Legal Medicine 26 (2017) 18–24

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Paternity testing at the Department of Forensic Medicine of Wroclaw Medical University (Poland)

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ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 4 January 2017
Accepted 23 February 2017
Available online 27 February 2017

Keywords:
Forensic science
Forensic genetics
DNA
Relationship
Paternity testing
Maternity testing

ABSTRACT

Evidentiary materials in court cases comprise the testimony of all relevant parties and witnesses, physical and biological evidence, and the stage of child's development at the moment of birth. In this study, an analysis was made of results obtained between 1966 and 2014 from paternity testing capable of providing biological evidence for court cases. During this time, 16,508 opinions were released, with exclusion of paternity in 4182 cases. The analysis of the total percentage of exclusions in individual years over the entire period of 49 years turned out to be very interesting. Initially, there was a high percentage of exclusions, which most likely resulted from women wrongfully accusing men; however, this figure stabilised in the twenty-first century at less than 20%.

1. Introduction

In Poland, according to Article 61^9 of the Family and Guardianship Code, 'the mother of a child is the woman who gave birth to it' [1]. Proof of motherhood consists of the child's birth certificate, presented in the course of the mother's testimony. According to the law applicable to a child born within matrimony, 'the father of a child born in marriage or before the expiry of 300 days from its termination or cancellation is the mother's husband. This presumption can be overturned by a court denial of paternity.' The father of an illegitimate child is a man who acknowledges it or whose paternity has been established through judicial proceedings. A man who has adopted a child may also be its legal father. Legal paternity does not always equate to biological paternity, which is based on a true blood relationship. A biological father, as a result of procreation, transmits his genes to his child, which can be confirmed through DNA testing [2]. Determining whether a man is the biological father of a child is essential for the good of the child as well as for a wrongfully accused man, since both legitimate and illegitimate children have the right to paternal property and child support. The method of establishing paternity depends on the child's legal situation. In the case of a legitimate child, it is based on presumption of the child's origin from the husband of its mother (art. 62§1 Family and Guardianship Code), whereas an illegitimate child may be acknowledged by its father, either voluntarily or through a court decision (Art. 73§1 Family and Guardianship Code). Establishment of paternity can be accomplished via civil judicial proceedings. According to the Family and Guardianship Code, paternity cases involve issues concerning the establishment of paternity (presumption of a child's origin from the husband of its mother, the acknowledgment of a child by its father, or court establishment of paternity), cases of denial of paternity, and cases of invalid acknowledgement [1]. Evidentiary materials include the testimony of the relevant parties and witnesses as well as physical and biological evidence, which since the 1920s has included the following studies: anthropological, studies of the most likely time of conception (calculation of the conception period), seminological (a study of the alleged father's fertility), and genetic.

The present paper is dedicated to genetic paternity testing now carried out in academic institutions and private laboratories. Two types of such analysis can be differentiated [3]. The first includes judicial tests conducted to meet the needs of justice (courts, offices of the public prosecutor) using strict procedures, including the simultaneous collection of material from the respondent, mother, and child, subject to the prior agreement of the involved parties and the preparation of an appropriate protocol for these activities. The agreement of both legal guardians is essential in cases where the child is underage. The second type of analysis is performed at

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http://dx.doi.org/10.1016/j.legalmed.2017.02.002
1344-6223/

the request of private individuals, using the same procedures. Private laboratories and some research institutes carry out examinations at the request of anonymous individuals without identification or fulfilment of the requirements listed above. Most often this type of analysis is done without the knowledge of the mother and child; the alleged father delivers biological material (e.g. hair, swab from the oral cavity) to the laboratory himself. Very often laboratories perform these services via Internet; in this situation packages for the collection of material, including instructions and biological samples, are sent by mail.

The research methods applied to date in examining blood relationships, including paternity testing, are based on genetic polymorphism, associated with differences between individuals. According to the universal definition, we can speak of DNA polymorphism when at least two alleles can be found in a single locus; in addition, the frequency of an allele appearing rarely in the population should be not less than 1% [4]. Polymorphism can also be based on genetic mutations, which must be present in germ cells for the mutation to be spread throughout the population. For example, mutations in microsatellite systems occur on average once in every 500 generations, but systems occur in which the frequency increases to 1 in 150 generations [4]. Polymorphisms observed in human blood used in testing can be classified into five types of blood groups [5]: red cell antigens; white blood cell antigens (human leukocyte antigens, or HLA); plasma protein blood groups and polymorphisms; isoenzyme polymorphisms; DNA polymorphisms (restriction fragment length polymorphisms, or RFLPs; short tandem repeats, or STRs; single nucleotide polymorphisms, or SNPs). Blood group system antigens, plasma proteins, and enzymes are encoded by genes located on chromosomes in the coding DNA. Serological examinations deal with phenotype analysis, i.e. antigens or enzymes resulting from gene expression. DNA polymorphisms used in forensic genetics generally relate to the non-coding parts of the genes.

The present study is based on my doctoral dissertation 'Paternity testing in the Department of Forensic Medicine at Wroclaw Medical University casuistry, analysis of results and errors, problems and suggestions', Wroclaw Medical University, 2015.

2. Materials and methods

The present paper offers an analysis of the results obtained in paternity testing on the basis of paternity test reports issued during the period from 1966 to 2014 at the Department of Forensic Medicine of Wroclaw Medical University, using various research methods. During this period, the following genetic tests were performed: first haemogenetic (serological, HLA), subsequently DNA polymorphism (RFLP, dot blot, STR, mitochondrial DNA, X and Y sex chromosomes).

In all laboratory techniques used in paternity testing, the basic material was blood. The characteristics of secretions were determined in saliva. In the DNA-test era, in justified cases (e.g. following a blood transfusion, or in cases involving religious beliefs), swabs were taken from the buccal mucosa. If there was no access to this type of material, tissues (e.g. paraffin blocks, bones, teeth, amniotic fluid) served as the source of DNA, as well as objects bearing deposits of genetic material (e.g. toothbrushes, razors, exhumed material). At first, serological examinations were performed exclusively on children three months of age or older, since only some blood group features (e.g. AB0) develop early in the interuterine period; later this age was altered to 6 months in accordance with the 1968 guidelines of the Polish Commission of Forensic Medicine [6]. RFLP or HLA analysis was performed on children aged 6 to 36 months due to the large volume of blood required for the test (approx. 15 ml). In the DNA era there are no age restrictions, in view of the minimal amount of blood necessary for the analysis. Collection of the material to be examined required compliance with strictly defined procedures, in an area adapted for this purpose and involving the participation of qualified personnel. The personal appearance of the parties the respondent (the putative father), mother, and child was essential. In cases where this was impossible, the closest relatives of the absent person (e.g. grandmother, brother, sister, grandfather) were called in as replacements. After confirming the identities of these individuals, an interview was carried out, with the aim of excluding the occurrence of a blood transfusion within the previous 3 months, a bone marrow transplantation involving one of the people being tested, and the possible existence of a twin brother of the respondent. On the basis of this information, a protocol was drawn up.

The collected material was used in paternity testing at the Wroclaw Department of Forensic Medicine. The subject of the analysis in this study was the documentation, including laboratory books, paternity test reports, and correspondence with judiciary entities, created in the years 1966 2014. In light of the different laboratory methodologies used during particular periods, the documentation has been divided into three groups: I (serological tests), II (DNA-RFLP tests), III (DNA-STR tests).

2.1. Serological tests (1966–2003)

In this period, polymorphisms of red blood cells (e.g. ABO, MNSs, Rh factors, Kell, Duffy, Kidd, P), white blood cells (HLA), serum proteins (e.g. Hp Glm, Km, Gc, C3), and isoenzymes (e.g. acid phosphatase ACP, phosmoglucomutase PGM, adenylate kinase AK, esterase D ESD, glyoxalase I GLOI, glutamate-pyruvate transaminase GPT, phosphoglycolate phosphatase PGP) were tested. The laws of blood group inheritance served as the basis for excluding respondents from fatherhood. The probability of exclusion was dependent on the frequency of the blood group system within the population and the range of testing. As a result of expanding the range of serological testing, the aggregate utility of the 16 blood group systems increased to 91%. This meant that 91 of 100 wrongfully accused men were excluded [7]. Apart from exceptional cases with very rare features, these tests were unable to confirm paternity. Therefore they could not fulfil the basic objective that should be aimed at in determining paternity: 'to find the father and to ensure the mother and child of his care' cit. [8].

2.2. DNA-RFLP tests (1991–2005)

The hybridisation-based RFLP method was used to study polymorphisms of minisatellite VNTR (variable number tandem repeats) sequences involving different numbers of repetitions of DNA units with a length of 10 100 bp occurring in the whole genome. Minisatellites which are characterised by a high level of polymorphism, estimated at 6×10^{-6}, whereas the polymorphism of 20 blood group systems studied hitherto, including HLA, was 3×10^{-3} [9] are characterised by great discriminative power; however, the analysis requires a large amount (about 10 50 μg) of high molecular weight DNA [10]. In RFLP analysis, as a result of cutting with restrictive enzymes in specific places and the use of SLP (single-locus polymorphism) or MLP (multi-locus polymorphism) DNA probes, fragments of varying length were obtained. A VNTR pattern is a characteristic feature of each men, with the exception of monozygotic twins possessing an identical genotype. A visual comparison of DNA profiles (pattern bands) was necessary in paternity testing, in which interpretation of the results was based on locating, in the genetic profile of the mother or alleged father, all of the DNA fragments obtained in a child's profile in cases of paternity confirmation or excluding the presence in the child of bands absent in the parents. According to the recommen-