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CASE REPORT

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Mother–child exclusion due to paternal uniparental disomy 6

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Abstract In a mother-child pair, false exclusions in markers on chromosome 6 have been observed. The genetic incompatibilities have been caused by paternal uniparental disomy. The consequences of such cases for investigations of parentage are discussed.

Introduction

In cases of clear-cut genetic incompatibilities between a man and a child, there is usually no dispute to consider such a situation as paternity exclusion. Exclusion of a mother from a child is much more problematic, as it was already formulated in the Roman legal conception "mater semper certa est, pater nunquam" (the mother is always certain, the father never). If an interchange of babies (e.g., in the postnatal care) can be ruled out, extensive genetic investigations have to be performed to clarify the basis of this constellation.

In this case report, we describe a paternal uniparental disomy (UPD) of chromosome 6, i.e., the inheritance of both homologs of chromosome 6 from the father, which caused multiple mother-child exclusions in markers coded for by chromosome 6.

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Materials and methods

Subjects

Three individuals, mother, child, and putative father, involved in a paternity case from the Institute of Legal Medicine of the University of Rostock were included. The mother and alleged father gave their informed consent to perform and to publish the results of the analyses.

Typing

Phenotypic markers: standard procedures (hemagglutination, microlymphocytotoxic assay).

HLA class II genotyping: DNA technology by using the polymerase chain reaction sequence-specific primer method (QIAGEN, Valencia, CA, USA).

Short tandem repeat (STR) typing: DNA extraction from EDTA blood—Chelex procedure (BioRad Laboratories, Hercules, CA, USA).

Investigation of the STR systems: commercial kits (AmpFlSTR Identifiler PCR Amplification Kit, Applied Biosystems, Foster City, CA, USA—see, e.g., Kubat et al. [10]; Geneprint Powerplex 16 System, Promega, Madison, WI, USA—see, e.g., Konjhodzic et al. [9]), for SE33 (ACTBP2) primers according to Polymeropoulos et al. [14] and the primer pairs designed for linkage mapping of chromosome 6 (ABI Prism Human Mapping Primers, Applied Biosystems). Panels 8, 9, and 10 were used to achieve a 10-cM resolution of chromosome 6. Additional individual primer pairs were included to map the SE33 (ACTBP2) locus on chromosome 6 [15].

Sizing of the amplification products and assessment of the alleles: ABI Prism 310 capillary electrophoresis instrument using POP-4 according to the manufacturer's instructions (Applied Biosystems).

Results

In the first investigation of paternity using the phenotypic markers AB0, MNS, RH, FY, JK, and HLA-ABC and the DNA STRs VWA, D3S1358, FGA, D8S1179, D21S11, D5S818, D7S820, SE33 (ACTBP2), D13S317, D16S539, D18S51, Penta E, Penta D, TPOX, and TH01, the child could be excluded from the mother in the systems HLA and SE33 (ACTBP2)(Table 1). In this respect, it is important to note that the mother–child exclusions affected two not closely linked regions of chromosome 6 (6p21.3 for HLA and 6q14 for SE33).

In all chromosomes tested with the exception of chromosome 6, the mother showed no exclusions to the child. The plausibility of maternity in these systems amounted to 99.99997%. The putative father could not be excluded from paternity of the child. Based on the data on the mother, child, and putative father (without data for chromosome 6 markers), the plausibility of paternity was 99.9975%; the analysis of the plausibility of paternity without the mother and including the chromosome 6 markers gave for the putative father a value of 99.999997%. The biostatistical computations, therefore, gave no evidence against the maternity of the mother and against the paternity of the putative father to the child.

These findings and the observation that the child was homozygous for the chromosome 6 markers tested (HLA and SE33), suggested that the genetic incompatibility between mother and child might be due to a paternal UPD6 (see [5, 6]).

For this reason, extensive typing of the mother, the child, and the putative father for chromosome 6 markers and tests to define heterozygosity of autosomal loci not coded for by chromosome 6 and X chromosomal loci in the child were added.

The typing results of chromosome 6 are listed in Table 2. In total, 42 loci on chromosome 6 were tested and 26 showed a mother–child exclusion. Furthermore, it was of great interest to note that the child was homozygous for all chromosome 6 markers tested. The father was heterozygous in 37 of the 42 chromosome 6 loci tested; the heterozygosity spanned the region from D6S1574 to D6S446, corresponding to the interval 6p25 to 6q27, i.e., practically the whole of chromosome 6.

In all other chromosomes of the child (1–5, 7–22, and X), heterozygosity of at least one locus was demonstrable (Table 3).

Table 1 Mother–child exclusions in the first investigation

System	HLA	SE33 (ACTBP2)
Mother	A2,29; B56,57; Cw1,w6; DRB1*01,*07; DQB1*03,*05	*17,*28.2
Child	A3; B13; Cw6; DRB1*07; DQB1*02	*14

Table 2 Chromosome 6 typing in mother, child, and father (from 6p25 to 6q27)

Locus	Mother	Child	Father
D6S1574	*156, 158*	*168*	160,168
F13A	**4,*6*	**7*	*7
D6S309	311, 321	321	321,323
D6S470	*124*	*132*	132,134
D6S289	*163, 167*	*171*	171,175
D6S422	302, 318	302	302,310
D6S276	211, 223	223	223
HLA-A	*A2, 29*	*A3*	A1,A3
D6S2960 (C3_4_13)	*286, 298*	*302*	302,310
D6S2906 (C3_3_6)	**9, *12*	**15*	*13,*15
D6S2939 (C2_4_4)	*10, *18	*10	*9,*10
HLA-C	Cw1, w6	Cw6	Cw6, Cw7
HLA-B	*B56,57*	*B13*	B8,B13
D6S2931 (C1_4_4)	*8, *9	*8	*8,*10
HLA-DR	B1*01, *07	B1*07	B1*07,*15
HLA-DQ	*B1*03, *05*	*B1*02*	B1*02,*06
D6S1610	*205, 207*	*201*	201,205
D6S1549	191, 199	199	191,199
D6S282	*119, 125*	*121*	121,127
D6S1650	*118, 120*	*122*	116,122
D6S452	*279*	*285*	273,285
D6S272	*185, 193*	*187*	183,187
D6S1573	*286, 290*	*296*	286,296
D6S257	*171, 177*	*161*	161,173
D6S460	*286*	*294*	278,294
D6S1609	*83, 99*	*97*	97
SE33	**17, *28.2*	**14*	*14,*28.2
D6S462	112	112	112,114
D6S300	*199*	*197*	197,209
D6S1671	*268, 284*	*267*	267,276
D6S434	206, 210	210	210,218
D6S1698	*174, 182*	*172*	172,180
D6S287	*124*	*122*	122,126
D6S262	*172, 180*	*182*	176,182
D6S292	158, 162	158	158,166
D6S308	342	342	342
D6S441	*171, 183*	*177*	177,181
D6S1581	263, 271	271	271,273
D6S264	115	115	113,115
D6S1697	252, 254	252	252,254
D6S446	216, 222	222	222,226
D6S281	*140*	*142*	142

The phenotypes or genotypes are designated as recommended by the "official" nomenclature (F13A, HLA, D6S2906, D6S2939, D6S2931, and SE33). In the other systems, the alleles are designated by the length of the amplification products in basepairs
Mother–child exclusions are printed in italics

Investigations of the child's karyotype produced evidence for a normal 46,XX type. The clinical evaluation of the phenotype of the child showed a macroglossia and a transient neonatal diabetes mellitus.

Table 3 Demonstrated heterozygosity of the child in chromosomes 1–5, 7–22, and X

Chromosome	Locus	Genotype/phenotype
1	D1S80	*22F, *24
	RH	CcDee
2	D2S1338	*17, *18
	TPOX	*8, *11
3	D3S1358	*16, *17
4	FGA	*21, *24
	MNS	MNs
5	D5S407	97, 99
	D5S644	92, 98
	D5S406	170, 182
7	D7S820	*10, *12
8	D8S1179	*11, *14
9	D9S1810	206, 208
	D9S1818	200, 206
10	D10S1649	136, 140
	D10S1655	252, 260
11	TH01	*9, *9.3
12	D12S391	*21, *22,
	VWA	*19, *20
13	D13S1322	96, 98
	D13S1243	253, 257
14	D14S990	141, 149
15	Penta E	*7, *12
16	D16S539	*11, *12
17	D17S30 (YNZ22)	*4, *8
18	D18S51	*15, *16
19	D19S433	*12, *13
20	D20S902	309, 313
	D20S906	96, 102
21	D21S11	*28, *33.2
	Penta D	*10, *13
22	D22S1170	203, 212
X	DXS1223	154, 158

The phenotypes or genotypes are designated as recommended by the "official" nomenclature (D1S80, RH, D2S1338, TPOX, D3S1358, FGA, D7S820, D8S1179, TH01, D12S391, VWA, Penta E, D16S539, D17S30, D18S51, D19S433, D21S11, and Penta D). In the other systems, the alleles are designated by the length of the amplification products in basepairs

Discussion

The results found in this case show that the child is homozygous for all chromosome 6 loci. The father, who is heterozygous for 37 of the 42 loci tested, must have transmitted two copies of the same chromosome 6 to the child. The results found in this case therefore demonstrate that the child must have inherited two copies of the same chromosome 6 from the father; thus showing a paternal UPD6 with isodisomy of chromosome 6. This finding is corroborated by the clinical symptoms (transient neonatal diabetes mellitus and macroglossia), which are indicative for a paternal UPD6 [6].

The mechanisms resulting in UPD are multiple [6]. The best one documented is that UPD arises from the loss of a chromosome from an initial trisomy (trisomy rescue), although other causes are also possible: monosomy duplication (a gamete nullisomic for one chromosome fuses with a normal gamete with a subsequent duplication of the single chromosome) or gamete complementation (a disomic gamete fuses with a gamete nullisomic for the same chromosome). In this case, the complete isodisomy of chromosome 6 is probably due to a mitotic nondisjunction.

For paternity testing, such cases can cause serious problems by the occurrence of genetic incompatibilities between a parent and the child. This fact was already described by Bein et al. [1] who observed pseudoexclusions of a father due to maternal UPD of chromosome 16.

If an interchange of the child can be ruled out, further possibilities for false parent–child exclusions besides UPD can be considered:

Null alleles Such alleles, which may have a heterogeneous background (mutations or deletions), exist in practically all systems and must always be taken into account [8].

Mutations In phenotypic markers, mutations are extremely rare (less than 1 in 10^6); so mutations in these systems can be disregarded. In STRs, the average mutation rate is around 0.14%; the mutations are due to replication slippage adding or removing one or more repeats [2]. Based on the mutation rates, it can be deduced that the existence of three genetic incompatibilities in STR systems are evidentiary for the nonpaternity of a putative father, especially in case that these incompatibilities cannot be explained by the gain or loss of one repeat (Mayr, unpublished observation). This conclusion corresponds to the considerations by Brinkmann et al. [3] who stated that false double exclusions might be found in very rare cases; for this reason, the requirement of three exclusions adds to the safety of the evidence of nonpaternity.

Chimerism In some individuals, more than one genetically distinct population of cells can be found. This phenomenon can be due to mosaicism (genetically different cells arising from a single zygote, usually by deletions of short pieces of DNA) or chimerism (originating from more than one zygote, see, e.g., [11]).

Blood (or twin) chimerism occurs in dizygotic twins due to the intrauterine passage of hematopoietic cells from one twin into the other and vice versa via vascular anastomoses, which can be found very rarely in dizygotic twin pregnancies. This form of chimerism can only be detected in blood samples containing cells originating from the hematopoietic stem cells of both twins by testing phenotypic markers and/or DNA polymorphisms. In chimera, the blood cells do not necessarily represent the true genotype of the individual. The genotype of blood chimera, however, can be identified by analyzing the DNA from hair follicles, which corresponds to the true genotype [7, 13].

In whole body (tetragametic) twins, all tissues contain a mixture of at least two lineages originating from different zygotes. Such individuals show in some systems up to four alleles, which can be detected in DNA samples obtained

from all tissues [4]. For this reason, whole body chimeras can be easily recognized in most cases. Nevertheless, two families were described in which mother–child exclusions could only be explained by the existence of an extreme tetragametic chimerism in which the germ cells showed genes different from the genes expressed in some lineages of somatic cells [12, 16].

In view of these considerations, one very important conclusion can be drawn for cases of paternity or maternity tests: an exclusion should only be formulated if at least three genetic incompatibilities in loci coded for by different chromosomes are present and if there is no indication of a possible chimerism. In all other cases, the above mentioned exceptions to the usually accepted rules of inheritance have to be considered and ruled out before postulating an exclusion between a parent and the child.

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