James Chun-I Lee,[1] *M.S. and Jan-Gowth Chang,*[2] *M.D.*

ABO Genotyping by Polymerase Chain Reaction

REFERENCE: Lee, J. C.-I. and Chang, J.-G., "**ABO Genotyping by Polymerase Chain Reaction,**" *Journal of Forensic Sciences*, JFSCA, Vol. 37, No. 5, September 1992, pp. 1269–1275.

ABSTRACT: ABO blood group system's genotyping by polymerase chain reaction in genomic DNA level is developed. The positions of nucleotide 258 and 700 of cDNA from A transferase were used to distinguish A, B, and O alleles by restriction enzyme digestion. To identify the 258th nucleotide, a 199- or 200-bp DNA fragment was amplified by PCR and digested with Kpn I. For the 700th nucleotide, a 128-bp PCR amplified fragment was designed and digested with Alu I. By examining the DNA fragment digested patterns, ABO genotypes were easily determined. Results obtained using this method on 20 ABO-known peripheral blood samples showed that this new technique could provide accurate ABO genotype. Biologic forensic samples, such as, blood stains, saliva stains, semen stains, hair, bone tissue, and semen contaminated with vaginal secretion were also successfully typed. This rapid, sensitive and reliable method should be applicable not only in forensic identification but also in medical examination.

KEYWORDS: pathology and biology, ABO blood group system, forensic DNA typing, human identification, polymerase chain reaction

Since 1901, when Karl Landsteiner [*1*] discovered the first human genetic marker system, ABO blood group has been widely applied in transfusion [*2*], paternity testing [*3*], anthropologic investigation [*4*], forensic identification [*5*], personality prediction etc. For applications, the scientists have spent a great deal of time in developing reliable methods to type various kinds of samples, especially after 1960s [*6,7*], ABO blood group substances were confirmed to be detectable in various human tissues. All the methods in determining ABO types are designed to detect antibody or antigen materials and have widely adopted for several decades, such as, Lattes Crust, absorption inhibition, absorption elution, mixed agglutinations [*5*] and enzyme-linked immunosorbent assay [*8*] etc. Most of them can get high ratio of correct results and great sensitivity. But even when these traditional methods are modified [*9,10*], they can only detect ABO phenotype. Since antigen substances are glycolipids or glycoproteins, there are several inherent problems in determining their activities that will affect the tests, such as, survival of antigen activity [*1*], contamination of microorganism [*11*], nonspecific absorption [*5*] etc. There are more serious influences in forensic typing, because samples like blood stains, semen stains, saliva stains, hair, bone, putrefied, or charred body etc., which are frequently encountered in forensic laboratory may be exposed under extreme conditions.

Received for publication 18 Nov. 1991, revised manuscript received 4 Jan. 1992; accepted for publication 28 Jan. 1992.

[1]Instructor, Department of Forensic Science, Central Police College, Kwei Shan, Taoyuan, Taiwan.

[2]Chief, Department of Molecular Medicine and Clinical Pathology, Jen-Ai Municipal Hospital, Taipei, Taiwan.

DNA analysis from human tissues has been described from a 2400-year-old Egyptian mummy [*12*], a 7000-year-old human brain recovered in Florida [*13*], human bones 300 to 5500-years-old recovered in England [*14*] etc. These studies indicate that DNA is very stable under some conditions and PCR (polymerase chain reaction) is a powerful tool for trace sample. In order to overcome the limitations of antigens, we use the much more stable genetic material within every nucleated cell in the body and developed ABO genotyping method in genomic DNA level by PCR and restriction enzyme digestion. The cDNA of A transferase (A gene) [*15*] was used to establish this approach.

Materials and Methods

DNA was isolated from twenty ABO-known peripheral blood samples, blood stains, saliva stains, hair, bone, and vaginal swab from the rape case according to the standard method described throughout the literature [*16–21*].

Two DNA fragments of ABO locus were designed to be amplified by two pairs of primers as described in Fig. 1. Amplification was accomplished in 100 μL of reaction mixture, which contained 50 ng extracted DNA, reaction buffer (10 mM Tris-HCl pH 8.3 at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.1% Triton X-100), 200 μM of dNTP, 2.5 units of Taq polymerase (Promega) and 15 pmole of each primer. A total of 35 cycles of amplification were carried out according to the following reaction cycles: primer 1 + 2, denaturation for 2 min at 94°C, annealing for 2 min at 55°C and extension for 3 min at 72°C; primer 3 + 4, denaturation for 2 min at 94°C, annealing for 2 min at 58°C and extension for 3 min at 72°C.

Products of primer 1 + 2 were digested with Kpn I and products of primer 3 + 4 were digested with Alu I. Ten microlitres of PCR products were digested with 5 units of Kpn I and Alu I (Promega) for 1 h. The digested amplified DNA were then run on a 3% Nusieve 3:1 agarose gel in TBE buffer (0.89 M Tris-HCl, 0.089 M borate, 0.002 M EDTA, pH 8.0) at 50 V for 1 h. The gel was stained for 15 min. in 0.5 μg/mL ethidium bromide and the bands were visualized by UV light.

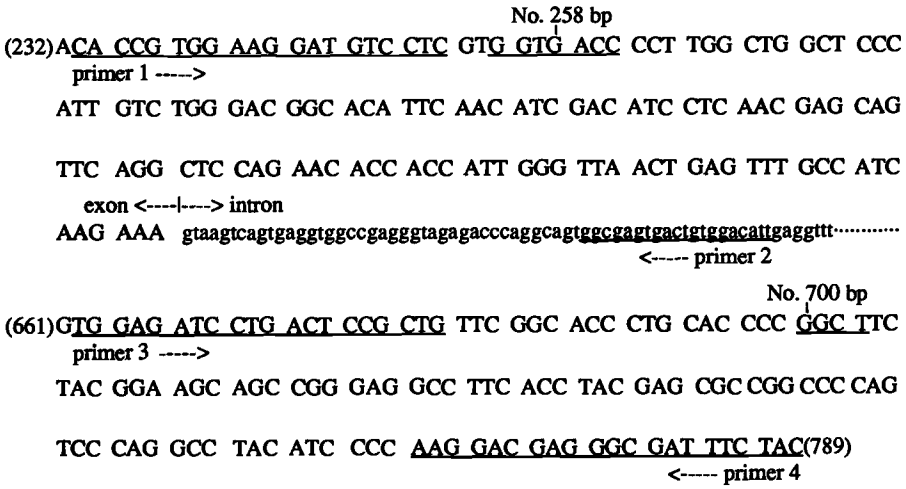

FIG. 1—*PCR mapping analysis of ABO genotyping direct from A transferase gene. No. 258 bp is deleted in 0 allele which creates Kpn I site as sequences underlined. No. 700 bp is A and B allele, which creates* Alu I site as sequences underlined. *cDNA sequences are as described by Yamamoto et al. The sequences of primer 2 is located at the intron area determined by the authors.*

Results

Primer 1 and 2 amplified a 199- or 200-bp DNA fragment containing the position of nucleotide 258 of cDNA from ABO locus. If the 258th nucleotide does not exist, the PCR product should be 199-bp O allele specific fragment, and which will create a Kpn I cleavable site on the O allele specific sequence. If the 258th nucleotide exist, the fragment should be 200-bp, and there is no Kpn I site. Therefore, if this fragment was completely digested with Kpn I, 171- and 28-bp fragments were found and recognized as homozygote OO, and 171-bp fragment was used as a 0 allele marker. If half digested, 200-, 171- and 28-bp fragments were found to be heterozygote AO or BO. If no digestion, no O allele was confirmed and genotype AA, AB or BB was possible. Primer 3 and 4 amplified a 128-bp DNA fragment containing the position of nucleotide 700 of cDNA from ABO locus. If the 700th nucleotide is A, this fragment should be B allele specific, and there is a Alu I cleavable site on the B allele specific sequence. Therefore, if this fragment was completely digested with Alu I, 88- and 40-bp fragments were found and recognized as homozygote BB, and 88-bp fragment was used as a B allele marker. If half digested, 128-, 88- and 40-bp fragments were found to be heterozygote AB or BO. If no digestion, no B allele was confirmed and genotype AA, AO or OO was possible. If neither 200- nor 128-bp fragments could not be digested, homozygote A allele was determined. By examining the digested patterns of these two fragments (Fig. 2), ABO genotypes were easily determined by Table 1.

The results of ABO genotype of twenty peripheral blood samples are shown in Table 2. DNAs from a blood stain, a saliva stain, a semen stain, hair, bone, and a vaginal swab were also successfully typed as shown in Fig. 3.

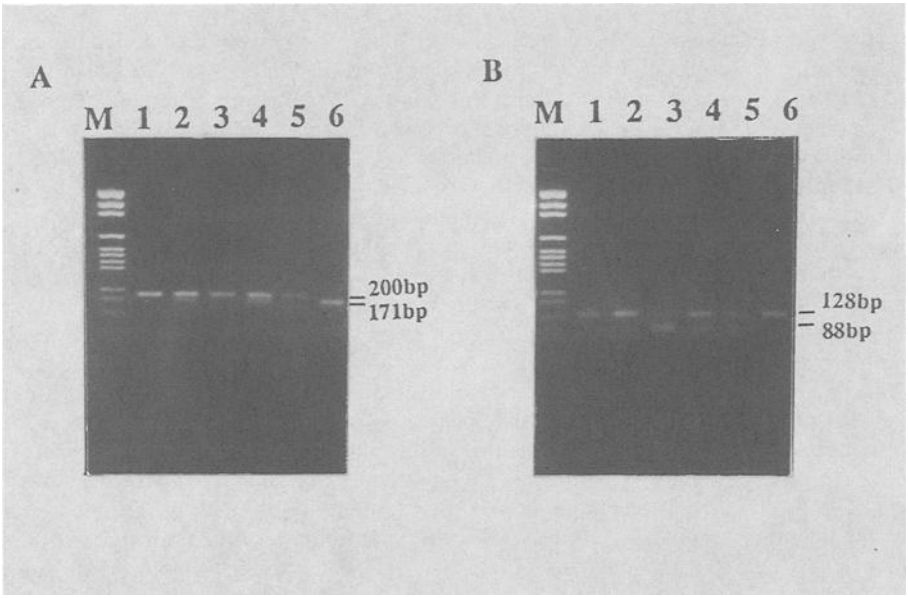

FIG. 2—*Restricted fragment patterns of ABO genotype obtained with digestion of the PCR-amplified products by Kpn I and Alu I from ABO locus. DNA samples were run in 3% Nusieve 3:1 agarose gel electrophoresis. Lane 1–6 are samples with genotype AA, AO, BB, BO, AB, and OO, respectively; M = pGEM marker. a, Products of primer 1 + 2 were digested with Kpn I. b, Products of primer 3 + 4 were digested with Alu I.*

TABLE 1—*ABO genotype[a] interpretation by RE digestion of PCR products.*

	Possible genotypes predicted by Kpn I	Alu I
Complete digestion	OO	BB
Half digestion	AO, BO	AB, BO
No digestion	AA, AB, BB	AA, AO, OO

[a]ABO genotype is determined by the intersection of possible genotypes predicted by Kpn I and Alu I digestion.

TABLE 2—*ABO genotypes determined by the positions of cDNA nucleotide 258 and 700 of ABO locus.*

Sample no.	Phenotype	Genotype	Sample no.	Phenotype	Genotype
1	A	AO	11	A	AA
2	B	BO	12	A	AO
3	B	BO	13	A	AO
4	A	AO	14	B	BB
5	A	AO	15	B	BO
6	B	BO	16	O	OO
7	AB	AB	17	O	OO
8	B	BO	18	O	OO
9	B	BO	19	A	AO
10	A	AO	20	A	AO

Discussion

This study demonstrates the applicability of molecular genetic techniques for ABO genotype analysis directly from the primary structure of DNA. We take advantage of the cDNA of A transferase to establish this rapid ABO genotyping method. According to Yamamoto et al. [*22*], for ABO polymorphism, there are four consistent nucleotide substitutions of A transferase leading to amino acid changes (residues 176, 235, 266, and 268) to form B transferase, B allelic cDNA. O allelic cDNA is formed due to the deletion of nucleotide position 258, which causes the loss of transferase activity.

In accurate tests, results on 20 ABO-known peripheral blood samples were in accordance with the phenotypes; besides, ABO genotypes were revealed. This test showed that the positions of nucleotide 258 and 700 were enough to determine ABO polymorphism. The results of stains, hair, bone and vaginal swab sample demonstrated that forensic samples were also applicable.

There are several advantages by using this approach for typing ABO blood group. First, ABO genotype can be determined. Since ABO blood group record is very prevalent, this approach obviously increases the value of this blood group system in suspect screening, paternity testing etc. Second, this genomic DNA typing method provides definite result. Results from this technique depend on the integrity of DNA in ABO locus. No matter how the sample is aged or exposed under extreme conditions, if there is intact target DNA left, it is possible to be typed. No possible contamination of microorganism will cause ambiguous result. Third, in rape cases, this approach can definitely determine the blood group of the rapist, which is unable to be determined by antigen test method. In addition, this result will not be interfered with secretor or nonsecretor problem. This is a great help for the investigator in first-step suspect screening by ABO blood group, because even if DNA profile bank is established in the near future, the investigator still can not read the DNA profile directly from the suspect and match it. ABO blood group

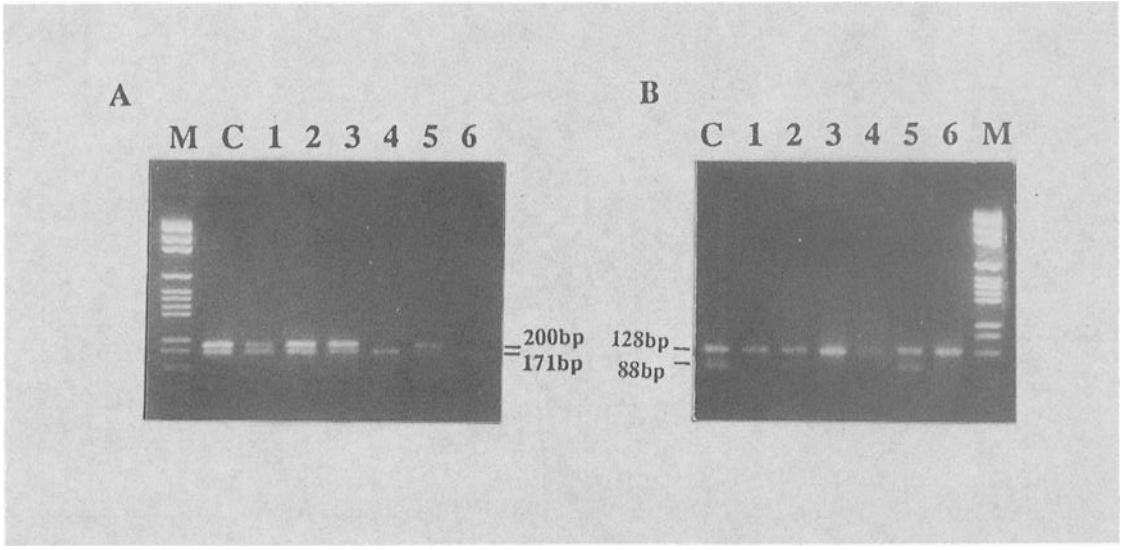

FIG. 3—*Restricted fragment patterns of ABO genotype from forensic samples. Restricted fragments were run in 3% Nusieve 3:1 agarose gel electrophoresis. M = pGEM marker; C = control sample of genotype BO; 1 = 2 mm × 2 mm blood stain; 2 = 1 cm × 1 cm saliva stain; 3 = single hair root in anagen phase stored for 2 months after plucked; 4 = 70 mg occipital bone fragments from skull bone tissue stored at room temperature for 40 years; 5 = epithelial cell from vaginal swab of a rape case; 6 = sperm cell from vaginal swab as 5. A, Products of primer 1 + 2 were digested with Kpn I. B, Products of primer 3 + 4 were digested with Alu I.*

typing by this method can provide the direct screening information for investigator in rape cases. Another major advantage of this approach is that the simple, fast, economic, sensitive, and reliable method make it possible to be adopted in forensic science. There are no dangerous radioisotope and tedious probe-hybridization work in this procedure.

Except for forensic application, this approach can also determine the ABO genotype of Bombay individual who is short of detectable A and B antigens. The mechanism of ABO blood group conversion from leukemia disease may be found by this approach.

References

[1] Gaensslen, R. E., Sourcebook in Forensic Serology, Immunology and Biochemistry, U.S. Government Printing Office, Washington, DC, 1983, pp. 261–328.

[2] Issitt, P. D., Applied Blood Group Serology, 3rd ed., Montgomery Scientific Publications, Miami, Florida, 1989, pp. 132–168.

[3] Melvin, J. R., Kateley, J. R., Oaks, M. K., Simson, L. R., and Maldonado, W. E., "Paternity Testing," *Forensic Science Handbook*, Vol. II, R. Saferstain, Ed., Prentice-Hall, Englewood Cliffs, New Jersey, 1988, pp. 273–346.

[4] Gaensslen, R. E. and Lee, H. C., "Genetic Markers in Human Bone Tissue," *Forensic Science Review*, Vol. 2, 1990, pp. 125–145.

[5] Lee, H. C., "Identification and Grouping of Bloodstains," *Forensic Science Handbook*, R. Saferstain, Ed., Prentice Hall, Englewood Cliffs, New Jersey, 1982, pp. 267–337.

[6] Swinburne, L. M., "The Identification of Skin," *Medicine, Science and the Law*, Vol. 3, 1962, pp. 3–12.

[7] Slavik, V. and Meluzin, F., "Bestimmung der Gruppenzugehörigkeit im System ABO aus Histologische Material: I. Histologische Präparate der Sezierten Fälle," *Zeitschrift für Rechtsmedizin*, Vol. 70, 1972, pp. 79–88.

[8] Bolton, S. and Thorpe, J. W., "Enzyme-Linked Immunosorbent Assay for A and B Water Soluble Blood Group Substances," *Journal of Forensic Sciences*, Vol. 31, No. 1, Jan. 1986, pp. 27–35.

[9] Lee, H. C., Gaensslen, R. E., Pagliaro, E. M., and Novitch, B., "Two-Dimensional Absorption-Inhibition," *Journal of Forensic Sciences*, Vol. 33, No. 5, Sept. 1988, pp. 1127–1138.

[10] Zhou, B., Gou, J., Wang, C., and Chen, J., "The Rapid Determination of the ABO Group from Body Fluids (or Stains) by Dot Enzyme-Linked Immunosorbent Assay (Dot-ELISA) Using Enzyme-Labeled Monoclonal Antibodies," *Journal of Forensic Sciences*, Vol. 35, No. 5, Sept. 1990, pp. 1125–1132.

[11] Hauser, R., Reszeja, S., Pawlowski, R., and Samet, A., "Mikrobille Kontamination der Antigene ABO im Knochengewebe," *Zeitschrift für Rechtsmedizin*, Vol. 92, 1984, pp. 189–197.

[12] Pääbo, S., "Molecular Cloning of Ancient Egyptian Mummy DNA," *Nature*, Vol. 314, 18 April 1985, pp. 644–645.

[13] Pääbo, S., Gifford, J. A., and Wilson, A. C., "Mitochondrial DNA Sequences from a 7000-year Old Brain," *Nucleic Acids Research*, Vol. 16, 1988, pp. 9775–9787.

[14] Hagelberg, E. and Sykes, B., "Ancient Bone DNA Amplified," *Nature*, Vol. 342, 30 November 1989, p. 485.

[15] Yamamoto, F., Marken, J., Tsuji, T., White, T., Clausen, H., and Hakomori, S., "Cloning and Characterization of DNA Complementary to Human UDP-GalNAc: Fucα1→2Galα1→3GalNAc Transferase (Histoblood Group A Transferase) mRNA," *Journal of Biological Chemistry*, Vol. 265, No. 2, 15 Jan. 1990, pp. 1146–1151.

[16] Kanter, E., Baird, M., Shaler, R., and Balazs, I., "Analysis of Restriction Fragment Length Polymorphisms in Deoxyribonucleic Acid (DNA) Recovered from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 403–408.

[17] Guisti, A., Baird, M., Pasquale, S., Balazs, I., and Glassberg, J., "Application of Deoxyribonucleic Acid (DNA) Polymorphisms to the Analysis of DNA Recovered from Sperm," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 409–417.

[18] Mullenbach, R., Lagoda, P. J. L., and Welter, C., "An Efficient Salt-Chloroform Extraction of DNA from Blood and Tissues," *Trends in Genetics*, Vol. 5, No. 12, December 1989, p. 391.

[19] Higuchi, R., von Beroldingen, C. H., Sensabaugh, G. F., and Erlich, H. A., "DNA Typing from Single Hairs," *Nature*, Vol. 322, 7 April 1988, pp. 543–546.

[20] Perry, W. L., Bass, W. M., Riggsby, W. S., and Sirotkin, K., "The Autodegradation of Deoxyribonucleic Acid (DNA) in Human Rib Bone and Its Relationship to the Time Interval Since Death," *Journal of Forensic Sciences*, Vol. 33, No. 1, Jan. 1988, pp. 144–153.

[21] Lee, H. C., Pagliaro, E. M., Berka, K. M., Folk, N. L., Anderson, D. T., Ruano, G., Keith, T. P., Phipps, P., Herrin, G. L., Garner, D. D., and Gaensslen, R. E., "Genetic Markers in Human Bone: I. Deoxyribonucleic Acid (DNA) Analysis," *Journal of Forensic Sciences*, Vol. 36, No. 2, March 1991, pp. 320–330.

[22] Yamamoto, F., Clausen, H., White, T., Marken, J., and Hakomori, S., "Molecular Genetic Basis of the Histo-Blood Group ABO System," *Nature*, Vol. 345, 17 May 1990, pp. 229–233.

Address requests for reprints or additional information to
James Chun-I Lee
Department of Forensic Science
Central Police College
56 Shu-Jen Road
Kwei Shan Taoyuan, 33334
Taiwan, ROC