedigrees). Many studies show that humans display surprising variation in recombination rates, either between males and females or among individuals of the same sex and also between different ethnic groups.

The aim of this study was to give an overview of the recombination pattern of the whole X chromosome in German 3-generation families including males with daughters and their sons. Early results for marker clusters in three linkage groups [13–15] were completed with additional STR markers spanning 4.7 to 150.9 Mb from the p-telomere to confirm observed recombination events within and between the marker clusters and to detect possible hot spots.

Methods

Buccal swabs for the 3-generation study were collected from 90 males with daughters and 135 grandsons. The people involved gave their consent for the investigation of STRs of forensic significance. In addition, before the STR typing was started, the samples were anonymised. The aim and the design of this study "measurement of the genetic distances between ChrX STRs" was evaluated and approved by the Otto-von-Guericke-Universität Magdeburg Ethics Commission. DNA extraction was carried out using the Chelex method or QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). For several markers, new primers were designed according to GenBank information using the Primer3 software. Table 1 lists references and new primers. The amplification was carried out in a 25-µl PCR reaction volume containing approximately 0.1–1 ng DNA, 200 µM each dNTP, 1.5 mM $MgCl_2$, 0.5 µM of each primer, 1 U Taq polymerase (Applied Biosystems, Foster City, CA) and 1× PCR buffer in a PTC-200 cycler (MJ Research, Watertown, MA). The following PCR cycle protocol was used: a 95°C–3 min soak; 94°C–30 s melting, 1 min annealing, 72°C–1 min extension, 30 cycles, 72°C–30 min final extension. Annealing temperatures of 54°C for DXS7133, 58°C for DXS6807, DXS9898 and DXS10103, 60°C for DXS9902, DXS6795, DXS9907, GATA144D04, DXS100160, DXS7424 and GATA172D05 and 62°C for DXS6800. Several markers were multiplexed.

Fragment analysis was done as previously described on an ABI prism 310 genetic analyzer [15].

LOD score functions were calculated according to Ott [16]. Recombination fraction in the 3-generation families was calculated with 95% confidence interval [17].

Results

First recombination study (mothers with several sons, 17 STRs)

Data for the pairwise linkage study (maximum LOD score analysis) with mothers and sons [2] are shown in detail in Table 2 with the additional marker DXS6809. Several marker pairs were found with significant linkage (maximum LOD scores >2). The numbers of informative meioses ranged from 88 to 204. Table 3 gives the maximum likelihood estimation of recombination fractions θ calculated from the LOD score functions for all the marker pairs with $Z_{max}>1$.

Second recombination study (3-generation families, 39 STRs)

A second approach of our recombination study considering 39 STRs involved 135 meioses in 90 3-generation families. The complete recombination data for all markers are given as ESM. Four clusters of closely localised markers were chosen as linkage groups: DXS148-DXS8378 at Xp21, DXS7132-DXS981 at Xq12, DXS10103-DXS10101 at Xq26 and DXS146-DXS10011 atXq28. Possible mutations were detected in some families, all with differences of only one repeat. The number of recombination events found on the short and long arms of the X chromosome in the male offspring is shown in Table 4. We detected that 15 female meioses did not produce a crossover event, and obviously, an unrecombined grandpaternal or grandmaternal ChrX was transmitted through two generations. A ChrX with more than two recombinations at one chromosomal arm along with a female meiosis was found in only two cases. Maternal age at the time of conception was 25 and 32 years.

Table 1 39 X chromosomal STR markers used in the 3-generation study

Locus (repeat length in bp)	Localisation[a] (distance from Xp-tel in kbp)	First reference	Primer or kit, if different from reference
DXS6807 (4)	4,753	[18]	HEX TGCATGCATGTTCACATAACTT AAAATACTCCCACCCCCAGT
DXS9895 (4)	7,387	[19]	[20]
DXS10148 (4)	9,198	[13]	
DXS10135 (4)	9,199	[13]	Mentype® Argus X-8
DXS8378 (4)	9,330	[19]	Mentype® Argus X-8
DXS9902 (4)	15,233	[19]	FAM-CTGGGTGAAGAGAAGCAGGA TGGGATCACCAGTGATTCTATC
DXS6795 (4)	23,154	[21]	FAM-CTGGTCCAACTGATGCACAC GAAATGCATCCATCCCCTAA
DXS9907 (4)	32,010	[22]	FAM-GATCGCTTGAGGCTAGCAGT GCTCAAACGATTCTCCCATC
GATA144D04 (4)	44,898	[23]	FAM-AAGATCTGCCAAGCCAGAAG CTTGCAGTGAGGGACAGAGA
DXS10076 (4)	48,194	[9]	
DXS10077 (3)	48,202	[9]	
DXS10078 (4)	48,207	[9]	
DXS100161 (4)	55,999	[24]	
DXS100160 (5)	56,506	S. Hering, GDB Acc.ID: 11525278	FAM-TCTGGGTTGAACACTTCTCTTTT TTTCCCTAGGTTAGGGGTATCA
DXS100159 (4)	56,766	[24]	
DXS100162 (4)	61,800	[24]	
DXS100163 (5)	62,000	[24]	
DXS100164 (5)	62,161	[24]	
DXS100165 (4)	63,994	[24]	
DXS7132 (4)	64,572	[19]	Mentype® Argus X-8
DXS10079 (4)	66,632	[15]	
HumARA (3)	66,682	[25]	[15]
DXS10074 (4)	66,894	[15]	Mentype® Argus X-8
DXS10075 (4)	66,915	[15]	
DXS981 (4)	68,114	[25]	
DXS6800 (4)	78,567	[19]	FAM-GCCTATTGTGGGACCTTGTG GAAATATTGGGGGCTGGTTC
DXS9898 (4)	87,682	[26]	HEX-CGAGCACACCTACAAAAGCT GTTTCGATTAGGTTCAGTTCCCA
DXS6789 (4)	95,336	[27]	
DXS7424 (3)	100,505	[19]	FAM-CCAGGTATTTGGGAAGCTGA GGAACACGCACATTTGAGAA
DXS7133 (4)	108,928	[19]	HEX CACTTCCAAAAGGGGAAAAA TCTTCCAAGAATCAGAAGTCTCC
GATA172D05 (4)	113,061	[19]	FAM-AGTGGTGATGGTTGCACAGA AAGCCCGGATTCAAAAAGAT
DXS10103 (4)	133,246	[28]	FAM CACACACACACACACACATGC GGAAACAGAACCAGGGGAAT
HPRTB (4)	133,443	[29]	Mentype® Argus X-8
DXS10101 (4)	133,482	[28]	Mentype® Argus X-8
DXS10146 (4)	149,335	[14]	
DXS10134 (4)	149,401	[14]	Mentype® Argus X-8

Table 1 (continued)

Locus (repeat length in bp)	Localisation[a] (distance from Xp-tel in kbp)	First reference	Primer or kit, if different from reference
DXS10147 (4)	149,414	[14]	
DXS7423 (4)	149,461	[19]	Mentype® Argus X-8
DXS10011 (4)	150,939	[30]	[31]

[a] Physical data obtained from NCBI v.36 (www.ensembl.org/Homo_sapiens)

The recombination fractions between the linkage groups in Xp21, Xq12, Xq26 and Xq28 are shown in Table 5 including LOD score calculation. We observed recombinations between linkage groups I/II and II/III, in approximately half of the chromosomes. Double crossover occurred 12 times between linkage group I/II and eight times between linkage group II/III. We found recombinations between linkage group III and IV reflecting the relatively low physical distance of 15.8 Mb only in one third of the chromosomes.

Recombination events between closely linked markers are very rare. We observed only one recombination event in linkage group I between DXS10148 and DXS10135 and two events in linkage group IV between DXS10146 and DXS10134 (Table 6). No recombinations were observed within linkage group II at Xq12 and within linkage group III at Xq26. We detected only one crossover within the long region around the centromere from DXS10161 (56 Mb from Xptel) to DXS981 (68.1 Mb) between DXS10165 and DXS7132.

Discussion

Human autosomal recombination maps can be constructed from high-resolution single-sperm analysis [32]. With regard to the gonosomes, this assay is applicable only when the pseudoautosomal regions are under investigation [33], and it does not work for the major part of the ChrX. Compared with autosomes, the ChrX recombination data pool are small as such maps can be accessed only by family studies counting the maternal crossovers. Some X-chromosomal recombination studies relied on CEPH family data. A study based on 349 CEPH meioses with STR markers demonstrated a repression of recombination across the X chromosome centromere [34]. However, CEPH cell lines are strongly affected by somatic STR mutations which can produce misleading results when recombination maps are constructed. This could be demonstrated in our own work for DXS8377 and DXS101 (J. Edelmann, unpublished data) and by earlier investigations [35, 36]. For this reason, our approach was to study ChrX recombination in samples of German families.

Kong et al. [37] presented a high-resolution recombination map analysing microsatellite markers for 146 Icelandic families with a total of 1,257 meiotic events. The ChrX genetic map also started with DXS6807 (4.7 Mb) and ended with DXS1073 (153.5 Mb). Calculated recombination rates showed regions with repressed recombination around the centromere (<1 cM/Mb) and high rates (>2 cM/Mb) near the telomeres, especially in Xq28 (markers localised 149–152 Mb from Xptel). Similar results for different recombination rates in the telomeric and centromeric regions were found by Myers et al. [38]. Recombination hotspots from SNP genetic variation data were detected in different ethnic populations. The overall recombination landscape and the extent to which it is dominated by hotspots were found to be similar between the X chromosome and the autosomes. A second-generation combined linkage physical map of the human genome (Rutgers Map v.2) was built by Matise et al. combining polymorphic information from mainly SNPs, STRs and some other markers in CEPH and deCODE families [39]. This high-density map facilitates interpolation of genetic position of further markers with known physical location. There is a shift of approximately 10 cM compared with the deCODE map.

Our starting situation for this genetic linkage study of ChrX STRs is a good knowledge of the physical marker localization which has been drawn electronically from the genome browser http://www.genome.ucsc.edu/.

Mothers with several sons (17 STRs)

Our first recombination study in German families including mothers with two or more sons resulted in information about linkage and the genetic distance of significant linked markers [1]. A clear pattern of linked marker groups is visible. Some combinations (e.g. DXS7132/DXS7424) failed to exceed the threshold LOD score of 2.0 for significant linkage on gonosomes. This may be due to the relatively low number of informative meioses depending on the heterozygosity of the STRs. In these cases, the support intervals for the recombination fraction are wide. Significant linkage was detected up to a recombination fraction of 0.198 (DXS6807/DXS9902). Exact counting of recombination events in the study was not possible. When two brothers inherited the same or the opposite marker combination, it had to be considered that

Table 2 Pairwise linkage analysis for 17 X chromosomal STR markers in German mothers with several sons

	DXS 6807	DXS 9895	DXS 8378	DXS 9902	DXS 7132	Hum ARA	DXS 6800	DXS 9898	DXS 6809	DXS 6789	DXS 7424	DXS 101	DXS 7133	GATA 172	HPRTB	DXS 8377
DXS9895	*13.34* (162)															
DXS8378	*3.83* (96)	*8.34* (112)														
DXS9902	*2.66* (142)	*5.20* (134)	*5.38* (88)													
DXS7132	(113)	(130)	(112)	(106)												
HumARA	(117)	(125)	(125)	(110)	*13.99* (140)											
DXS6800	(152)	(141)	(88)	(112)	*3.99* (95)	*5.57* (106)										
DXS9898	(165)	(197)	(104)	(157)	*5.60* (128)	*4.51* (122)	*15.58* (144)									
DXS6809	(141)	(146)	(83)	(128)	*2.21* (93)	*3.97* (102)	*9.34* (119)	*15.32* (159)								
DXS6789	(172)	(189)	(114)	(173)	*3.21* (127)	*3.01* (127)	*9.81* (141)	*12.33* (204)	*15.51* (156)							
DXS7424	(104)	(125)	(73)	(99)	*1.63 (83)*	*1.04 (90)*	*2.86* (88)	*5.46* (118)	*7.06* (108)	*6.31* (116)						
DXS101	(163)	(173)	(102)	(140)	*2.83* (117)	*1.09 (115)*	*5.91* (149)	*9.82* (174)	*12.55* (158)	*12.22* (159)	*9.98* (109)					
DXS7133	(144)	(182)	(108)	(148)	*3.91* (119)	*2.37* (126)	*5.5* (140)	*8.3* (187)	*10.76* (150)	*15.36* (188)	*11.3* (107)	*16.64* (155)				
GATA172	(164)	(169)	(104)	(151)	*3.10* (120)	*2.74* (119)	*5.27* (142)	*7.96* (179)	*15.81* (156)	*11.03* (169)	*8.32* (145)	*8.87* (178)	*9.66* (152)			
HPRTB	(95)	(103)	(94)	(91)	(103)	(102)	(81)	(107)	(128)	(109)	(64)	(104)	*1.69 (154)*	*3.99* (149)		
DXS8377	(189)	(182)	(121)	(173)	(141)	(138)	(185)	(191)	(173)	(189)	(130)	(187)	(181)	(194)	(119)	
DXS7423	(88)	(93)	(79)	(91)	(104)	(107)	(80)	(99)	(74)	(106)	(60)	(86)	(94)	(83)	(79)	*7.10* (105)

Maximum LOD scores >1 are listed; significant scores (>2) are in italicised letters, and number of informative meioses in brackets

Table 3 Pairwise linkage analysis for 17 X chromosomal STR markers

Marker-Kombination	θ values	($Z_{max}-1$)
DXS6807–DXS9895	0.059	0.027–0.112
DXS6807–DXS8378	0.130	0.062–0.240
DXS6807–DXS9902	0.198	0.121–0.315
DXS9895–DXS8378	0.067	0.026–0.139
DXS9895–DXS9902	0.149	0.088–0.237
DXS8378–DXS9902	0.099	0.041–0.196
DXS7132–HumARA	0.045	0.016–0.096
DXS7132–DXS6800	0.122	0.056–0.230
DXS7132–DXS9898	0.124	0.065–0.212
DXS7132–DXS6809	0.182	0.097–0.325
DXS7132–DXS6789	0.169	0.096–0.281
DXS7132–DXS7424	0.180	0.088–0.349
DXS7132–DXS101	0.168	0.092–0.289
DXS7132–DXS7133	0.148	0.080–0.252
DXS7132–GATA172	0.160	0.085–0.276
HumARA–DXS6800	0.107	0.050–0.199
HumARA–DXS9898	0.141	0.076–0.240
HumARA–DXS6809	0.136	0.067–0.245
HumARA–DXS6789	0.172	0.010–0.281
HumARA–DXS7424	0.220	0.129–0.465
HumARA–DXS101	0.240	0.141–0451
HumARA–DXS7133	0.196	0.155–0.322
HumARA–GATA172	0.173	0.096–0.295
DXS6800–DXS9898	0.036	0.012–0.081
DXS6800–DXS6809	0.063	0.024–0.130
DXS6800–DXS6789	0.077	0.036–0.141
DXS6800–DXS7424	0.146	0.070–0.272
DXS6800–DXS101	0.131	0.074–0.214
DXS6800–DXS7133	0.132	0.073–0.220
DXS6800–GATA172	0.136	0.076–0.225
DXS9898–DXS6809	0.046	0.018–0.095
DXS9898–DXS6789	0.092	0.052–0.149
DXS9898–DXS7424	0.114	0.057–0.204
DXS9898–DXS101	0.096	0.052–0.160
DXS9898–DXS7133	0.119	0.070–0.189
DXS9898–GATA172	0.120	0.069–0.191
DXS6809–DXS6789	0.033	0.010–0.075
DXS6809–DXS7424	0.081	0.033–0.160
DXS6809–DXS101	0.061	0.027–0.116
DXS6809–DXS7133	0.072	0.033–0.134
DXS6809–GATA172	0.040	0.014–0.086
DXS6789–DXS7424	0.097	0.045–0.182
DXS6789–DXS101	0.067	0.031–0.124
DXS6789–DXS7133	0.062	0.030–0.111
DXS6789–GATA172	0.083	0.042–0.144
DXS7424–DXS101	0.048	0.015–0.110
DXS7424–DXS7133	0.039	0.011–0.096
DXS7424–GATA172	0.106	0.056–0.179
DXS101–DXS7133	0.033	0.011–0.076
DXS101–GATA172	0.106	0.060–0.174
DXS7133–GATA172	0.094	0.041–0.152
DXS7133–HPRT	0.230	0.148–0.372
GATA172–HPRT	0.167	0.099–0.266
DXS8377–DXS7423	0.082	0.034–0.162

Maximum likelihood estimation of recombination fraction θ with the ($Z_{max}-1$) support interval for marker pairs with maximum LOD scores >1 (2-generation study)

crossover had occurred in both meioses or markers had segregated together. Accurate estimation of small genetic marker distances (<3 Mb) is also not possible with this method [40].

Recently, results of a similar study testing some 2-generation families from Sweden and Somalia with Mentype® Argus X-8 kit were reported [41]. The recombination frequencies between DXS10101 and DXS10134 (15.9 Mb distance) were calculated to be 0.29 (0.19–0.495, 33–55 informative meioses) in the European population. The mathematical model used was similar to the classical LOD score analysis, but the number of informative meioses was low. In comparison, our LOD score function for recombination between HPRTB and DXS8377 (the nearest markers available at this time with 15.9 Mb distance) was very flat and reaches its maximum at 0.12. This is not significant for linkage on the X-chromosome, but this method does not prove the opposite scenario that these markers are absolutely independent. The most likely recombination fraction was found to be 0.37 (0.23–0.5; 119 informative meioses).

3-generation families (39 STRs)

In contrast, our second family study in 3-generation families gave more precise information about the X

Table 4 Number of recombination events detected on the short and long arm of the X chromosome (3-generation study, 135 males)

	Xp, 0	Xp, 1	Xp, 2	Xp, 3
Xq, 0	15	22	4	1[a]
Xq, 1	22	29	10	0
Xq, 2	14	16	1	0
Xq, 3	0	0	0	0
Xq, 4	1[b]	0	0	0

[a] Maternal age at time of conception, 25 years

[b] Maternal age at time of conception, 32 years

Table 5 Recombinations between the four marker clusters (3-generation study)

Linkage group	Physical distance	Recombinations/ informative meioses	Maximum LOD scores	Recombination fraction (95% confidence interval)
I (DXS10148-DXS8378)/II (DXS7132-DXS981)	55.3 Mb	74/135	<0	0.548 (0.465–0.632)
II (DXS7132-DXS981)/III (DXS10103-DXS10101	65.1 Mb	71/135	<0	0.526 (0.442–0.610)
III (DXS10103-DXS10101)/IV (DXS10146-DXS10011)	15.8 Mb	46/134	2.907	0.343 (0.263–0.423)

chromosomal recombination. At this time, the exact physical marker position on the X chromosome is known, and more markers were included to compensate for the situation that several meioses did not supply information because of homozygosity of the mothers. Hence, the remaining gaps between informative haplotypes are small. We observed four double crossovers between linkage groups I/II and II/III confirming the independent segregation. The lowest physical distance between the four postulated linkage groups is given for DXS10101 and DXS10146 with only 15.8 Mb. LOD score analysis between linkage group III/IV resulted in a maximum LOD score of 2.9. This is in contrast to the low values in our first recombination study. The difference can be explained due to the phase information in the 3-generation study compared with the first study, where, in nonrecombinant phenotypes, double crossover had to be considered. The estimated recombination fraction from 0.263 to 0.423 is in good agreement with our first results. Combination of the deCODE markers with the forensically used X chromosomal markers enables the estimation of genetic distances and confirms that the genetic distance between linkage group III and IV is approximately 34 cM. Similar results were obtained by interpolating the genetic distances from Rutgers map v.2 [42].

Special attention was paid to the closely localised markers within the four linkage groups and around the centromere. Tillmar et al. [41] found only two recombination events within linkage groups II and IV, whereas the four loci pairs used in the Mentype® Argus X-8 kit were in linkage disequilibrium testing 718 males. This and our own results were in agreement with our assumption that clusters of close markers are inherited as stable haplotypes. Extended haplotypes can be found especially in the region from Xp11 to Xq12 because of repressed recombination around the centromere. When using haplotypes from telomeric regions for kinship analysis, the much higher recombination rate has to be considered. Furthermore, mutations can occur, sometimes detectable in the change number of repeats. Analysis of additional flanking markers can help to decide whether a crossover or a mutation is more likely. In general, our results gave no indications for the existence of recombinational hot spots within the linkage groups in Xp21, Xq12, Xq26 and Xq28.

Table 6 Allele combinations in three families with recombination within the marker clusters in Xp21 and Xq28

Marker	Grandfather	Daughter	Grandson
DXS10148	19	19/23.1	19
DXS10135	22	22/27	27
DXS10146	15	15/34.2	34.2
DXS10134	38	34/38	38
DXS10146	15	15/19	19
DXS10134	37	34/37	37

Counting the recombination events in this study resulted in a relatively large number of short and also long ChrX arms with no crossover observations. This seems to contradict the general rule that, in humans, at least two crossover events occur on each metacentric chromosome (one on each arm [40]). But, there is a limit in the crossover detection by the indirect recombination assay using genotype data. Because only two of the four chromatids are involved in the exchange process and only one of the four is transmitted to the gametes, only 50% of all recombination events are detected [12]. Furthermore, possible recombinations in the pseudoautosomal regions could not be examined in this study.

Conclusions

Our recombination results in Germans in 2- and 3-generation families are generally in agreement with other studies estimating the relationship between physical and genetical distance along the X chromosome. We presented four groups of closely localised STR markers with genetic distances of at least 34 cM between the linkage groups. Within the groups, crossing over events are very rare, especially in the regions Xq12 and Xq21. Therefore, linkage disequilibrium can be assumed, and marker combinations are inherited as stable haplotypes over generations. The frequencies of such haplotypes are very low. When, in a deficiency kinship case, two possibly related individuals share a rare ChrX STR haplotype, a strong hint on kinship can be assumed. A website (http://

www.chrx-str.org) presents first haplotype frequencies of several populations for selected STR clusters. Independent segregation between linkage groups I/II and II/III was proven using two different family studies. The genetic distance between groups III and IV was found to be too small to assume independence. This should be in mind when calculating complex pedigrees. In kinship cases with several generations, a loose linkage can produce misleading results.

Recombination studies of makers with very short distances are sufficiently informative only when a very large number of meioses can be observed. Hence, an international cooperation study considering tightly linked markers within the linkage groups I–IV will soon follow.

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