

Up to Date Investigation Methods in Hereditary Cases

Györgyi Bujdosó and Péter Sótonyi

Institute of Forensic Medicine of Semmelweis University Budapest and Research Unit Sponsored by the Hungarian Academy of Science, 1091 Budapest, Hungary

KEY WORDS Hereditary; chromosome; DNA; expertism.

ABSTRACT The authors about 30 years ago in the Institute of Forensic Medicine of Semmelweis University were given the task of assisting the Court to clear up the natural child's legal status with evidences based on natural science. In the present paper, a few special cases are demonstrated with a decisive chromosome or DNA test result. The chromosomes are obtained from the blood following a 72-hour culture. The used method were: G-, and C banding. The uses of DNA polymorphism were the following: HUMTH01, HUMvWF, S33, D21S11, D18S51, FGA, D20S85, D3S1359, D5S2360, AMG. It is a good thing that there is an increasing number of males, who would like to know out of court, whether the children born are their descendants or not. But in their practice it occurred on request by the police, that the mother's identity was questionable due to the possibility of exchange of babies in hospitals. Due to the results of chromosome tests in some cases gave support to genetic counsel as well as in pre-natal diagnoses too.

INTRODUCTION

In human societies it is natural that a part of the population is born out of wedlock. This fact has been reflected by the various societies in the different historical ages. The various civilisations have applied different regulation methods. In the present Hungarian society the women having children out of wedlock do not meet with condemnation, and these children are protected from all types of discrimination. However, the taking over of a natural child is rarely the consequence of a premeditated decision. For the most part it is the acceptance of an unexpected pregnancy.

About 30 years ago in the Institute of Forensic Medicine of Semmelweis University I was given the task of assisting the Court to clear up the natural child's legal status with evidences based on natural science.

It is a good thing that there is an increasing number of males, who would like to know out of court, whether the child/children born out of or even in wedlock is/are their descendants or not. There are adults who want to find their ge-

netic fathers. Moreover, several men, having the intention of putting matters straight in the autumn of their life, have asked for my assistance to clear up the genetic status of children originated from relations 20,30 or 50 years ago. In one or two cases the client has deposited the result of his DNA test at a lawyer or a notary and imposed a condition that the inheritance can only be accrued following the child's positive DNA test.

Following an entrepreneur's sudden death a mother's lawyer has come up with the claim that the man should not be buried until the clearing up of the child's origin born out of wedlock.

In a family meeting a man and woman found it surprising that they resemble each other very nearly. The woman lived in family. The man was brought up by her single mother and did not know his father. The possibility of a common father did emerge. They had a settled life, however, both of them felt lonely and would have been glad to be consanguineous sister/brother. Therefore, they asked for chromosome analysis to clear up their origin. The result seemed to confirm their suspicion.

A case in which I gave an expert opinion about 25 years ago can demonstrate the importance of identity for a child. I could verify the genetic paternity of the male concerned by chromosome analysis first time in international relations for the seven-year old boy, the mother and the putative father. It was of scientific significance at that time. No news was received from them for a long time. Then the former boy as a grown up person has visited me before his marriage and introduced his fiancée. Although his father has ignored him. He knows his origin due to the expert opinion, and this is important for his future family.

In the present paper a few special cases are demonstrated with a decisive chromosome or DNA test result.

MATERIAL AND METHOD

Chromosome Analysis

Materials for chromosome analysis can be obtained from every cell having nucleus. Therefore, any cell of the organism is suitable including blood cell, fibroblast, bone marrow, amniotic cell, hair bulb, etc.

In paternity investigations the examination material is obtained by an one-off and simple taking of blood. The chromosomes are obtained from the blood taken under sterile circumstances following a 72-hour culture.

The examination of chromosome polymorphism has become significant in the past 25 years. Since the introduction of various band techniques /G, Q, C, R/ in the '70s the normal variants of chromosomes can be identified, systematised or determined much easier (Caspersson et al. 1970; Summer et al. 1971; Vogel 1977; Budowle 1991).

In G-band staining following a 72-hour culture the mitotic chromosomes, stopped in metaphases, are examined by light microscope in normal lighting. Following a proper pre-treat-

ment /e.g. digestion/ the chromosomes stained by Giemsa show dark or light bands.

The G-band staining is seldom used in paternity tests, however, it is applied if a change in the morphology of somatic chromosomes is detected at the child or the putative father. The result can be conclusive for paternity (Gebauer et al. 1988).

In my 25-year practice the above change could be observed only in five cases. In the first case we were the first to prove paternity world-wide by joint balanced translocation. [t(2term.q-; 13 q term+)] (Bujdosó 1972; Bujdosó and Somogyi 1975).

C-band staining /centromer staining/ is frequently used in practice. Following a pre-treatment different from the one used in G-band staining /e.g. hydrolysis, heat/ the chromosomes are stained by Giemsa. The stain molecules bind to a different site and stain only the centromere /middle/, centromeric heterochromatin, of the chromosomes concerned. Chromosomes 1, 9, 16 and Chromosome Y - if it is available - are taken out of the 46 Chromosomes. The centres of chromosomes /centromeres/, the variations in their

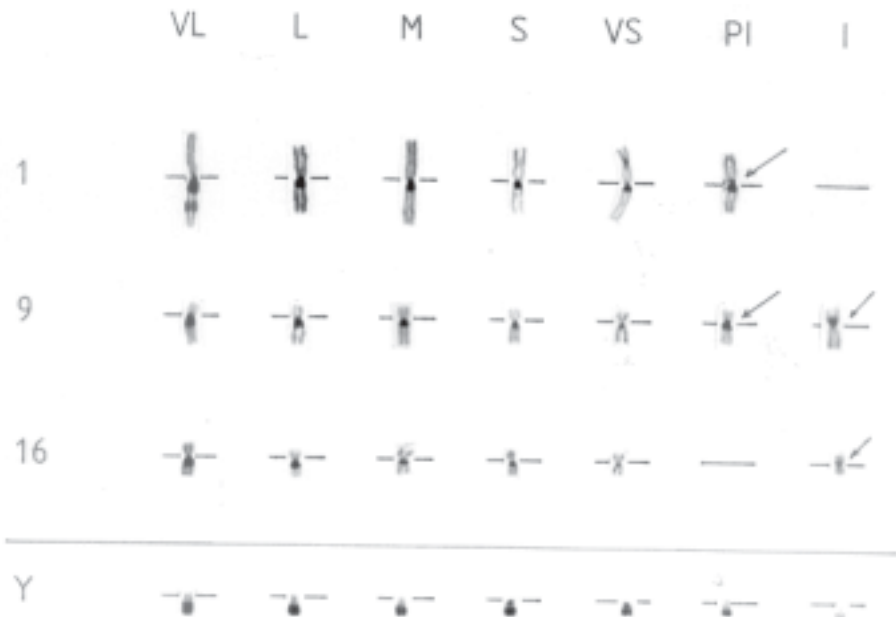


Fig. 1. C-band polymorphism

length, are investigated (Craig-Holmes and Shaw 1971; Baliček et al. 1978).

If a partial or total pericentric inversion is found on the centromer of a chromosome concerned it is a very valuable and positive feature for the probability of genetic paternity for the male in question (Wahran et al. 1972) (Fig. 1).

Q-band staining is another frequently used method in which the mildly, moderately or highly fluorescent or non-fluorescent bands characteristic to the chromosomes concerned /Chromosomes 3, 4, 13, 14, 15, 21, 22 or Y/ are investigated with ultra violet lightning following a pre-treatment by fluorescent stains /atebrin or quenacrin/ (Schnedl 1971). All chromosomes are matched by these methods and evaluated according to Mendel's Law. The descendant's genome is inherited in parts from his/her mother and father. /Normal number of chromosomes: female = 46 XX, male = 46 XY/. In special /male/ cases the characteristic Chromosome Y can be decisive for the elaboration of expert opinion (Bender and Gooch 1961) (Fig. 2).

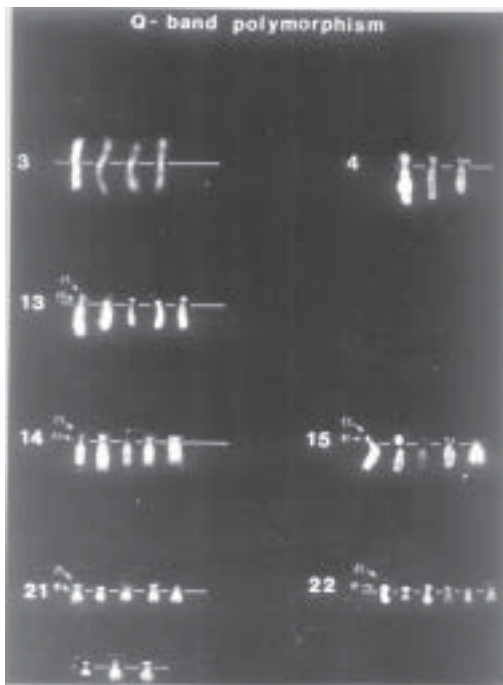


Fig. 2. Q-band polymorphism

DNA Analyses

Materials for DNA test can be obtained from every cell having nucleus in the same way as for chromosome analysis. DNA tests for the identification of paternity are performed from peripheral blood taken from living persons. The development of molecular genetics and the testing of DNA band samples have rendered the identification possible similar to fingerprinting. This method is known as DNA fingerprint.

The method was described first by Jeffreys in 1985, and he recommended its introduction in paternity testing. By the discovery of increasing number of enzymes the several meters long DNA molecules consisting of billions pairs of basis can be split into parts with a size of few thousands basis pairs (Nathans 1979). These so-called micro-satellites can be investigated in 14 sites of chromosomes by Jeffrey's DNA gene probe (Jeffreys 1987). The DNA molecules fragmented by special enzymes and split by electrophoresis can be separated by gene electrophoresis on the basis of their various length and molecular weight (Southern 1975).

The heterozygotes and homozygotes can be distinguished by the stripes obtained by electrophoresis /two stripes or one stripe/. The multiloci contain several unknown stripes /MLS/ (Lubjuhn et al. 1996).

A child inherits half of his/her bands from the mother as the genetic information stored in human cells is half-and-half of maternal and paternal origin. Therefore, the stripes not corresponding to maternal ones are certainly of paternal origin.

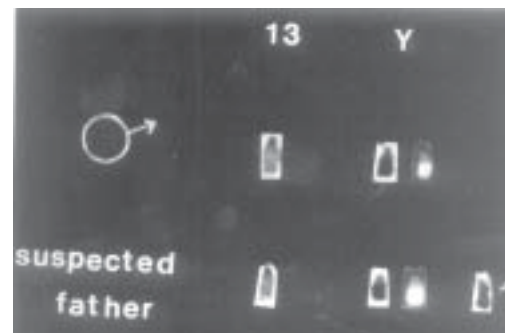


Fig. 3. Very large Y-chromosomes (Q-band)

As the alleles show various frequency in population level and have mutations several systems with low mutation rate are used in DNA tests (Budowle et al. 1991; Morling and Hansen 1993, 1997; Lins et al. 1996).

RESULTS

Under normal circumstances the Chromosome Y is found in the range of small acrocentric chromosomes /21, 22/. In some cases the biological father could be determined on the basis of size and fluorescence of Chromosome

Y. In a case the size of Chromosome Y was the same as of the largest acrocentric Chromosome 13. The same observation was found at the putative father. The genetic paternity was proved (Fig. 3).

In another instance Chromosome Y was very small and non-fluorescence both at the child and the alleged father. The issue of paternity was solved regarding the expert opinion (Fig. 4).

The advantage of chromosome analysis is that the chromosome similarly to blood group is constant and characteristic for the individual in the whole life. The chromosome analysis can

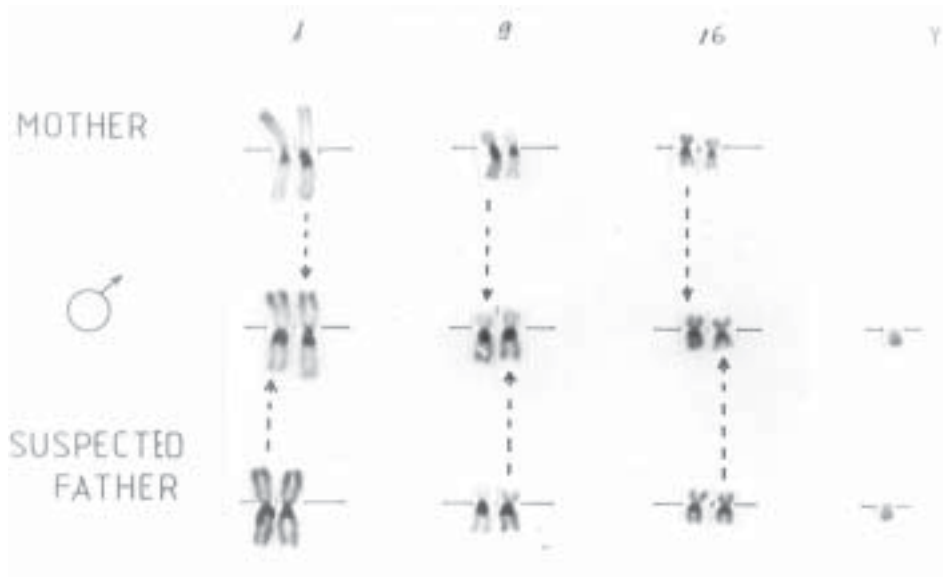


Fig. 4. Very small Y-chromosomes too. (C-band)

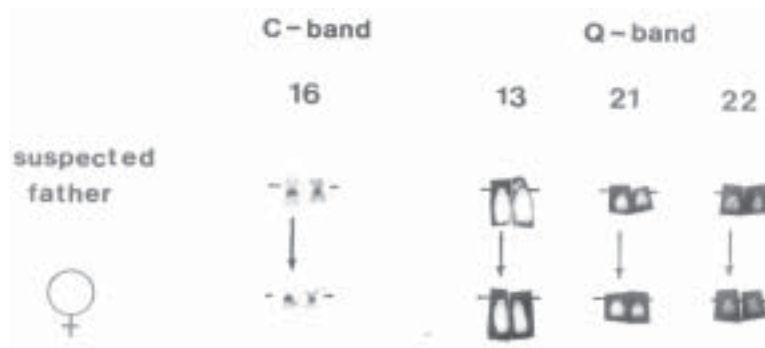


Fig. 5. Father and daughter

give direct proof for the origin even in fetal life (Koske-Westphal and Passarge, 1977; Friedrich-Schöler et al. 1979; Bujdosó et al. 1984; Bujdosó 1985; Szilvassy 1990; Csiky et al. 1997; Bujdosó and Arnold 1999).

There are cases in which the mother's chromosome analysis cannot be performed. In one of our cases the mother was a heavy drug user, therefore, blood could not be taken from her. However, the genetic paternity was rendered probable by the characteristic chromosome polymorphisms (Fig. 5).

The chromosome analysis is suitable in lawsuits or out of court procedures in which the father or mother died in the meantime, and only few family members /the ascendants, the collaterals/ can be included in the investigation. This method is effective also in the increasing number of inheritance proceedings.

In some cases the alleged father could not be examined as he had died. Therefore, the expert

opinion regarding his paternity was given following the testing of his ascendants or collaterals. In one of our cases the mother suffered a traffic accident, thus her parents were tested. The result of the chromosome analysis verified the paternity of her common-law husband in Paris. The male's parents were happy to have the child from a Hungarian mother in their family (Fig. 6).

In chromosome analysis a disease can be detected, and in some instances this information can be forwarded to the persons concerned in time. I would highlight the case in which the putative father denied his paternity regarding the girl born in wedlock. There was a pending suit to overcome the presumption of paternity, however, the concordant results of blood group test and anthropological opinion did render his paternity probable. The chromosome analysis put an end to the case and confirmed that the plaintiff is the girl's genetic father as in addition to

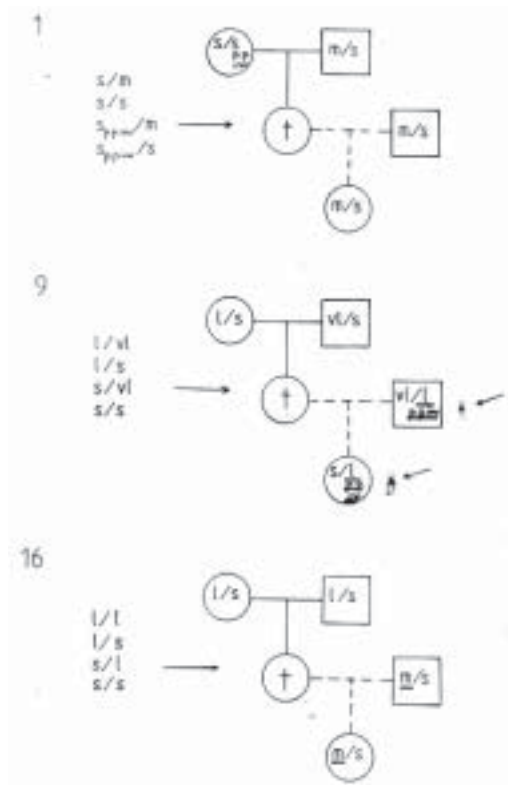


Fig. 6. The mother died. (family tree with C-band)

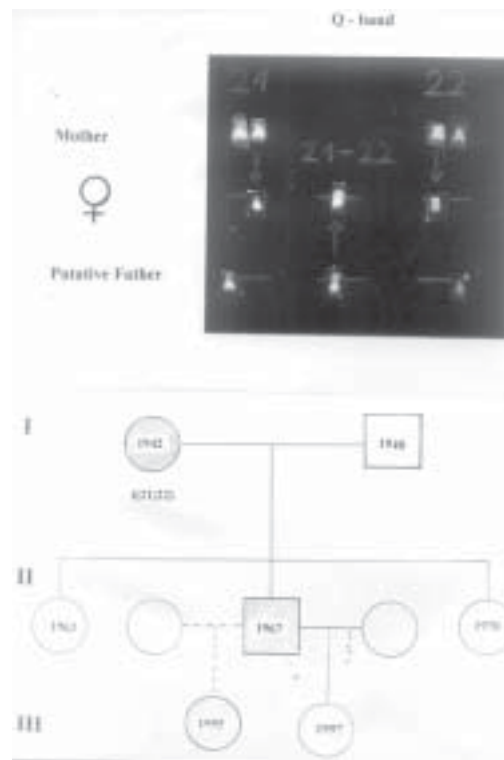


Fig. 7. t (21,22) with Q-band ad family tree



Fig. 8. Same like 7 with G-band

the normal variants the translocations 21 and 22 were found both at the child and the plaintiff. Due to these translocations the male's genetic paternity regarding the minor was verified. The parties concerned did realise that this fact demands genetic counselling. Therefore, performance of chromosome analysis for the other

family members were requested. The change seemed to be stable as the male inherited it from his mother, however, he did not transmit it to her daughter born in his second marriage (Fig. 7, 8).

The chromosome analyses were performed for the identification of the mother upon the request of the Scientific Council of Health and the decision of the police. It was definite in every case that woman with the debated maternity was the mother of the child concerned. In the police proceedings the child was born at home with multiple developmental anomaly and taken to hospital later. The possibility of the child's change in the hospital emerged. The chromosome analysis definitely proved that the child was not changed in the hospital concerned. The maternity was proved even by the DNA test performed later. The cause of the multiple developmental anomaly could be that the procreator of the child was highly probable the mother's close relative (Fig. 9).

Earlier we have had similar cases in higher number in which the father had sexual intercourse with her daughter, and the child originated from this relation. In the past 30 years we examined only one and healthy new-born who originated from a fraternal relation.

Examples of DNA Polymorphism Examinations

Recently the following polymorphisms are used from the available ones: HUMTH01, HUMvWF, S33, D21S11, D18S51, FGA, D20S85, D3S1359, D5S2360, AMG.

Our practice is demonstrated in the following.

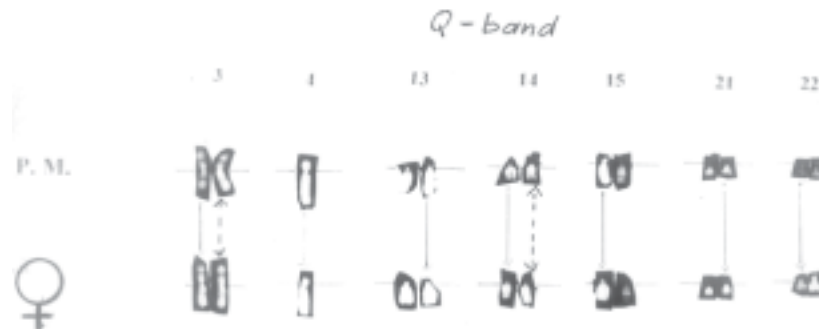


Fig. 9. Possible mother and daughter with Q-band

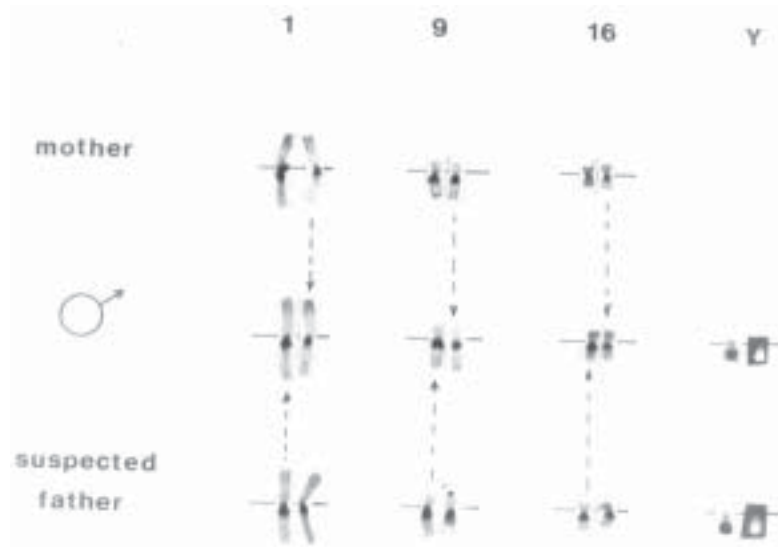


Fig. 10. C-band investigated on 1, 9, 16, Y chromosomes + Y chromosome Q-band too within a family

1. A 35-year old man has requested for an investigation publicly to clear up his paternity regarding the 6-year-old boy born from a mother ten years older than our client. Even the result of chromosome analysis was “highly probable” for his paternity including rare polymorphisms and a long Chromosome Y (Fig. 10).

In addition to chromosome analysis the DNA polymorphism was also tested (Table 1).

One common allele at the least could be detected in the investigated DNA system regarding the 6-year-old-boy and the putative father. The value of Essen-Möller probability /EM/ (Hummel 1979) was 99.9999 per cent. This means that the genetic paternity of the alleged father is “practically proven” for the minor as to find another similar concordance in the every above investigated feature is 1:100 000.

Table 1: The DNA polymorphism test includes the genotypes

	Mother	Child	Putative father
HUMTH01	6/9	6/9	6/6
HUMvWF	15/16	14/15	14/16
D18S51	12/18	12/15	15/15
FGA	22/23	22/23	23/23
D5S2360	6/7	6/11.1	6/11.1
D3S139	12/13	3/13	3/3
D21S11	45.2/48	45.2/45.2	45.2/48
SE33	20/27.2	20/23.2	23.2/31.2

2. In this case the mother could not be tested. The 41-year-old man had already owned the 10-year-old boy, however, he wanted to have evidence for his fatherhood within a private request. The outcome of the chromosome analysis definitely rendered the genetic fatherhood of the man concerned probable (Fig. 11).

One common allele at the least could be detected in every investigated DNA system regarding the 10-year-old-boy and the alleged father.

The test of DNA polymorphism performed by 7 DNA markers of significant information practically proved (99.999%) the paternity of the male concerned (Table 2).

In the above demonstrated cases the results of DNA tests confirmed the paternity.

In the following case the chromosome analysis was performed upon the court’s order to clear up the origin of two children. The former

Table 2: The DNA tests include the following DNA systems

	Child	Putative father
HUMTH01	7/7	7/8
D18S51	13/14	14/17
FGA	19/20	20/22
D6S965	13/14	13/19
D3S1359	3/16	15/16
D5S2360	15.1/18	15.1/20
SE33	17/30.2	20/30.2

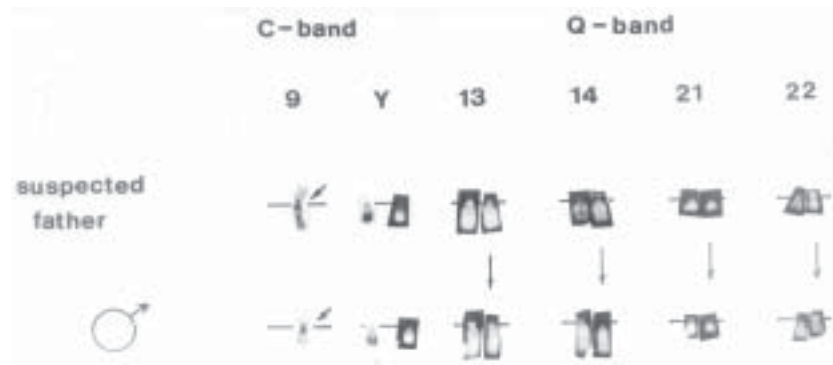


Fig. 11. Investigation without mother

husband as plaintiff has stated:” I would own the children but my former wife has told me that they are not mine.” The mother said that they were divorced, however, both of them wanted their children. She did not have sexual intercourse with another person at the time in question. The result of the blood group test excluded the plaintiff’s genetic paternity regarding the girl. The results of the chromosome analysis confirmed the exclusion for the girl and excluded the plaintiff’s genetic paternity regarding the boy too.

Then the following DNA polymorphism tests were performed demonstrating an outcome that

the plaintiff’s genetic paternity was excluded at both children (Table 3).

Table 3: The DNA polymorphism tests show the plaintiff’s genetic paternity excluded at both children

	Mother	Minor K.B.	Minor A.B.	Putative father
HUMTH01	6/6	6/8 K!	6/9.3	9.3/9.3
FGA	5/7	7/11 K!	7/13 K!	5/7
D20S85	2/6	6/8	6/7	7/8
D3S1359	6/12	3/12 K!	12/13 K!	10/15
D21S11	45.2/46.2	45.2/47.2	46.2/47 K!	47.2/47.2
SE 33	64.2/66.2	65.2/66.2 K!	65.2/66.2 K!	53/61.2

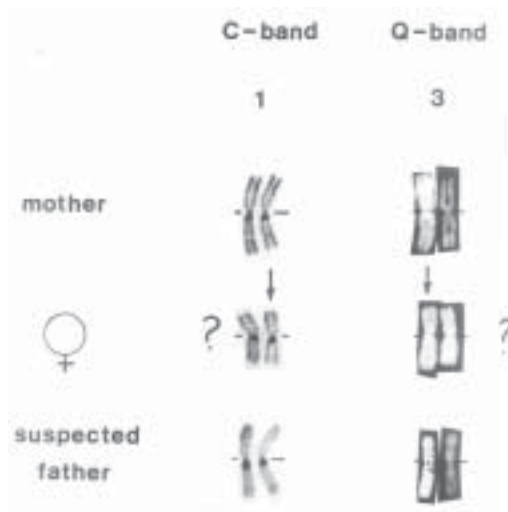


Fig. 12. Exclusion. Chromosome no. 1 with C, chromosome no. 3 with Q-banding in the daughter

As a conclusion it can be established that the various test systems /blood group - chromosome; DNA - chromosome/ should be combined in the investigation of origin.

The development of cytogenetical methods, primarily the fluorescent in situ hybridisation / FISH/ technique, can present a basis for DNA tests. The in situ hybridisation can assist the mounting of cytological specimens that can be examined under microscope as simple chromosome analysis. The fluorescent signal gives exact information about the place of the probe on the chromosome (Sacchetti et al. 1999).

The biological father can be identified by the above methods now. However, “the indisputableness of the true father and the perfect certainty of origin are rather the outcome of a common confidence even in the best family relations than based on scientific facts. And this is not altered by the fact the fathers believed to be

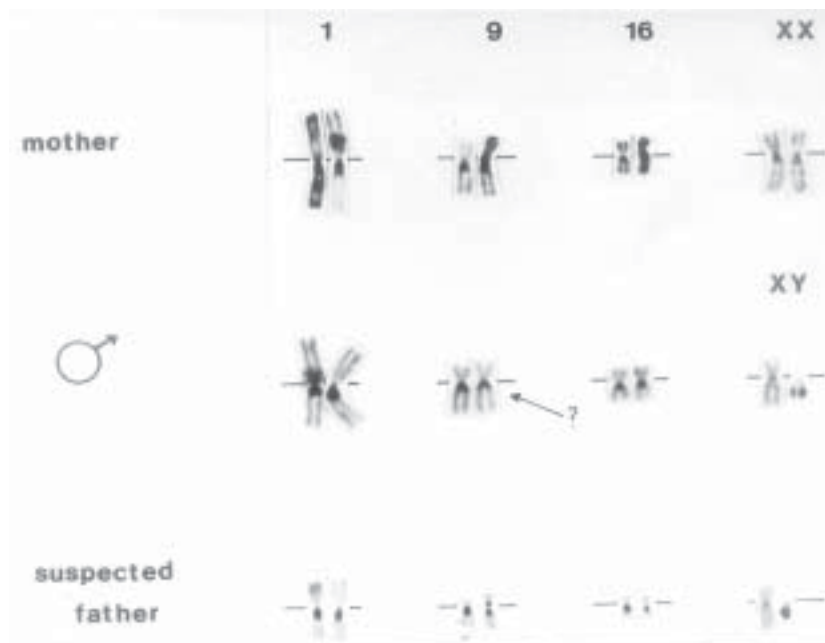


Fig. 13. Exclusion with C-banding in the boy

fathers are the true fathers in majority.” The scientific evidences have gained an increasingly important role in lawsuits.

REFERENCES

- Baliček P, Zizka J, Skalská H 1978. Variability and Familial Transmission of Constitutive Heterochromatin of Human Chromosomes Evaluated by the Method of Linear Measurement. *Hum Genet*, **42**: 257-265.
- Bender MA, Gooch PO 1961. An unusually long human Y chromosome. *Lancet*, **II**: 463-464.
- Budowle B et al 1991. *Am J Hum Genet*, **48**: 137-144.
- Bujdosó Gy 1972. A₂D transzlokációval bizonyított származásmegállapítás. *Biol Közl*, **XX**: 81-86.
- Bujdosó Gy 1985. *X vagy Y, apaságvizsgálat, igazságszolgáltatás*. Budapest: Medicina Könyvkiadó.
- Bujdosó Gy, Somogyi E 1975. Chromosomenuntersuchung in Prozessen zur Vaterschaftsfeststellung. *Acta medicinae legalis et socialis*, **XXIV**: 599-604.
- Bujdosó Gy, Somogyi E, Bergou V 1984. The use of chromosomes in paternity actions. *Forensic Science*, **25**: 35-43.
- Bujdosó G, Arnold J 1999. *Genetischer Atlas der Anthropologie und Erbbiologie*. GENLAB. Weiss und Hameier GmbH Co Ludwigshafen.
- Caspersson T, Zech L, Johansson C 1970. Quinacrine mustard fluorescence of human chromosomes. *Exp Cell Res*, **61**: 474-475.
- Craig-Holmes AP, Shaw M 1971. Polymorphism of human constitutive heterochromatin. *Science*, **174**: 702-704.
- Csiky O, Bujdosó, Györgyi M, Váczy Zsuzsa H 1997. *Apasági perek, apaságvizsgálat*. HVGORAC Budapest.
- Friedrich-Schöler E, Herbich J, Szilvássy J 1979. Zur zytogenetischen Vaterschaftsdiagnose. *Ärztl Lab*, **25**: 299-303.
- Gebauer-HJ, Scheil-HG, Rohrborn-G 1988. Partielle Deletion am kurzen Arm eines Chromosoms 13 als Hinweis auf Vaterschaft im Rahmen eines Gutachtens. *Z-Rechtsmed*, **99**: 249-251.
- Hummel K 1979. *Biostatistische Abstammungsbegutachtung*. Tabellenband I und II. Freiburg, i. Br.
- Jeffreys AJ 1987. Genetic typing and confirmation of parenthood. *Lancet*, 806.
- Jeffreys AJ, Wilson V, Thein SL 1985. Hypervariable “minisatellite” regions in human DNA. *Nature*, **314**: 67-73.
- Jeffreys AJ, Wilson V, Thein SL 1985. Individual-specific “fingerprints” of human DNA. *Nature*, **316**: 76-79.
- Koske-Westphal T, Passarge E 1977. Cytogenetic paternity test in father-daughter incest (case report) author’s transl. *Dtsch Med Wochenschr*, **102(27)**: 999-1000.
- Lins Am, Sprecher Cs, Puers C 1996. Multiplex sets for the amplification of polymorphic short tandem repeat loci-silver stain fluorescence, detection. *Biotechniques*.
- Lubjuhn T et al. 1996. Multilocus fingerprinting and single locus analysis in the great tit for paternity determination. *Electrophoresis*, **17**: 1555-1558.
- Morling E, Hansen H 1993. Paternity testing with VNTR DNA systems. *J Leg Med*, **105**: 189-196.
- Nathans D 1979. Restriction endonucleases, simian virus

- 40 and the new genetics. *Science*, **206**: 903-909.
- Rodewald A 1992. Chromosomale Polymorphismen: geographische und ethnische Variabilität. *Homo*, **43/1**: 72-96.
- Sacchetti L, Caleugno G, Coto I 1999. Efficiency of two different nine-loci short tandem repeat systems for DNA typing purposes (multicolor-Fish). *Clin Chem*.
- Schnedl W 1971. Unterschiedliche Fluoreszenz der beiden homologen Chromosomen Nr. 3. beim Menschen. *Hum Genetik*, **12**: 59.
- Southern EM 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Mol Biol*, **98**: 503-517.
- Summer AT, Evans HJ, Buckland R 1971. New technique for distinguishing between human chromosomes. *Nature New Biology*, **31**: 232.
- Szilvássy J 1990. Die Methoden der medizinisch-biologischen abstammungs- begutachtung und ihr Beweiswert Der. *Österreichische Amtsvormund*, 31-36.
- Vogel W 1977. Chromosomenpolymorphismen und Vaterschaftsbegutachtung. *Ärztl Lab*, **23**: 268
- Wahran J, Atidia J, Goitein R, Cohen T 1972. Pericentric inversions of chromosome 9 in two families. *Cytogenetics*, **11**: 132-144.