Int J Legal Med (1996) 109:216–217

SHORT COMMUNICATION

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An improved method for MN genotyping by the polymerase chain reaction

Received: 29 February 1996 / Received in revised form: 30 July 1996

Abstract A novel method of human MN blood group genotyping is reported using the polymerase chain reaction. Genotyping is based on two base substitutions characteristic of M and N alleles in the 2nd exon of the glycophorin A gene. Using a newly designed primer trio, PCR products for M (255 bp) and N (270 bp) alleles are rapidly and simultaneously detected by a single PCR procedure and subsequent polyacrylamide gel electrophoresis. This method enables MN genotyping from not only minute but also degraded DNA samples.

Key words MN blood group system · Genotyping · Forensic evidence

Introduction

MN phenotypes are serologically determined by the existence of M and N antigens encoded by several glycophorin genes [1, 2]. Of these, glycophorin A (GPA) encodes both M and N determinants and has three nucleotide differences, which lead to two amino acid substitutions at positions 1 (M: serine for *TCA*; N: leucine for *TTA*) and 5 (M: glycine for *GGT*; N: glutamic acid for *GAG*) [3], of the M and N alleles in exon 2. Corfield et al. [4] developed a method of MN genotyping by the polymerase chain reaction (PCR) using two allele-specific oligonucleotide primers (ASOP), M ASOP and N ASOP, based on the nucleotide substitutions at position 5, and a reverse primer common to both M and N alleles. They detected each allele with the amplified size of 781 bp. We describe here an improved MN genotyping method where shorter amplified fragments (M: 255 bp; N: 270 bp) are detected with a primer trio composed of two newly designed primers and the M ASOP. We also applied this method to some degraded DNA samples derived from forensic casework.

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Materials and methods

Whole blood was collected from 120 unrelated individuals and tested by routine MN blood grouping procedures. DNA from peripheral blood was isolated by the phenol-chloroform method. Bloodstains and a vaginal secretion prepared on gauze were stored at room temperature for 1–3 years. DNA isolation from 4 mm^2 pieces of each stain was performed either by the phenol-chloroform method or using InstaGene DNA purification matrix (BIO-RAD).

For amplification of M and N alleles, three primers were prepared: M ASOP [3] (5′-GCATC AAGTA CCACT GGT-3′), primer N (5′-ATTGT GAGCA TATCA GCATT-3′) which recognizes a substitution at the 2nd nucleotide in exon 2 of GPA, and a reverse primer MN (5′-AGAGG CAAGA ATTCC TCC-3′) which anneals to the intron sequence. The reverse primer is common to M and N alleles, but the 3′ end nucleotide is not complementary to those of other glycophorin groups which have nearly identical sequences [2]. The PCR reaction mixture (25 µl) was composed of 2 m*M* Tris-HCl (pH 8.8), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.1% Triton X-100, 0.1 m*M* tetramethylammonium chloride, 100 µ*M* each dNTP, 10 p*M* each primer, 0.5 U DNA polymerase (Gene Taq, Nippon-Gene) and 0.5–20 ng of genomic DNA. PCR conditions were as follows: denaturation (94° C – 30 s), annealing (60° C – 30 s) and extension (72° C – 60 s) for 30 cycles and an additional extension (72° C – 120 s). The PCR products were electrophoresed in 8% polyacrylamide gels (2% C, 80 × 60 × 1 mm) at a constant 120 V for 1 h, and visualized by silver staining.

Results

Figure 1 A shows the amplified bands detected from M, N and MN individuals. The M and N bands appeared at 255 bp and 270 bp, respectively. The sizes were identical with those based on the reported GPA genomic DNA sequence [2] and the primer designs. The size difference of 15 bp between two bands was easily discernible in 8% polyacrylamide gels. Both bands were simultaneously detectable with no unspecific bands even though three primers

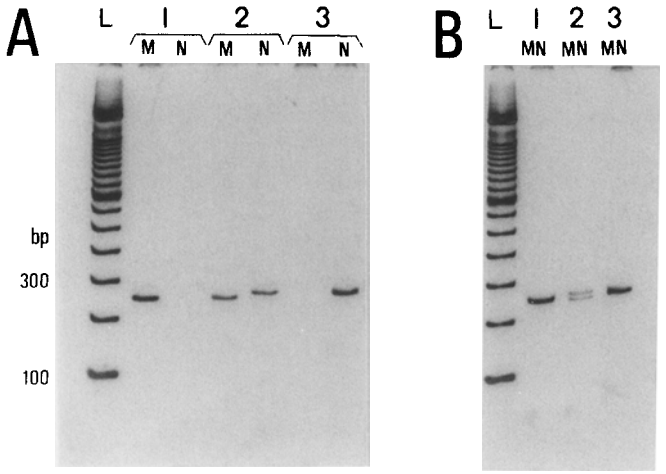

Fig. 1 M and N alleles in genomic DNAs from 1 (serological type M), 2 (type MN) and 3 (type N) individuals were amplified with either M or N primer pairs (**A**) and MN primer trios (**B**). L, 100 bp DNA ladder (GIBCO BRL)

Table 1 Comparison of MN types between serotyping and genotyping

		n		*n*		*n*	Total
Serotyping	M	40	MN	47	N	33	120
Genotyping	MM	40	MN	47	NN	33	120

n, number of individuals typed

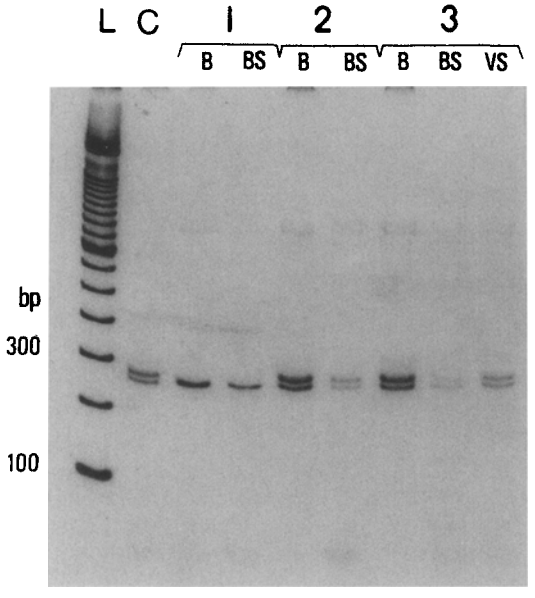

Fig. 2 Detection of M and N alleles from forensic stains. 1 (type M), one-year-old material; 2 (type MN), two years old; 3 (type MN), three years old. B, blood sample frozen at –40° C; BS, bloodstain; VS, vaginal stain; C, control (type MN)

(each 10 p*M*) were added in one PCR mixture (Fig. 1 B). From 120 individuals tested, no incompatibility was observed between the results of conventional serotyping and those of this genotyping (Table 1). The MN genotyping was also successful on some degraded DNA samples from forensic casework including bloodstains up to 3 years old, a vaginal swab, and blood samples frozen at –40° C (Fig. 2).

Discussion

The MN blood group system has been used a routine test in forensic identification from blood and bloodstains. A MN genotyping method from genomic DNA using the PCR technique by Corfield et al. [4], however, required two amplifications of different annealing temperatures for the M (51° C) and N (49° C) alleles, and sometimes failed to amplify one allele especially from old bloodstains [5]. Moreover, both amplified products were of the same size (i.e. 781 bp), so that two lanes on an electrophoresis gel were necessary. A commercial typing kit including GYPA (= GPA), which is able to amplify six loci in one PCR, is available from Perkin Elmer (Amplitype PM Kit). The typing protocol of this kit, however, seems to be somewhat complicated, because a subsequent hybridization procedure is required for the detection of alleles in each locus. In comparison, our improved MN PCR genotyping is simple and time saving because short fragments of different size for M and/or N PCR products can be detected. Successful MN typing from bloodstains and a vaginal stain extract up to three years old suggested that our method would be suitable for the typing of forensic stains exposed to various conditions.

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