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Determination of ABO genotypes with DNA extracted from formalin-fixed, paraffin-embedded tissues

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Summary. The gene encoding the specific glycosyltransferases which catalyze the conversion of the H antigen to A or B antigens shows a slight but distinct variation in its allelic nucleotide sequence and can be divided into 6 genotypes when digested with specific restriction enzymes. We extracted DNA from formalin-fixed, paraffin-embedded tissues using SDS/proteinase K treatment followed by phenol/chloroform extraction. The sequence of nucleotides for the A, B and O genes was amplified by the polymerase chain reaction (PCR). DNA fragments of 128 bp and 200 bp could be amplified in the second round of PCR, using an aliquot of the first round PCR product as template. Degraded DNA from paraffin blocks stored for up to 10.7 years could be successfully typed. The ABO genotype was deduced from the digestion patterns with an appropriate combination of restriction enzymes and was compatible with the phenotype obtained from the blood sample.

Key words: ABO blood group – Genotyping – Paraffin-embedded tissue – Polymerase chain reaction – Glycosyltransferase

Zusammenfassung. Das Gen wird für die spezifischen Glykosyltransferasen kodiert, welche die Konversion des H-Antigens in das A- oder B-Antigen katalysieren, weist eine leichte, aber definierte Variation seiner allelischen Nukleotid-Sequenz auf. Auf diese Weise lassen sich 6 Genotypen unterscheiden, nach Spaltung mit spezifischen Restriktionsenzymen. Wir haben DNA von formalinfixierten, paraffineingebetteten Geweben extrahiert, indem wir SDS-Proteinase-K-Behandlung mit nachfolgender Phenol-Chloroform-Extraktion anwandten. Die Sequenz der Nukleotide für die A-, B- und O-Gene wurde amplifiziert mit Hilfe der Polymerase-Kettenreaktion (PCR). DNA-Fragmente mit 128 Bp und 200 Bp konnten in der zweiten PCR-Phase amplifiziert werden, indem ein Aliquot der ersten PCR-Phase eingesetzt wurde. Degradierte DNA von Paraffin-Blöcken, welche bis zu 10,7 Jahren gelagert waren, konnte erfolgreich typisiert werden. Der ABO-Genotyp wurde dann abgeleitet von den Mustern nach Spaltung mit einer geeigneten Kombination von Restriktionsenzymen und war kompatibel mit dem Phänotyp, der von der Blutprobe erhalten wurde.

Schlüsselwörter: ABO-Blutgruppe – Genotypisierung – paraffineingebettetes Gewebe – PCR – Glykosyltransferase

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Introduction

The A and B phenotypes are expressed after conversion of the H antigen to A and B antigens by N-acetylgalactosaminyltransferase (A transferase) and galactosyltransferase (B transferase), respectively [1, 2]. A single base substitution in the A and B genes leads to the expression of functionally different A and B transferases, respectively. Deletion of a single base in the O gene gives rise to no transferase activity, yielding the O blood type [3, 4].

We have previously reported [5] the determination of ABO genotypes with leukocyte DNA extracted from fresh blood, depending on the polymorphism of the gene encoding the transferase [3, 4]. We have amplified the regions including a single-base deletion site of the O allele (248 bp) and a single-base substitution site in the A and B alleles (467 bp), among the ABO polymorphic sites of the cDNA at the ABO gene locus, with the polymerase chain reaction (PCR) and treated with restriction enzymes.

Here we report details for ABO genotyping with degraded DNA extracted from formalin-fixed, paraffin-embedded tissue. The primers used in the previous study [5] were not suitable for amplification from paraffin block samples. The 248 bp fragment was successfully amplified in the second round of PCR, using an aliquot of the first round PCR product as template, however, the 467 bp fragment was amplified in some of the samples. Either pairs

of primers designed for shorter fragments (128 bp and 200 bp) [6] or reamplification gave better results from degraded DNA.

Materials and methods

Specimens. Paraffin blocks of tissues (Table 1) were used which had been prepared by routine histopathological procedures. The fixation periods were mostly 1 week or less. The ABO phenotype was determined serologically.

DNA preparation. Approximately 20 mg of embedded tissue was excised from each block and minced with a surgical razor. After washing with xylene, samples were suspended in 200 µl of TEN (100 m*M* Tris-HCl buffer, pH 8.0, 40 m*M* EDTA, 10 m*M* NaCl) containing 1% SDS and 0.5 mg/ml proteinase K (Boehringer Mannheim, Germany), followed by incubation at 50°C for 16 hrs. SDS and proteinase K were added to a final concentration of 2% and 1 mg/ml, respectively, and the mixture was incubated for another 24 hrs. Following extraction with phenol/chloroform, the DNA was treated with 20 µg/ml ribonuclease A (Sigma, USA) and suspended in 100 µl TE buffer (10 m*M* Tris-HCl buffer, pH 8.0, 1 m*M* EDTA).

The amount of DNA was estimated spectrophotometrically [7]. An aliquot of 15 µl was applied to 1.5% agarose (NuSieve GTG, FMC, USA) gel electrophoresis and the DNA was visualized by ethidium bromide staining. Either HindIII digested λ DNA (Takara, Japan) or HaeIII digested ϕ x174 DNA (Toyobo, Japan) was run concurrently as DNA molecular weight markers.

PCR amplification. Amplified regions that include nucleotide 258 (a single-base deletion site of the O allele) and nucleotide 700 (a single-base substitution site in the A and B alleles) of cDNA at the ABO gene locus [4, 6] were designated as frag-258 and frag-700, respectively.

Sequences of the primers used are as follows; a) 5′-CACCGTGGAAGGATGTCCTC, b) 5′-AATGTCCACAGTCACTCGCC, c) 5′-TGGAGATCCTGACTCCGCTG and d) 5′-GTAGAAATCGCCCTCGTCCTT [6]. Primers a) and b) were used for amplification of frag-258 and c) and d) for frag-700.

Amplification with Taq DNA polymerase (Toyobo, Japan) was performed [6] in a 100 µl reaction mixture containing 1 µl of sample DNA solution for 35 cycles. After the reaction, 1 µl aliquot was then subjected to another 35 cycles of amplification (second round PCR) under the same conditions.

Enzyme digestion of the PCR products. Two aliquots of amplified frag-258 DNA were treated with BstEII or KpnI and an aliquot of frag-700 DNA with AluI (Toyobo, Japan) for 16 hrs.

Electrophoretic analyses of the PCR products and of the enzyme digests. DNA was electrophoresed on 10% polyacrylamide gels and the bands were visualized by silver staining [8]. BioMarker Low (BioVentures, USA) was used as a size marker on the gels.

Table 1. ABO genotypes determined with the DNA extracted from formalin-fixed, paraffin-embedded tissues

Sample no.	Organ	Death-to-fixation interval (days)	Time after embedding (years)	Phenotype	Genotype
1	Testis	0.5	0.5	O	OO
2	Liver	0.5	1	A	AO
3	Liver	0.5	1	B	BO
4	Liver	0.8	1	O	OO
5	Lung	1.2	1	A	AO
6	Liver	1.2	1.2	O	OO
7	Liver	1.2	1.2	A	AO
8	Liver	0.5	1.5	AB	AB
9	Liver	0.5	1.5	B	BO
10	Lung	0.5	2	O	OO
11	Kidney	2.5	2	A	AO
12	Thymus	0.5	2.2	B	BO
13	Lung	1	2.5	O	OO
14	Lung	0.2	3.7	A	AO
15	Spleen	0.5	3.7	A	AO
16	Kidney	0.5	4	A	AO
17	Spleen	0.5	4	O	OO
18	Thymus	0.5	8.7	O	OO
19	Thymus	2	9.5	A	AO
20	Thymus	0.5	10.7	B	BO

Results and discussion

Approximately 1–10 µg of DNA was isolated from 20 mg of paraffin-embedded tissue. The DNA was degraded with molecular weights ranging mostly from 100 bp to 2 kbp (Fig. 1). The degraded DNA, however, could be utilized to amplify the 128 bp and 200 bp fragments. In the first round of PCR, 3 frag-258 and 6 frag-700 DNAs from 20 samples were successfully amplified. The second round of PCR gave a sufficient amount of the DNA fragment for analysis in all the samples.

Genotypes were determined according to the method previously described [6]. In brief, the frag-258 DNA of the A/B and O alleles is cleaved by BstEII and by KpnI, respectively. The frag-700 DNA of the A/O and B allele is cleaved by HpaII and by AluI, respectively. KpnI and AluI digestions are indispensable and sufficient for genotyping [6], however, we carried out three digestions with BstEII, KpnI and AluI to confirm the results. HpaII is unsuitable for the analysis since it recognizes the non-polymorphic sites in the fragment [6]. Fig. 2a shows the presence of the A/B allele in the samples 8, 19 and 20 and the

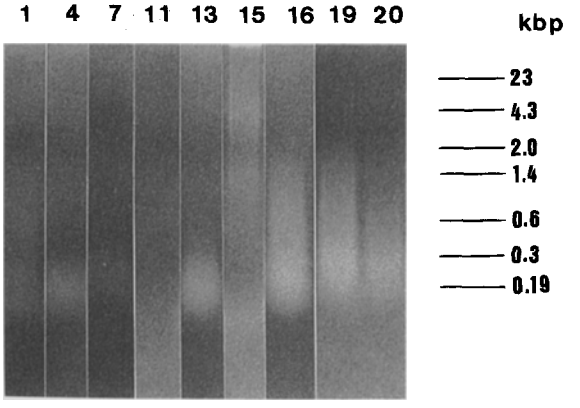

Fig. 1. Examination of DNA isolated from formalin-fixed, paraffin-embedded tissue. DNA was electrophoresed in 1.5% agarose gels and visualized by UV light after ethidium bromide staining. Lane number corresponds to the sample number

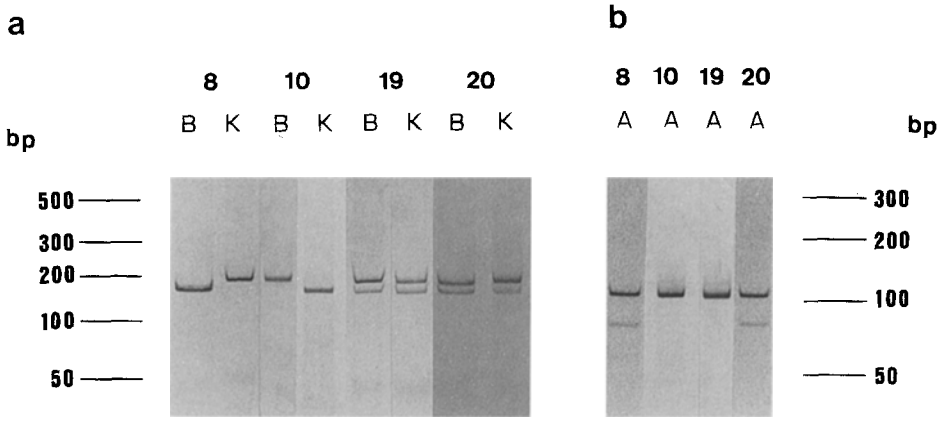

Fig. 2a, b. Electrophoretic pattern of DNA samples digested with restriction enzymes. **a** Frag-258 analysis. The amplified frag-258 DNA was digested with BstEII (*B*) or KpnI (*K*). Fragments of 23 bp and 28 bp produced by BstEII and KpnI digestions, respectively, are not visible. **b** Frag-700 analysis. The amplified frag-700 DNA was digested with AluI (*A*). Fragment of 40 bp produced by the digestion is not visible. The genotypes of the samples 8, 10, 19 and 20 were determined as AB, OO, AO and BO, respectively. Number described above the lane represents the sample number

O allele in the samples 10, 19 and 20. Fig. 2b shows that the samples 8 and 20 also possess the B allele. The genotypes of the samples 8, 10, 19 and 20 were determined as AB, OO, AO and BO, respectively. In all the samples the genotype determined by this method matched the ABO blood group phenotype (Table 1).

The procedure described can be applied to determine the ABO genotype from a trace amount of decomposed sample of forensic interest. Moreover, it may be available for retrospective investigations for the genetic diagnosis of diseases or the improvement of breeds in animals.

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