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ORIGINAL ARTICLE

G. Bein · B. Driller · M. Schürmann · P. M. Schneider
H. Kirchner

Pseudo-exclusion from paternity due to maternal uniparental disomy 16

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Abstract The investigation of a case of disputed paternity revealed indirect exclusion of the alleged father in the haptoglobin system and in the DNA single-locus system D16S309/Hinf I (MS205). The paternity index for the non-exclusion systems was > 10^6. Since both exclusion systems (HP and MS205) are located on chromosome 16, we investigated 10 microsatellite loci covering this chromosome with 10–20 cM resolution. Analysis of the child's chromosome showed only alleles of maternal origin and lack of inheritance of paternal alleles for five informative loci. The markers close to the centromere of chromosome 16 were heterozygous, whereas distal loci were either heterozygous or homozygous for maternal alleles. This is consistent with a maternal meiosis I nondisjunction of chromosome 16 leading to maternal uniparental heterodisomy. This case emphasizes that the opinion of non-paternity should be based on the absence of paternal alleles at genetic systems located on at least two different chromosomes.

Key words Uniparental disomy · UPD · Chromosome 16 · Parentage testing

Introduction

Uniparental disomy (UPD) is a rare phenomenon leading to the presence of two chromosomes from one parent in individuals with otherwise normal disomic cells (Engel 1993). We describe a paternity case where an opinion of non-paternity would have been given on the basis of two indirect exclusions. Both genetic systems are located on chromosome 16 and the analysis of several dinucleotide repeat markers revealed only maternal alleles of this chromosome in the child. To our knowledge this is the first published report on a pseudo-exclusion of an alleged father in a case of disputed paternity due to UPD. Many cases of UPD appear to have a completely normal phenotype (Ledbetter and Engel 1995) and the exact frequency of this phenomenon in the population is unknown. Parentage testing without knowledge of this cause of aberration from Mendelian segregation may lead to the erroneous opinion of non-paternity.

G. Bein (✉)
Institute of Clinical Immunology and Transfusion Medicine,
Justus-Liebig-University Langhansstrasse 7,
35392 Giessen, Germany
Tel. + 49-641-99-41500; Fax + 49-641-99-41509
e-mail: gregor.bein@immunologie.med.uni-giessen.de

B. Driller · H. Kirchner
Institute of Immunology and Transfusion Medicine,
University of Lübeck Medical School, Lübeck, Germany

M. Schürmann
Institute of Human Genetics,
University of Lübeck Medical School, Lübeck, Germany

P. M. Schneider
Institute of Legal Medicine,
Johannes Gutenberg University, Mainz, Germany

Material and methods

Biochemical phenotyping and restriction fragment length polymorphism (RFLP) testing

The biochemical phenotypes and DNA single-locus genotypes of the mother, child and putative father involved in a case of disputed paternity were determined employing standard procedures and included the systems AB0, MNSs, Rh, Group-specific component (GC), alpha-1-antitrypsin (PI), plasminogen (PLG), transferrin (TF), acid phosphatase (ACP), glyoxalase (GLO), phosphoglucomutase 1 (PGM1), D1S7/MS1 (Hinf I), D2S44/YNH24 (Hinf I), D7S21/MS31 (Hinf I), D7S22/G3 (Hinf I), D12S11/MS43 (Hinf I), and D16S309/MS205 (Hinf I). Haptoglobin typing followed essentially a published procedure (Scherz et al. 1990). Sizing of the DNA fragments and calculation of the paternity index were done using the DNA VIEW software (C.H. Brenner, Berkeley, California, USA).

DNA isolation

DNA was isolated using the salting out method (Miller et al. 1988) and the concentration was determined by spectrophotometry.

Multilocus profiling

Hinf I digested DNA was probed with the multi-locus probe MZ 1.3 (Schacker et al. 1990). Sizing of the DNA fragments, determination of band sharing and calculation of paternity probability were done employing a previously published algorithm (Brenner et al. 1994).

Mapping of chromosome 15 and 16

Typing of dinucleotide repeat markers of chromosome 15 and 16 followed essentially the protocol of the ABI PRISM linkage mapping set (panel 21 and 22, ABI, Perkin Elmer, Weiterstadt, Germany). The fluorescent dye-labeled PCR products were run on an automated sequencing system (ABI 377, Perkin Elmer, Weiterstadt, Germany) and were sized using the GeneScan Analysis software version 2.0.2. The arbitrary allele sizes were calculated employing the Gene Scan 350 TAMRA size standard (Perkin Elmer, Weiterstadt, Germany). A match was considered if the size of the parental alleles fell into a window of 0.25 base pairs of the respective alleles of the child.

Results

The biochemical phenotypes and DNA single-locus genotypes of the mother, child and putative father for the genetic systems AB0, MNSs, Rhesus, GC, PI, PLG, TF, ACP, GLO, PGM1, D1S7, D2S44, D7S21, D7S22 and D12S11 were consistent with paternity of the alleged man. However, the investigation of the HP system and the D16S309/MS205 (Hinf I) DNA single-locus system revealed inconsistencies. The mother and child were heterozygous at the HP locus and shared both alleles (phenotype HP 2FF,2FS), whereas the man possessed neither of these alleles (phenotype HP 1S). Furthermore, the child possessed only one fragment at the locus D16S309 matching one of the maternal fragments but neither of the two paternal fragments (genotypes: mother D16S309 2.90 kb, 3.64 kb, child D16S309 3.60 kb, man D16S309 2.00 kb, 2.54 kb). The combined paternity index for the non-exclusion systems was > 10^6.

Investigation of this case employing the multi-locus probe MZ 1.3 and hybridization of Hinf I digested DNA revealed nine fragments > 4 kb which were informative, i.e. present in the child but absent in the mother. All nine fragments were also present in the alleged father. The band sharing between child and man was 73% and thus characteristic for first degree relatives (Schneider et al. 1992). The paternity index calculated from this multi-locus profile was 10^6.

Since both systems which revealed indirect exclusion from paternity (HP and D16S309) are located on chromosome 16, we studied a panel of dinucleotide repeat markers for this chromosome and chromosome 15 for control purposes. All markers which were analysed at chromosome 15 revealed genotypes which were consistent with paternity (data not shown). However, analyses of the child's chromosome 16 showed alleles only of maternal origin and lack of inheritance of paternal alleles for five informative loci (Table 1). The markers close to the centromere of chromosome 16 were heterozygous, whereas distal loci were either heterozygous or homozygous for maternal alleles (Fig. 1). At least three cross-over events took place in meiosis I (Fig. 1).

Table 1 Genotypes of the mother, child and the alleged father at 10 dinucleotide repeat loci covering chromosome 16. The map position (Kosambi centi Morgans, KcM, data from the Généthon human genetic linkage map) and the arbitrary size of the alleles are indicated. Alleles of the same size (0.25 base pairs) are written underneath in columns

Locus	KcM	Arbitrary size of PCR products [base pairs]			
D16S423	9				
Mother			134.79	136.80	
Child				136.80	
Father		132.82			149.72
D16S405	30				
Mother		132.43	140.59		
Child		132.35	140.59		
Father		132.47			
D16S420	47				
Mother			257.32	266.73	
Child			257.32	266.73	
Father		255.42	257.30		
D16S401	49				
Mother		171.84		179.57	
Child		171.79		179.58	
Father		171.86	177.63		
D16S411	62				
Mother		215.65	219.59		
Child		215.72	219.59		
Father		215.66		221.62	
D16S415	71				
Mother			226.64		
Child			226.63		
Father		224.69		228.66	
D16S503	88				
Mother		299.84	303.75		
Child		299.84	303.66		
Father			303.66	305.62	
D16S515	98				
Mother				338.00	343.93
Child				338.07	
Father		328.14	336.00		
D16S511	118				
Mother		182.01		204.01	
Child		182.01			
Father			203.99		218.33
D16S520	133				
Mother		147.61	149.79		
Child		147.61	149.79		
Father				153.57	155.50

Discussion

In the present case we observed two indirect exclusions from paternity and thus non-paternity of the alleged man was assumed. However, the matching of DNA fragments for several DNA single-locus systems and a DNA multi-locus system between the child and the man, led to the suspicion of a biological relationship. An aberration of chromosome 16, where both exclusion systems are located was postulated. The analysis of dinucleotide repeat markers of this chromosome revealed alleles only of maternal origin in the child. Lack of paternal alleles was found for five informative loci whereas the same analysis

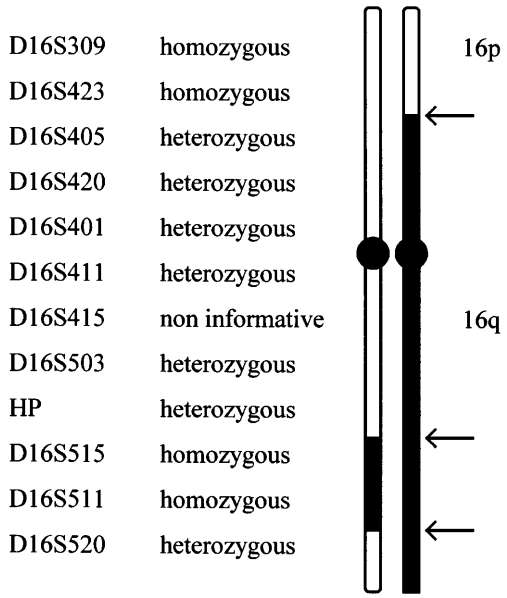

Fig. 1 Schematic map of the child's chromosome 16. Homozygosity and heterozygosity for the respective loci are indicated in black and white. The arrows indicate cross-over events

of chromosome 15 was consistent with paternity. The loci close to the centromere of chromosome 16 were heterozygous for maternal alleles which is consistent with maternal meiosis I non-disjunction of chromosome 16 leading to maternal combined uniparental hetero- and isodisomy.

The first case of UPD in humans was described in 1988 (Spence et al. 1988). Shortly thereafter it was discovered that maternal UPD 15 leads to Prader-Willi syndrome (Nicholls et al. 1989) and paternal UPD 15 to Angelman syndrome (Malcolm et al. 1991). Since then, UPD of chromosomes 2, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, 20, 21, 22, XXmat, XXpat and XYpat (reviewed in Ledbetter and Engel 1995) and chromosome 8 (Benlian et al. 1996) has been reported. The most common mechanism of UPD is "rescue" of a trisomic zygote caused by meiotic nondisjunction events by later loss of one copy of the supernumerary chromosome. In many cases, trisomic cell lines are present in the placenta, but not in the fetus (confined placental mosaicism). Normal postnatal development has been reported for UPD of some chromosomes. However, in other cases, UPD leads to intrauterine growth retardation (IUGR) and this may be caused by imprinting effects that are confined to placental tissue (Robinson et al. 1997). UPD for chromosome 16 is the most frequent and may result in fetal death, IUGR or normal development of the newborn (Kalousek et al. 1993). The propositus of this case presented an apparently normal phenotype and consequently, a physical examination did not take place.

Parentage testing must consider UPD as a phenomenon leading to indirect exclusions. PCR short tandem repeat systems (PCR-STR) are increasingly used as a single technique for the purpose of parentage testing. In contrast to conventional RFLP single-locus systems (VNTR systems), PCR-STR systems do not provide direct evidence for biological relationships. In particular, cases where the child presents with maternal uniparental heterodisomy may lead to the false opinion of non-paternity. In those cases, the child may receive two alleles at a given locus from the mother, while neither of these alleles is present in the alleged father. Two or more indirect exclusions of this type may lead to the opinion of non-paternity without considering the rare phenomenon of UPD. Polymorphic loci used for parentage testing should therefore be located preferably on different chromosomes to reduce the potential of false exclusions at numerous loci.

In conclusion, the opinion of non-paternity should be given only on the basis of at least two exclusions in genetic systems which are located on two different chromosomes. Current standards of parentage testing should be adapted accordingly.

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