นิพนธ์ต้นฉบับ

DNA profiling: applications in paternity testing and monitoring bone marrow transplantation

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DNA was extracted from 3 μl of whole blood utilizing Chelex 100 resin and used in PCR-based amplification of four genetic loci (D1S80, D4S43, D17S30 and APO B) in order to determine the paternity in 50 families by comparison of alleles between the father, mother and child. The father was not excluded in 40 families. Moreover, the DNA profiling also provided a powerful method for monitoring bone marrow transplantation patients with chronic myeloblastic leukemia (CML).

Key words: *DNA, PCR, CML.*

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ดีเอ็นเอที่สกัดจากเลือด 3 ไมโครลิตรโดยใช้สารเรซิน Chelex 100 สามารถนำมาใช้เพิ่มปริมาณดีเอ็นเอที่ตำแหน่งต่างๆ จำนวน 4 ตำแหน่งได้แก่ D1S80, D4S43, D17S30 และ APO B โดยใช้ปฏิกิริยาลูกโซ่โพลีเมอเรส เพื่อนำมาตรวจสอบความเป็น พ่อ-แม่-ลูก ในครอบครัว จำนวน 50 ครอบครัว จากการศึกษาเปรียบเทียบลักษณะของ alleles ระหว่าง พ่อ-แม่-ลูก ทั้ง 50 ครอบครัว พบว่า 40 ครอบครัว ที่ไม่มีข้อขัดแย้งสำหรับความเป็นพ่อ-แม่-ลูก อย่างแท้จริง นอกจากนั้น ลายพิมพ์ดีเอ็นเอยังเป็นวิธีการที่มีประสิทธิภาพสูง ในการติดตามผลหลังการทำการปลูกถ่ายไขกระดูกในคนไข้ที่ป่วยเป็นโรคมะเร็งเม็ดเลือดขาวอีกด้วย

DNA profiling or DNA fingerprinting is now widely used in many countries. Recently, it has gained increasing interest and importance in Thailand because of the publicity concerning a well-know Buddhist monk who was accused of having had affairs with several women followers and had supposedly fathered a child. In the U.S.A DNA profiling was featured in the trial of football star O.J. Simpson who has been alleged to have brutally stabbed his ex-wife and her boy friend to death in Los Angeles and who supposedly left his own blood at the scene of the crime.

The use of the polymerase chain reaction (PCR) in the amplification of variable number of tandem repeat (VNTR) sequences has been a valuable technique for determining allele frequency distribution,[1-5] in paternity testing[6,7] and in forensic examinations of biological samples.[8-10]

In this paper, we report the results of PCR amplification of DNA in the paternity testing of 50 families.

Materials and Methods

DNA extraction from blood

DNA from 3 µl of whole blood was isolated using the Chelex-100 resin extraction method as described by Walsh et al.[11]

PCR amplification

Amplification was performed in a final volume of 25 µl in a Perkin Elmer thermocycler. The primer sequences for the four genetic loci examined were as follows:

D1S80 : AACTGGCCTCCAAACACTGCC
CTTGTTGGAGATGCACGTGCC
D4S43 : CACAGAGAGCTTAGTGGAGCTT
CACTTCACTGACATCCACATCT
D17S30 : CGAAGAGTGAAGTGCACAGG
CACAGTCTTTATTCTTCAGCG
APO B : AAATGGAAACGGAGAAATTATG
CCTTCTCACTTGGCAAATAC

The cycle temperature and electrophoresis conditions were as described by Kasai et al.[12], Hom et al.[13], Ivey et al.[14] and Boerwinkle et al.[15] for the locus D1S80, D4S43, D17S30 and APO B, respectively.

Results and Discussion

Following agarose gel electrophoresis separation of the amplified DNA products, the sizes of the D1S80, D4S43, D17S30 and APO B alleles, respresenting the number of DNA repeat units, were determined by comparison with standard size markers. By this means, paternity determination was conducted by comparing the alleles of the child with both the mothers and putative father's alleles.

Family 1

A 68 year old man was accused by a 41 year old woman that her 19 year old male off spring was his son since, at the time of the pregnancy, they were living together. He learned from the newspaper that DNA profiling was able to prove paternity; if the claim was true he agreed to

support both mother and child as well as making a will benefiting the young man. The results of amplifying D4S43 and D17S30 loci alleles (Fig. 1) excluded paternity.

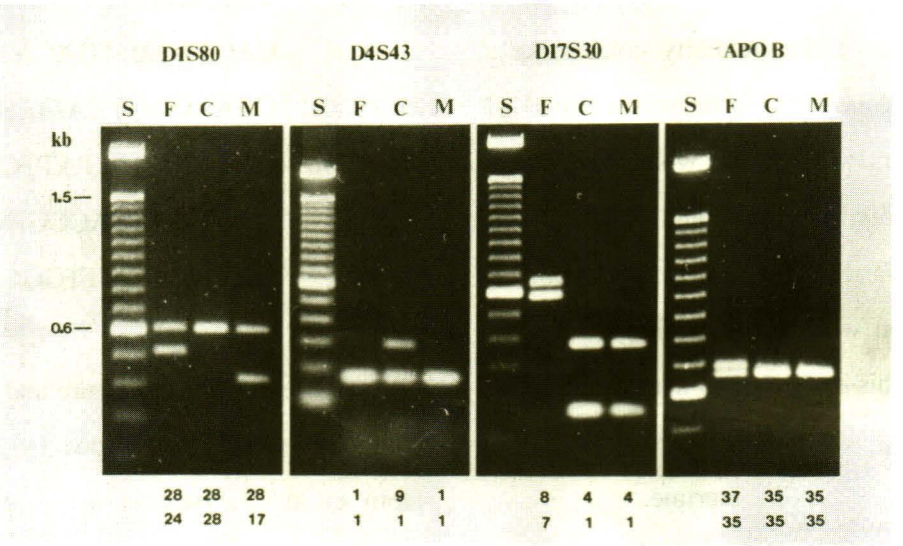

Figure 1. Determination of paternity using DNA amplification of 4 VNTR loci

lane S = 100 bp DNA ladder

lane F = Putative father

lane C = Child

lane M = Mother

Family 2

The Provincial Court of Nonthaburi, Juvenile and Family Sections, requested paternity identification of a 9 year old boy in a claim for legal support. The alleged father was shown to have identical DNA profiles with the child in the 4 genetic loci (Fig. 2).

Family 3

A legally married couple lived separately for 10 years without obtaining a divorce. During this period the wife lived with another man and a boy was born, but legally the father was recognized as being the undivorced husband. The putative father requested the Office of Civil Rights Protection and Legal Aid, Office of the Attorney General, to have DNA testing to prove his claim. The testing showed that he could not be excluded.

Family 4

A 23 year old Dutchman visited Thailand eight times during which he lived with a 20 year old woman from Nakhorn Ratchasima Province. The woman claimed that a five-week old boy was his son. They consulted the Forensic Unit at Ramathibodi Hospital, Mahidol University, which referred the subject for DNA profiling. The results showed that the Dutchman could not be excluded

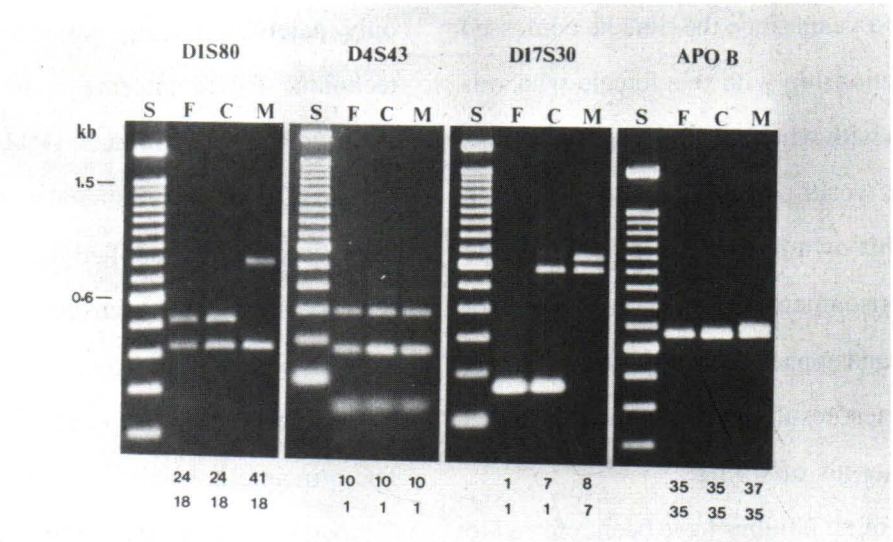

Figure 2. Determination of paternity using DNA amplification of 4 VNTR loci

lane S = 100 bp DNA ladder

lane F = Putative father

lane C = Child

lane M = Mother

as the father of the child and he was happy to bring his son to Holland.

Family 5

A US immigrant couple to Thailand were both type A blood group. Of their three sons, the oldest and the youngest had type A blood, but the middle son had type O blood. They suspected that son has been mistakenly switched for another at the hospital following birth and they consulted the Forensic Unit at Ramathibodi Hospital, Mahidol University, who referred the case to our laboratory. DNA profiling could not exclude the paternity of the father.

Family 6

The Institute of Forensic Medicine of the Police Department requested paternity and maternity testing for a couple and two children of 9 and 12 years of age, as the couple were suspected of bringing illegal immigrants from Vietnam as child labour. The results of DNA profiling revealed that one child was their offspring whereas the other was unrelated.

Family 7

Our laboratory was asked to determine by DNA profiling the paternity of a prison convict and his alleged 19 year old daughter. The prisoner has

been in jail for 3 years since the time he confessed to a sexual relationship with this female who was born to woman with whom he had lived for a short period some 22 years prior. The mother accused him of raping his own daughter and he was given a 16 year imprisonment. His relatives heard of DNA profiling and appealed to the court for testing. DNA profiling test results showed that the alleged daughter was not his offspring.

A total of 50 families have been referred to us for DNA profiling, and the father could not be excluded in 40 cases. These results were confirmed using the M13 multilocus probe.[16,17]

Since DNA profiling studies concern in heritable characteristics that are highly specific to an individual, this has led to the development of not only paternity testing but also the use of this technique for monitoring reconstitution in bone marrow transplantation. DNA profiling using D1S80, D4S43, D17S30 and APO B loci has also been successfully applied to study engraftment after bone marrow transplantation in a patient with chronic myeloid leukemia who wasreferred to our laboratory from Ramathibodi Hospital. The principle is based on observations of cells in the patient before and after marrow transplantation. If DNA samples from the blood of the patient after transplantation showed similar DNA profiling as the donor's at the locus that is an indication that the transplantation was successful. In our study, 7 donor-recipient pairs were referred from allogeneic bone marrow transplant. Figure 3

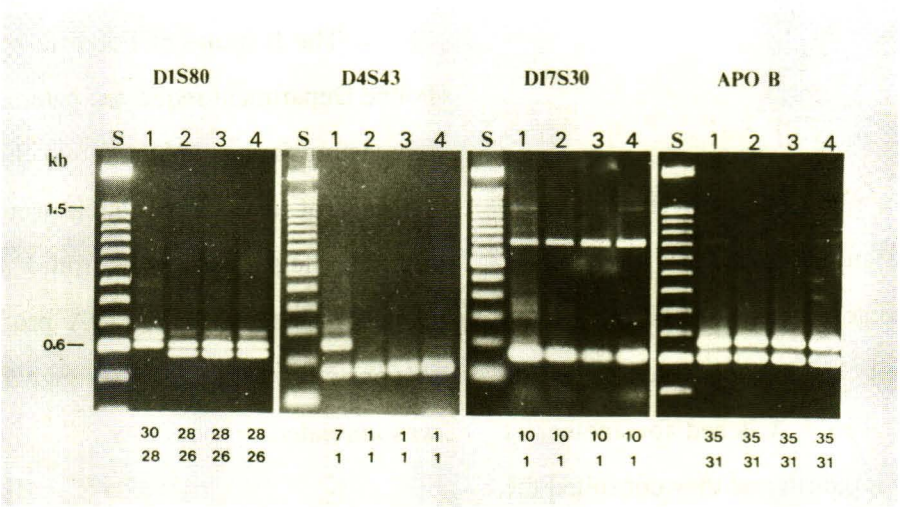

Figure 3. Ethidium bromide staining patterns of complete chimerism detected by DNA amplification of 4 VNTR loci on samples from donor and recipient pairs.

lane S = 100 bp DNA ladder

lane 1 = Recipient before BMT

lane 2 = Donor

lane 3 = Recipient 1 month after BMT

lane 4 = Recipient 1 year after BMT

shows the ethidium bromide staining of complete chimerism detected by PCR amplification of 4 VNTR loci on peripheral blood samples of the CML patients. The results obtained using these loci produce DNA profiles more rapidly and which were simpler to interpret than those obtained from M13 mutilocus profiling.

A hoped-for future development of forensic analysis is to other simple repeat polymorphisms. Such a procedure may involve the use of fluorescence-tagged PCR products for analysis after polyacrylamide gel electrophoresis.

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