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Two loci 'exclusion' of true paternity is due to genetic disorder in a child

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ABSTRACT

We describe a paternity case with three genetic incompatibilities between a three-year-old boy and his putative father.

STR analysis of 2 out of 25 markers revealed the absence of paternal alleles and presence of two maternal alleles at D2S441 and D2S1338 loci in the child. The rest 23 STR markers served to confirm paternity. In addition, we analyzed Y-STRs and determined the same haplotype in the child and his putative father.

With massive parallel sequencing on HID Ion GeneStudio S5 System using Precision ID GlobalFiler NGS STR Panel v2 (Applied Biosystems) we confirmed the presence of two alleles of maternal origin at D2S441, D2S1338 loci and identified two maternal alleles at additional locus D2S1776 located on chromosome 2 in the child.

Finally, we confirm paternity. Three loci 'exclusion' was due to maternal uniparental disomy of chromosome 2 in the child.

1. Introduction

Uniparental disomy (UPD) is a phenomenon in which an offspring receives both copies of a particular chromosome or a part of a chromosome from only one parent. UPD may result either in isodisomy or in heterodisomy [1]. To date it is widely discussed that UPD can cause trouble for interpretation of parentage testing results [2–4].

In this study, we report a paternity case with three genetic incompatibilities between a putative father and a child due to maternal uniparental disomy of chromosome 2.

2. Materials and methods

We discovered a case with maternal uniparental disomy of chromosome 2 during paternity testing. Saliva and EDTA blood samples were obtained from a Caucasian trio involving a mother, a child and a putative father. Genomic DNA was extracted from above mentioned samples using one of the following methods: Chelex-100 method (Bio-Rad, USA) for STR analysis and using Prepfiler BTA Forensic DNA Extraction Kit (Applied Biosystems) for massive parallel sequencing.

Autosomal STRs and amelogenin were amplified with GlobalFiler and Verifiler Plus Kits (Applied Biosystems), PowerPlex Fusion 6C System (Promega). Y-STRs were analyzed with the use of Yfiler and Yfiler Plus Kits (Applied Biosystems). Analysis of X-STR markers including amelogenin was performed using Investigator Argus X-12 QS kit (Qiagen).

DNA isolation and PCR amplification of above-mentioned groups of markers were performed according to manufacturer's recommendations. PCR amplifications were carried out on ProFlex PCR System (Applied Biosystems) and amplification products were separated by capillary electrophoresis on the ABI PRISM 3500 Genetic Analyzer (Applied Biosystems). Fragments were analyzed using GeneMapper ID—X Software Version 1.4 (Applied Biosystems). To quantify the total amount of human DNA in a sample Quantifiler Trio DNA Quantification Kit and QuantStudio™ 5 Real-Time PCR Instrument (Applied Biosystems) were used.

Library preparation was performed on HID Ion Chef System using Precision ID GlobalFiler NGS STR Panel v2 and Precision ID DL8 Kit. For template preparation and sequencing HID Ion Chef System and HID Ion GeneStudio S5 System were used (Ion 530 Chip and Ion S5 Precision ID Chef & Sequencing Kit). The data were analyzed using Torrent Suite Software (plugin HIDGenotyper and software Converge).

2.1. Clinical presentation and cytogenetic analysis of the child

The child is a three-year-old boy born from apparently healthy parents of European origin. The child has a diagnosis of Miller-Dieker syndrome, but the child's metaphase FISH results showed the absence of a specific 17p13.3 deletion with Miller-Dieker/lissencephaly region probe - LS1 LIS1 Miller-Dieker probe 17p13.3 (Vysis, Abbott

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Table 1
Three genetic incompatibilities at loci D2S441, D2S1776, D2S1338 between the child and the alleged father. Noninformative status of the locus TPOX.

Locus	Cytogenetic location	Mother	Child	Alleged father
TPOX	2p25.3	8/11	8/8	8/8
D2S441	2p14	11/11.3	**11.3/11.3**	11/15
D2S1776	2q31-32	10/11	**10/10**	11/12
D2S1338	2q35	23/23	**23/23**	17/20

Genotypes of autosomal STR loci D2S441, D2S1338, TPOX for a trio paternity case involving a mother, a child and a putative father were generated with GlobalFiler and Verifiler Plus Kits, PowerPlex Fusion 6C System. D2S1776 genotypes were obtained with massive parallel sequencing. Child's alleles of maternal origin are highlighted in bold.

Table 2
Massive parallel sequencing data on HID Ion GeneStudio S5 System using Precision ID GlobalFiler NGS STR Panel v2.

Locus	Sample	Genotype	Coverage	Sequence
TPOX	mother	8	12721	[AATG]8
		11	12127	[AATG]11
	child	8	25743	[AATG]8
		8		[AATG]8
	alleged father	8	23166	[AATG]8
		8		[AATG]8
D2S441	mother	11	8413	[TCTA]11
		11.3	10787	[TCTA]4 TCA[TCTA]7
	child	11.3	14206	[TCTA]4 TCA[TCTA]7
		11.3		[TCTA]4 TCA[TCTA]7
	alleged father	11	6937	[TCTA]11
		15	4852	[TCTA]12 TTTA[TCTA]2
D2S1776	mother	10	11366	[AGAT]10
		11	13477	[AGAT]11
	child	10	22945	[AGAT]10
		10		[AGAT]10
	alleged father	11	9891	[AGAT]11
		12	10689	[AGAT]12
D2S1338	mother	23	8899	[TGCC]7 [TTCC]13 GTCC[TTCC]2
		23		[TGCC]7 [TTCC]13 GTCC[TTCC]2
	child	23	7322	[TGCC]7 [TTCC]13 GTCC[TTCC]2
		23		[TGCC]7 [TTCC]13 GTCC[TTCC]2
	alleged father	17	3323	[TGCC]6 [TTCC]11
		20	3123	[TGCC]7 [TTCC]13

Laboratories). Cytogenetic analysis of the child revealed a normal 46, XY karyotype. Magnetic resonance imaging of the brain determined the presence of cortical dysplasia with lissencephaly. The child suffers from seizures.

3. Results

Firstly, DNA samples isolated from a mother, a child and a putative father were typed with PowerPlex Fusion 6C System. Microsatellite analysis revealed the absence of paternal alleles and presence of maternal ones in homozygous state at D2S441, D2S1338 in the child (Table 1). Since the presence of two father-child exclusion loci is usually not a sufficient basis for paternity exclusion, we analyzed our trio case with GlobalFiler and Verifiler Plus Kits. This step did not reveal any additional genetic inconsistency: all loci except for D2S441, D2S1338 were informative and served to confirm paternity.

The study of Y-chromosomal markers with Yfiler Plus Kit showed that the child and his putative father have identical haplotypes. The profile data of 12 X-*STRs* obtained with Investigator Argus X-12 QS kit demonstrated the absence of any genetic alterations in the child.

Maternal origin of two child's alleles at D2S441, D2S1338 was confirmed with massive parallel sequencing on HID Ion GeneStudio S5 System using Precision ID GlobalFiler NGS STR Panel v2 (Table 2). At D2S1776 the child showed homozygote state (10/10), his mother had heterozygote state (10/11) and his putative father had heterozygote state (11/12). Both child's alleles at D2S1776 were also inherited from his mother (Tables 1 and 2). Massive parallel sequencing data did not clarify the question related to the origin of the child's TPOX alleles also situated at chromosome 2.

4. Discussion

In the paternity case described here we observed three incompatibilities between the child and the alleged father at loci D2S441, D2S1338, D2S1776. Our results showed that the child has a homozygous state for all three markers D2S441, D2S1338, D2S1776 spanning the whole chromosome 2. With massive parallel sequencing, we confirmed the maternal origin of the child's alleles at loci D2S441, D2S1338, D2S1776. Thus, our typing and sequencing data pinpoint to the presence of maternal uniparental disomy of chromosome 2 in the child. Moreover, our generated data let us confirm the paternity of the alleged father. The identification of maternal and paternal isoalleles at D7S820 and D8S1179 gave us strong evidence to prove paternity.

5. Conclusion

In summary, our data indicate that for those paternity cases where genetic inconsistencies are observed on the same chromosome, extended typing approach including autosomal STR-makers, X-STRs, Y-STRs, mitochondrial markers has to be used to avoid false exclusion of paternity or maternity.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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