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Sex and gender issues in competitive sports: investigation of a historical case leads to a new viewpoint

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Supplementary Data

Experimental Methodology

The samples from Foekje Dillema's clothing were collected and DNA analysis was performed under conditions and with methods commonly applied in forensic laboratories.[1-3] We carried out multilocus autosomal as well as X- and Y-chromosomal short tandem repeat (STR) profiling used for forensic human identification purposes, as well as sex chromosome quantification analyses targeting several X and Y regions.

Biological materials (putatively skin cells, sebaceous oils and sweat) were recovered from three items of clothing known to belong to Dillema using the double swab method.[3] Specific areas of the clothing that were most likely to contain only Dillema's biological material (namely the inside armpit seam, the inside back collar, and the inside seam of the sleeves) were targeted for sampling, to reduce the potential for contamination. DNA was extracted using the QIAamp DNA Micro Kit (Qiagen), in accordance with the manufacturer's recommendations, and six independent samples contained DNA of sufficient quality for further analysis. Each sample was analysed in triplicate, for each quantification and genotyping system employed.

Autosomal STR profiling was performed with the AmpF*l*STR Identifiler PCR Amplification kit (Applied Biosystems) and the PowerPlex 16 system (Promega) commonly used for forensic human identity testing. The two commercially available kits amplify a total of 17 autosomal loci, as well as the X-chromosomal amelogenin gene *AMELX* and the Y-

chromosomal *AMELY*. Y-chromosomal STR genotyping was performed with the AmpF*l*STR Yfiler PCR Amplification kit (Applied Biosystems) amplifying 17 loci scattered around the chromosome. Eight X-chromosomal STRs, encompassing 4 linkage groups, and *AMELX* and *AMELY*, were amplified with the Mentype Argus X-8 PCR Amplification kit (Biotype). Each kit was used strictly in accordance with the manufacturer's validated protocols. Peak height ratio of the *AMELX/AMELY* loci from each of the commercial kits allowed relative X:Y quantitation.

All STR profiles were interpreted in accordance with guidelines proposed for low copy templates.[1] Specifically, any allele must have been replicated in at least 3 independent amplifications to be considered genuine. This is considered standard operating practice for amplifications of less than 100pg of template DNA, and/or highly degraded DNA. Stutter alleles in the n-1 position were not called if the peak area was less than 15% of the parent peak area. Any peak below 50 RFU was not called as genuine, to prevent spurious alleles being included.

Real-time PCR quantification of autosomal (specifically the *hTERT* gene locus on 5p15.33) and Y-chromosomal (specifically the *SRY* gene locus) DNA was performed with the Quantifiler Duo DNA Quantification kit (Applied Biosystems). Relative X:Y quantitation was performed investigating the *PCHD11X* and *PCDH11Y* homologous loci, with a 90bp deletion on the X chromosome compared to the Y chromosome, using a described real-time PCR assay,[2] although 10µg of BSA was added to the reaction to overcome inhibition seen in the Dillema samples. Standard curves were created from triplicate measurements of known DNA samples: XY (male control), XXY (confirmed Klinefelter's control), and XXXY (created by mixing equal quantities of XX female and XY male DNA). Four of Dillema's DNA samples were measured in duplicate in each assay.

Results and Conclusions

Only low quantities of degraded DNA were recovered, as expected, from the material used for sample collection. Autosomal STR genotyping with the Identifiler and PowerPlex 16 commercial STR kits showed a single autosomal profile (Supplementary Tables 1 and 2), consistent with a single non-chimeric donor, in this case presumed to be Foekje Dillema from family accounts. The lack of additional alleles at the autosomal loci also indicated that DNA obtained was from a single donor (presumed to be Dillema), and had not been contaminated with another individual's DNA. The probability that a chimeric individual would display the same allelic profile at all 17 loci examined was estimated at 1.97×10^{-8}, by calculating the probability that two full siblings (as most chimeras are formed from two zygotes) would share all observed alleles. As such, the possibility that Dillema was a chimera can effectively be ruled out, at least for her skin cells.

Although autosomal loci showed approximately equal quantities of each allele (indicating a 1:1 ratio between homologous chromosomes), the *AMELX* and *AMELY* loci from both PCR kits displayed ratios of 2.85-4.5:1. In contrast, XY male control samples had ratios of 0.7-1.3:1 in all amplifications, as expected. Y-chromosomal STR genotyping with the commercial AmpF*l*STR Yfiler PCR Amplification kit showed a single and complete Y chromosome profile with all Y-STR loci amplified (Supplementary Table 3), and with peak heights between the loci consistent with degraded DNA. The presence of all Y-STRs analysed in allele numbers consistent with a single Y-chromosome indicates that an entire, single and intact Y chromosome was present. X chromosome STR genotyping using the commercial Mentype Argus X-8 PCR Amplification kit provided information about 5 STRs in 4 linkage groups. The remaining 3 loci could not be amplified due to the extremely degraded nature of the DNA samples. Two amplifications showed a second allele at two loci (DXS10074, HPRTB), but these could not be replicated in other amplifications and therefore are

interpreted as artefacts of amplifying low levels of DNA. The consensus profile showed that only a single allele at each locus was amplified (Supplementary Table 4), indicating that only one type of X chromosome was present. Indirect *AMELX/AMELY* quantification from the Mentype Argus X-8 PCR Amplification kit gave an X:Y ratio of 3.63 (2.18 - 5.29), in agreement with *AMELX/AMELY* results obtained from the Powerplex 16 and Identifiler kits.

Real-time PCR quantification of the *PCHD11X/PCDH11Y* loci in four of Dillema's samples resulted in a mean ratio of 3:1 for the loci tested, with ratios ranging from 2.32 to 3.66, and six of the eight replicates falling within the XXXY range (Supplementary Figure 1A). Triplicate standard curves gave ratios of 0.8-1.2 for XY controls, 1.76-2.29 for XXY controls, and 2.53-3.39 for XXXY controls (Supplementary Figure 1A). Notably, this ratio, estimated by direct quantitative PCR, was found to be in the range of the more indirect quantification measures for *AMELX/AMELY* obtained via fragment length analysis and peak height inspection (see above). Real-time PCR quantification of autosomal and Y chromosomal loci of Dillema's DNA using the Quantifiler Duo kit gave a 4:1 ratio for the loci tested (Supplementary Figure 1B). Control DNA samples with 1 Y chromosome per autosomal pair gave an average ratio of 1.94 (range 1.37 – 3.01) autosomes per Y chromosome, while controls with 4 autosomes per Y chromosome had ratios of 4.02 (3.23 – 5.98). Dillema's samples ranged from 3.59 to 6.05, with an average of 4.37 (Supplementary Figure 1B), consistent with the presence of one Y chromosome per two diploid sets of autosomes, and an 46,XX/46,XY genotype from a single donor. The presence of a few 45,XO or 47,XXY cells is not excluded, but a significant contribution by such aneuploid cells would be in disagreement with the overall 4:1 ratio.

From the combined genotyping and DNA quantification results, we conclude that Foekje Dillema had a 46,XX/46,XY mosaic genotype, with equal numbers of both genetic cell types at least in her skin, which may have originated from a 47,XXY zygote with one

type of maternal X chromosome. The formation of such a zygote would require fertilization by a normal 23,Y sperm of an aneuploid 24,XX oocyte carrying two sister chromatids from an X chromosome that has not undergone crossing-over in meiotic prophase, followed by nondisjunction of the sister chromatids in the second meiotic division. Alternatively, mitotic nondisjunction might have occurred in a 46,XY embryo, giving rise to a 47,XXY (and the lethal karyotype 45,YO) blastomer. In both scenarios, loss of X and Y by nondisjunction events at mitotic divisions of 47,XXY blastomers must have resulted in clones of 46,XY and 46,XX cells. The above-described events are all known, or at least have been suggested to occur,[4-5] but a series of events leading to a 46,XX/46,XY mosaic individual with one set of autosomes and one type of maternal X chromosome must be extremely rare, as this has been described, to our knowledge, in only two previous case reports.[6-7]

Supplementary References

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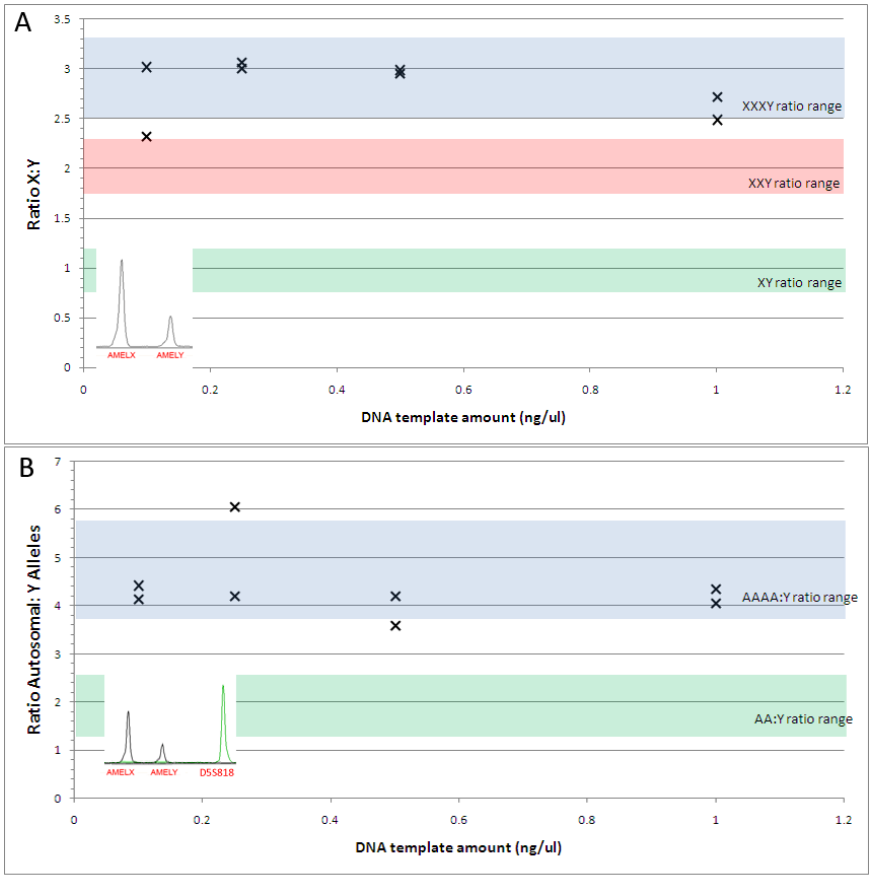

Supplementary Data Figure 1.

Sex chromosome and autosome quantification

A. Quantitative real-time PCR targeting the *PCHD11X* and *PCDH11Y* homologous loci. Triplicate standard curves of XY, XXY, and XXXY DNA were used to define the expected X:Y ratio ranges for each genotype. Six of 8 of Dillema's DNA samples fall within the XXXY range. The inset shows the imbalance between the *AMELX* and *AMELY* peaks in the PowerPlex 16 amplification system, which supports the 3:1 ratio in Dillema's DNA samples.

B. Quantitative real-time PCR targeting an autosomal locus (*hTERT*) and a Y-chromosomal locus (*SRY*). Triplicate standard curves of 46,XY (AA:Y) and 46,XY+46,XX (AAAA:Y) were compared to Dillema's samples, with six of eight samples falling within the 4:1 autosome:Y range. The inset displays the imbalance between the *AMELX* and *AMELY* loci, and an autosomal locus D5S818, further supporting the 4:1 ratio observed.

Table 1. PowerPlex 16 autosomal STR genotypes and consensus profile

sample	D3S1358	TH01	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	vWA	D8S1179	TPOX	FGA	Penta_D	Penta_E	AMELX/AMELY
1_08	14,17	7			11	12,*14*		*14.2*	12	17,20				14		X
3_08	14,17	6,7	32		11	10,12	12		12	17,20	12	9	23	12,14	10,15	X,Y
5_08	14,17	6,7	28,32	14,17	11	10,12	10,12	12,13	12	17,20	12,13	8,9	21,23	12,14	10	X,Y
10_08	14,17	6,7	28,32	14,17	11	10,12	10,12	12,13	12	17,20	12,13	8,9	21,23	12,14	10,15	X,Y
19_08	14,17	6,7	28,32	14,17	11	10,12	10,12	12,13	12	17,20	12,13	8,9	21,23	12,14	10,15	X,Y
20_08	14,17	6,7	28,32	14,17	11	10,12	10,12	12,13	12	17,20	12,13	8,9	21,23	12,14	10,15	X,Y
consensus	**14,17**	**6,7**	**28,32**	**14,17**	**11**	**10,12**	**10,12**	**12,13**	**12**	**17,20**	**12,13**	**8,9**	**21,23**	**12,14**	**10,15**	**X,Y**

Table 2. Identifiler autosomal STR genotypes and consensus profile

sample	D3S1358	TH01	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	vWA	D8S1179	TPOX	D19S433	D2S1338	FGA	AMELX/AMELY
1_08	14,17	6,7	28,32	14	11	10,12	10,12	12,13	12	17,20	12,13	8,9	14,14.2		21	X,Y
3_08	14,17	7	28		11	10,12		12,13		17,20	12,13	8,9	14,14.2		21,23	X,Y
5_08	14,17	6,7	28,32	14	11	10,12	10,12	12,13	12	17,20	12,13	8,9	14,14.2	18	21,23	X,Y
10_08	14,17	6,7	28,32	14	11	10,12	10,12	12,13	12	17,20	12,13	8,9	14,14.2	18	21	X,Y
19_08	14,17	6,7	28,32	14	11	10,12	10,12	12,13	12	17,20	12,13	8,9	14,14.2	18	21,23	X,Y
20_08	14,17	6,7	28,32	14	11	10,12	10,12	12,13	12	17,20	12,13	8,9	14,14.2	18	21,23	X,Y
consensus	**14,17**	**6,7**	**28,32**	**14**	**11**	**10,12**	**10,12**	**12,13**	**12**	**17,20**	**12,13**	**8,9**	**14,14.2**	**18**	**21,23**	**X,Y**

Table 3. Yfiler Y-STR genotypes and consensus profile

sample	DYS 456	DYS 389I	DYS 390	DYS 389II	DYS 458	DYS 19	DYS 385	DYS 393	DYS 391	DYS 439	DYS 638	DYS 392	YGATA H4	DYS 437	DYS 438	DYS 448
1_08	16	14			18		14	12	10		23	13	12	15	12	19
3_08	16								10	11						
5_08	16	14	24	31	18	14	11,14	12	10	11	23	13	12	15	12	19
10_08	16	14	24	31	18	14	11,14	12	10	11	23	13	12	15	12	19
19_08	16	14	24	31	18	14	11,14	12	10	11	23	13	12	15	12	19
20_08	16	14	24	31	18	14	11,14	12	10	11	23	13	12	15	12	19
consensus	**16**	**14**	**24**	**31**	**18**	**14**	**11,14**	**12**	**10**	**11**	**23**	**13**	**12**	**15**	**12**	**19**

Table 4. Mentype Argus X-8 X-STR genotypes and consensus profile

sample	**DXS10135**	**DXS8378**	**DXS7132**	**DXS10074**	**HPRTB**	**DXS10101**	**DXS10134**	**DXS7423**	**AMELX/AMELY**
1_08	20	11		16				15	X,Y
3_08									X,Y
5_08		11		*15*,16	14			15	X,Y
10_08	20	11		16	*13*,14			15	X,Y
19_08		11		16	14			15	X, Y
20_08	20	11		16	14			15	X,Y
consensus	**20**	**11**		**16**	**14**			**15**	**X,Y**

Footnote to Tables 1-4:
The above tables display Dillema's STR profiles at a total of 18 autosomal STR loci, 17 Y-chromosomal STR loci, and 8 X-chromosomal STR loci. With reference to Tables 1 and 2, a maximum of two alleles are expected at each autosomal locus. For example, at the locus D3S1358 on chromosome 3, Dillema possessed alleles with 14 and 17 STR repeats (one on the paternal chromosome, one on the maternal). At other loci, the two homologous chromosomes contained STR repeats with the same number of alleles, for example at the locus D5S818 on chromosome 5 both alleles had 11 repeats. For both the Y and X chromosome STRs, only a single allele is expected if a single type of the chromosome is present. In all tables, alleles shown in italics represent drop-in events: alleles could not be replicated in separate amplifications. According to standard interpretation guidelines, these alleles are not included in the consensus profile, as they likely represent random contamination events and/or errors in amplification.