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Incorrect opinions in disputed paternity cases – causes of discrepancies. A case report

Summary

In the report we have presented cases where false opinions concerning disputed paternity and blood relationship establishment were issued. In case 1a wrong assumption resulted in a false opinion in which paternity was excluded. The genetic profile of the child's deceased defendant father was generated from genetic profiles of the child's grandparents. In the issued opinion it was concluded that the deceased is not the child's father. Based on analysis of grandparents' genetic profiles we can only conclude that the child is not their grandchild. In disputed paternity cases where DNA from the child's relatives (grandparents, siblings etc.) but not its parents is examined, many autosomal STR loci should be determined and if needed, STR loci on chromosome Y and X. Moreover, statistical analysis should be carried out. In case 2 the opinion was issued based on the fact that there were no excluding traits between defended relatives. In case 3 sampling was not done according to the proceedings, which did not allow to establish whether the sent samples were from the people mentioned in the protocol and finally a false opinion was issued.

The report confirms a great role of a forensic genetics expert in issuing a true opinion concerning disputed paternity or blood relationship.

Keywords loci STR, disputed paternity, false opinion, blood relationship, statistical analysis

Introduction

In the Department of Forensic Medicine and Toxicology, Medical University of Silesia, Katowice genetic examinations in cases of disputed paternity and identification of unknown (NN) people have been done for 20 years.

The scope of genetic research has been expanding systematically and now 23 autosomal STR loci are determined. When a boy is examined, 16 STR loci on chromosome Y are determined. For opinions where paternity is excluded there is a requirement that a common trait between the child and the defendant father should not be in 4 loci. In opinions done in our Department this requirement is usually exceeded and ranges from 5 to 20 loci. In opinions where paternity is confirmed, the value of paternity probability calculated for a trio: mother – child – defendant using a DNA Stat program 2.1 must be 99,9999%.

A wide range of genetic markers determined in our laboratory allows for confirming paternity with much higher probability. The value is often 99,99999999%. Genetic examinations are usually commissioned by family courts, public prosecutor's offices or private persons.

Long – standing experience in this kind of examinations causes that to the Department of Forensic Medicine and Toxicology, Medical University of Silesia, Katowice opinions concerning cases of disputed paternity where the prosecutor or a private person is doubtful whether the opinion is true are sent to be verified. According to the ruling genetic examinations are done again.

In the report there have been presented 3 cases in which the new examinations and detailed analysis of the obtained results resulted in issuing opinions different than those from the previous laboratory.

The aim of the report was to show the reasons of false opinions in cases of disputed paternity by carrying out the detailed analysis of opinions sent to our Department and then comparing the results obtained in our laboratory with those from the studied opinions.

Material and methods

Opinions in cases of disputed paternity as well as identification of unknown person NN in which the prosecutor or a private person was doubtful whether the opinion was true or not were sent to the Department of Forensic Medicine and Toxicology, Medical University of Silesia, Katowice. According to the ruling genetic examinations were done again. Samples of biological material from the parties were collected according to the proceedings binding in our Department, which meant: presence of two qualified persons while sampling, identity check – up of the examined persons, answer to protocol questions concerning blood transfusion and bone marrow transplantation, buccal swabs collection and finally the protocol sign.

DNA isolation from buccal swabs was performed with the use of a Sherlock AX kit (A & A Biotechnology) [1]. DNA concentration was determined with the use of a Nano Drop ND – 1000 spectrophotometer (Thermo Fisher Scientific TK Biotech). PCR reaction was performed using a Power Plex ESX 17 and Power Plex HS 16 kit (Promega) in a Gene Amp PCR System 9700 thermocycler (Applied Biosystems) according to manufacturer's instructions [17]. Amplification products were separated towards a DNA CC5 ILS 500 and CC5 ILS 600 standard (Promega) using a 3130AB Prism Genetic Analyzer (Applied Biosystems). Analysis of DNA profiles towards the following loci: AMEL, D3S1358,THO1, D21S11, D18S51, D10S1248, D1S1656, D2S1338, D16S539, D22S1045, VWA, D8S1179, FGA, D2S441, D12S391, D19S433, SE33, D5S818, D13S317, D7S820, CSF1PO, TPOX, Penta D, Penta E was carried out by determining them according to international nomenclature using a Gene Mapper IDv. 3.2 software (Applied Biosystems, Inc.). Analysis of DNA profiles towards loci on chromosome Y was carried out with the use of a Y filer test [18]. Frequencies of alleles in the population were found in literature [3, 4, 10, 11, 12, 14, 15, 16, 19]. In the case of linked markers such as D12S391 and VWA to calculate the total paternity index D12S391 was used as a more informative marker [8]. Both the probability of paternity and the probability of maternity were calculated using a DNA Stat 2.1 program [5]. Moreover, siblings likelihood was calculated with the use of a Familias program 3.1.9.3 [13].

Results and discussion

Case 1

Two false opinions concerning paternity of the deceased male, where biological material from his parents was analyzed have been presented. In another opinion concerning the same case biological material from the alleged sister recognized by the deceased before he died as well as biological material from the disputed child who had already been examined and its grandparents were investigated. The results of the research have been presented in Table 1 and 2.

Based on the obtained results of DNA polymorphism examinations in mother, disputed child and its alleged grandparents (parents of the deceased alleged father) the following conclusion was presented: "…….based on the obtained results and assuming that the mother of defendant deceased alleged father XY (grandmother of the disputed child) and the father of defendant deceased alleged father XY (grandfather of the disputed child) are biological parents of the deceased defendant XY and that the biological mother of underage child XX 1 is XX, the paternity exclusion of deceased defendant XY to underage child XX 1 has to be confirmed. Exclusion of the biological paternity of defendant XY is confirmed by analysis of the obtained results, which shows incompatibility in DNA fragments of 5 loci in the genetic profile of child XX 1 and among DNA fragments in the genetic profile of deceased defendant XY (alleged father)……" Moreover, it was concluded that "…… according to the guidelines of Forensic Hemogenetics Committee of Polish Association of Forensic Medicine and Criminology of 2013 it is assumed that the exclusion of paternity happens when there is incompatibility (lack of trait segregation) in at least 4 examined loci". In the studied case incompatibility was found in at least 5 of the 15 examined loci.

Based on the examinations of DNA polymorphism in mother, disputed child XX 1 and her sister XX 2 (recognized by the deceased before he died) and the DNA profile of deceased alleged father XY generated owing to the previous analysis the following conclusions were presented: " ……. based on the obtained results and assuming that the biological mother of underage XX 1 and XX 2 is XX, which the comparative analysis confirms and that XX 1 and XX 2 have the same biological father, DNA fragments in the incomplete genetic profile of the biological father were appointed. Analysis of XX's genetic profile and the incomplete genetic profile of alleged father XY as well as child XX 2 (sister of XX 1) showed incompatibility of traits in 10 of the examined loci. In other 5 loci it was impossible to issue an opinion on inheritance because of the fact that unknown alleles are difficult to be verified towards the examined material.

In the studied case due to the lack of knowledge on the genetic profile of the biological father (determined directly or indirectly) it is not possible to decide whether XX 1 and XX 2 have the same father or not ……."

Moreover, we report that the comparative analysis of genetic profiles in XX 2 (recognized child) and the supposed biological parents of deceased defendant XY (grandmother – mother of the defendant and grandfather – father of the defendant) exclude paternity towards XX 2, too.

Table 1. Polymorphic autosomal sequences in mother, disputed child and parents of the deceased alleged father done in other laboratory.

Marker	Mother XX	Child XX1	Genetic profile of defendant XY (possible DNA fragments from the alleged father of the disputed child) generated based on his parents' examinations.	Mother of alleged father XY (grandmother)	Father of alleged father XY (grandfather)
D8S1179	13/13	13/13	12/14 lub 13/14	14/14	12/13
D7S820	10/12	10/12	10/11 lub 10/12 lub 11/12 lub 12/12	10/12	11/12
TH01	9/9	9/9	7/8 lub 7/9 lub 8/9.3 lub 9/9.3	8/9	7/9.3
D13S317	8/12	8/9	9/12 lub 9/14 lub 11/12 lub 11/14	9/11	12/14
D16S539	11/12	11/12	9/9 lub 9/12	9/12	9/9
D19S433	13/14	12/14	12/12 lub 12/15 lub 15/15	12/15	12/15
VWA	16/18	16/17	14/16 lub 14/17 lub 15/16 lub 15/17	16/17	14/15
TPOX	8/10	8/10	8/10 lub 8/11 lub 10/12 lub 11/12	8/12	10/11
D18S51	13/14	14/14	12/14 lub 12/19 lub 14/15 lub 15/19	14/19	12/15
FGA	20/25	20/21	21/23 lub 21/25 lub 22/23 lub 22/25	21/22	23/25
D21S11	29/30	29/**30.2**	28/30 lub 30/30	28/30	30/30
CSF1PO	10/11	**10**/**11**	9/12 lub 9/13 lub 12/14 lub 13/14	9/14	12/13
D3S1358	17/17	**15**/17	14/17 lub 16/17	14/16	17/17
D2S1338	19/26	**17**/19	21/22 lub 21/23	22/23	21/21
D5S818	11/12	**9**/12	11/12 lub 12/13	12/12	11/13

Alleles which are in child XX1 but not in the alleged father are underlined and in bold.

Table 2. Polymorphic autosomal sequences in mother, disputed child and sister of the disputed child in other laboratory.

Marker	Mother XX	Child XX1	Genetic profile of defendant XY (possible DNA fragments from the alleged father of the disputed child) generated based on his parents' examinations.	Child XX2 – sister of child XX1
D8S1179	13/13	13/13	12/14 lub 13/14	13/14
D7S820	10/12	10/12	10/11 lub 10/12 lub 11/12 lub 12/12	10/12
TH01	9/9	9/9	7/8 lub 7/9 lub 8/9.3 lub 9/9.3	9/9
D13S317	8/12	8/9	9/12 lub 9/14 lub 11/12 lub 11/14	8/9
D16S539	11/12	11/12	9/9 lub 9/12	12/12
D19S433	13/14	12/14	12/12 lub 12/15 lub 15/15	12/13
VWA	16/18	16/17	14/16 lub 14/17 lub 15/16 lub 15/17	18/**18**
TPOX	8/10	8/10	8/10 lub 8/11 lub 10/12 lub 11/12	8/8
D18S51	13/14	14/14	12/14 lub 12/19 lub 14/15 lub 15/19	13/14
FGA	20/25	20/21	21/23 lub 21/25 lub 22/23 lub 22/25	21/25
D21S11	29/30	29/**30.2**	28/30 lub 30/30	29/30
CSF1PO	10/11	**10**/**11**	9/12 lub 9/13 lub 12/14 lub 13/14	9/10
D3S1358	17/17	**15**/17	14/17 lub 16/17	**15**/17
D2S1338	19/26	**17**/19	21/22 lub 21/23	**17**/26
D5S818	11/12	**9**/12	11/12 lub 12/13	12/12

Alleles which are parent in child XX1 and XX2 but not in the alleged father are underlined and in bold.

In Table 3 we have presented the results of genetic examinations of bone fragments from the exhumed deceased alleged father of girls XX 1 and XX 2 as well as biological material collected again from girl XX 1 and her mother which were carried out in the Genetic laboratory in the Department of Forensic Medicine and Toxicology, Medical University of Silesia, Katowice. Genetic profiles of the parents of the deceased defendant (children's grandparents) have been compared. Analysis of the obtained results towards blood relationship excluded explicitly paternity of the children's grandfather to their alleged father. This conclusion invalidated the previous opinion questioned by the child's mother.

DNA polymorphism analysis carried out in the Department of Forensic Medicine allowed for issuing the following opinion: " ….. assuming that child XX 1 is XX's daughter and that none of these persons did not have blood transfusion in the last three months as well as bone marrow transplantation before the examination, the opinion is issued: … analysis of DNA polymorphism did not give the rise to the exclusion of paternity of deceased defendant XY to child XX 1. Defendant XY with probability boarding on certainly is the father of child XX 1. The value of paternity probability is 99, 9999999995325628%". Additionally, the value of paternity probability calculated for child XX 2 was

Table 3. Polymorphic autosomal sequences done based on the examination of biological material from the exhumed defendant in the laboratory in the Department of Forensic Medicine and Toxicology, Medical University of Silesia, Katowice.

Marker	Mother XX	Child XX1	Child XX2 – sister of child XX1	Defendant – alleged father XY	Mother of the defendant (grandmother)	Father of defendant (grandfather)
D8S1179	13/13	13/13	13/14	13/14	14/14	12/13
D7S820	10/12	10/12	10/12	10/12	10/12	11/12
TH01	9/9	9/9	9/9	9/9.3	8/9	7/9.3
D13S317	8/12	8/9	8/9	9/**9**	9/11	12/14
D16S539	11/12	11/12	12/12	**11**/12	9/12	9/9
D19S433	13/14	12/14	12/13	12/12	12/15	12/15
VWA	16/18	16/17	18/18	17/**18**	16/17	14/15
TPOX	8/10	8/10	8/8	8/10	8/12	10/11
D18S51	13/14	14/14	13/14	14/**16**	14/19	12/15
FGA	20/25	20/21	21/25	21/22 no common trait with father	21/22	23/25
D21S11	29/30	29/30.2	29/30	30/**30.2**	28/30	30/30
CSF1PO	10/11	10/11	9/10	9/**11**	9/14	12/13
D2S1338	19/26	17/19	17/26	**17**/23	22/23	21/21
D5S818	11/12	9/12	12/12	**9**/12	12/12	11/13
D3S1358	17/17	15/17	15/17	**15**/16	14/16	17/17
D10S1248	14/16	16/16	non examined	14/16	non examined	non examined
D1S1656	13/17.3	14/17.3	non examined	14/16	non examined	non examined
D22S1045	11/16	15/16	non examined	15/15	non examined	non examined
D2S441	11/11.3	11/11.3	non examined	11/14	non examined	non examined
D12S391	17/17.3	17.3/23	non examined	18/23	non examined	non examined
SE33	23.2/27.2	18/27.2	non examined	17/18	non examined	non examined
Penta E	5/11	5/14	non examined	14/16	non examined	non examined
Penta D	11/13	13/13	non examined	12/13	non examined	non examined

Alleles which are present in the defendant but not in his father (grandfather of child XX1). There is no common trait in *locus* FGA.

Profiles of gandmother, grandfather and child XX2 were determined in other laboratory.

Paternity probability for a trio: mother – child XX1 and alleged father XY was **99.9999999995325628%** in duo: father – child XX1 99.9999997769%.

Probability that child XX1 and XX2 are siblings (15 STR loci) **99.76897399%** and probability of half-siblings **99.61905841%**.

99,99676600486068% in duo: father – child and 99,999966414608% in trio: mother – child – father. The value of siblings likelihood for XX 1 and XX 2 was 99, 76897399% and the value of half – siblings likelihood was 99,61905841%.

CASE 2

The next opinion issued by other laboratory concerned identification of unknown person NN and so determination of blood relationship based on the investigation of personal belongings of missing person NN and her alleged daughter.

The results of the examination concerning only 15 STR *loci* have been shown in Table 4. With no calculation of the value of maternity probability the following conclusions were reached: " ….. sent in to evidence (personal belongings of NN person) revealed DNA from a woman who had already had a broad genetic profile determined. The profile of the woman shows incompatibility with the profile in alleged daughter ZZ, so the person whose the profile was determined may be the mother of ZZ …."

According to the guidelines of Forensic Hemogenetics of Polish Association of Forensic Medicine and Criminology relatedness probability a posteriori should be 99,9999% [2]. In the cited paper a clear tendency to a constant increase in the number of the examined markers was mentioned. The value of maternity probability was only 99,039121787621%. After marking 15 STR loci in cases where there is biological material only from 2 individuals (in the studied case biological material from: alleged mother and alleged child was examined), the value of relatedness probability is rarely 99,9999%. A lower value does not give us the right to issue an uncompromising opinion.

Table 4. Polymorphic autosomal sequences in cases of identification of person NN done in other laboratory.

Marker	Child ZZ	Genetic profile of the alleged mother established bases on the personal belongings investigation
D10S1248	13/14	13/14
D1S1656	16/17.3	13/17.3
VWA	17/17	17/18
D16S539	11/12	11/11
D2S1338	17/20	17/23
D8S1179	13/14	13/15
D21S11	28/29	29/30.2
D18S51	14/15	14/18
D22S1045	15/15	15/16
D19S433	14/14.2	14/14
TH01	9.3/9.3	6/9.3
FGA	20/21	21/23
D2S441	11/14	11/11
D3S1358	15/16	15/18
D12S391	18/21	17/18

The value of maternity probability is **99.0391217876**.

CASE 3

The biggest group makes cases where analysis was carried out in laboratories other than forensic. In these cases the analysis was done again in our laboratory. In a few cases with paternity exclusion where one of the parties questioned the opinion, the new examination allowed for confirming the paternity probability. In each of these cases, the examination of a sample or samples of biological material collected in our Department showed convergences with the results obtained for the biological material delivered by mail to the laboratory verifying the questioned opinion. In two cases a biological material sample from the alleged father was delivered by mail. In 1 case there were samples of biological material from a child and alleged father. In other cases biological samples from: mother, child and alleged father were delivered. Sampling without participation of the parties whose should be the witnesses of the proceedings finally resulted in a false opinion. In each of these cases the new analysis carried out in our Department allowed for issuing a quite different opinion, the opinion where paternity was confirmed with the probability bordering on certainly. Analyses in disputed paternity cases where proceedings concerning sampling were not restricted do not allow for claiming whether the sent samples come from the individuals mentioned in the court form or not. The presence of the witness while sampling was not substantiated in the protocol.

DISCUSSION

In the course of guardianship and family proceedings there is a possibility to question opinions concerning the establishment or denial of paternity as well as establishment of fatherhood futility. The opinions mentioned in the report can be questioned by mother on her child's behalf, an underage or adult child, a defendant father, the mother's husband or a prosecutor.

The person questioning the opinion should level essential charges concerning possible mistakes or errors. Sampling contrary to proceedings is one of the most frequent mistakes. Differences and convergences found when the new examinations were done always resulted from the fact that the parties did not participate in sampling proceedings. Samples of biological material

were collected in different places, at different time or the parties collected their biological material themselves and then sent it to the laboratory. In each of these cases the opinion should raise doubts. It should also be mentioned that there is no information on possible blood transfusion or bone marrow transplantation in these cases.

Expert's knowledge about bone marrow transplantation gives a possibility to collect alternative biological material and carry out a simultaneous DNA examination in order to exclude possible mistakes. It seems essential to establish regulations concerning sampling for examinations in disputed paternity cases including information about blood transfusion, bone marrow transplantation, close blood relationship of the parties as well as possible in vitro fertilization.

To question a forensic opinion and level essential charges, at least basic knowledge in this field is needed so that the charges to be legitimate. In the case where sampling was at variance with the proceedings questioning the opinion seems to be unquestionable. To find mistakes or errors in opinions from Table 1, 2 or 3 specialist knowledge is needed.

In case 1 the wrong point was proposed which resulted in issuing a false opinion with paternity exclusion. The genetic profile of the defendant father of the child was determined pursuant to the grandparents' genetic profiles. In the opinion it was explicitly but erroneously concluded that the deceased is not the father of the child. Based on the grandparents' profiles we can only conclude that the child is not their granddaughter. In another opinion paternity of the deceased towards the disputed daughter was erroneously excluded based on the generated profile. Moreover, another daughter recognized by the deceased before he died was excluded. Having the genetic profiles of these two girls it was necessary to calculate sibling likelihood and broaden genetic examination in a range of autosomal STR *loci*. To confirm that the girls have the same father it was necessary to analyze STR *loci* on chromosome X.

In case 2 based on DNA examinations it was concluded that there is a close blood relationship between the alleged daughter and private evidence from unknown person NN. To study DNA polymorphism 15 STR *loci* were only examined. Statistical analysis was not carried out to decide about blood relationship. The value of maternity probability we calculated appeared to be too low to confirm a close relationship between the examined evidence and the alleged daughter [2, 7]. The question is whether the opinion with the confirmed maternity is true in the case of such a low value of maternity probability. A similar problem happens when the child's mother is not examined [6].

We have decided to mention the case of disputed paternity [9] we had already published, which should warn forensic experts against mistakes or errors they can make while issuing opinions about blood relationship. In the mentioned case genetic analysis allowed for getting a high probability of paternity (99,9999%) determining 10 STR *loci*. None exclusions were found. When the examination was expanded it appeared that the defendant man is not the father of the disputed child (there were 5 exclusions when 26 STR *loci* were determined). Detailed analysis of genetic profiles in the child and the excluded man suggested a close blood relationship between the man and the child. The case shows that each opinion concerning establishment of blood relationship should be based on analysis of many STR *loci*. Moreover, the issue of in vitro fertilization (semen from an unknown man or egg cell from a unknown woman) should be taken into consideration when blood relationship is to establish.

CONCLUSIONS

- Opinions issuing in cases of disputed paternity or blood relationship establishment should have clear and standing proceedings concerning sampling, the number of STR *loci* to determine and statistical calculations.
- Each opinion should include a wide analysis of many STR *loci* as well as be expanded in non – typical cases where the defendant man is not examined but his close relatives (grandparents, siblings etc.) or when there is a possibility of blood relationship between the defendant fathers.
- Issuing opinions should be always based on statistical calculations and the right conclusion about blood relationship.

Source of tables: authors

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