



Distribution of MN genotypes detected by PCR-SSCP analysis

N. Nakayashiki^{a,*}, J. Kanetake^a, Y. Sasaki^a, I. Yuasa^b, A. Miyoshi^c,
M. Hashiyada^d, Y. Aoki^a

^aDepartment of Legal Medicine, Iwate Medical University School of Medicine, 19-1 Uchimaru,
Morioka 020-8505, Japan

^bDepartment of Legal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8503, Japan

^cDepartment of Forensic Medicine, Fukuoka University School of Medicine, Fukuoka 814-0180, Japan

^dDepartment of Forensic Medicine, Tohoku University School of Medicine, Sendai 980-8575, Japan

Abstract

Genotyping of the MN blood system was performed by means of PCR-single strand conformation polymorphism (PCR-SSCP) analysis. Twelve band patterns corresponding to each MN genotype composed of alleles M^G, M^T, N¹, N² and N^V were detected. In general, M^G or N¹ > M^T > N² in order of decreasing frequency was observed as four common alleles in five Japanese, two Chinese and a German populations. PCR-SSCP analysis provides more discriminative classification to the MN genotyping.

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1. Introduction

Molecular basis of the MN blood group system revealed that the expression of M and N antigens is based on two amino acid substitutions encoded on exon 2 of the glycophorin A (GPA) gene [1]. PCR technique was applied to the MN genotyping by using allele-specific amplification [2,3]. Recently, several nucleotide substitutions around exon 2 of the GPA gene have been demonstrated using PCR-restriction fragment length polymorphism (PCR-

* Corresponding author. Tel.: +81-19-651-5110x3383; fax: +81-19-622-2826.

E-mail address: nnakaysk@iwate-med.ac.jp (N. Nakayashiki).

RFLP) [4] and PCR-single strand conformation polymorphism (PCR-SSCP) [4,5]. In this study, we investigated the distribution of the MN genotypes by PCR-SSCP analysis in Japanese, Chinese and German populations.

2. Materials and methods

2.1. DNA samples

DNA samples were collected from unrelated Japanese donors residing in northern Japan, Aomori ($n = 106$) and Iwate ($n = 140$), and in southern Japan, Miyazaki ($n = 99$), Kagoshima ($n = 53$) and Okinawa ($n = 100$). Foreign DNA samples from two Chinese populations in Shenyang ($n = 99$) and Nanjing ($n = 113$), and one in Germany ($n = 71$) were also tested.

2.2. PCR-SSCP

Amplification was performed according to the previous report [5] with slight modifications. The reaction mixture (25 μ l) was composed of $1 \times$ PCR reaction buffer, 200 μ M of each dNTP, 20 pM of each primer (5' -GAG GGA ATT TGT CTT TTG CA-3' and 5' -GGT CCC CTA AAA TAG GGT TA-3'), 0.5 U of *Taq* DNA polymerase, and 10–20 ng of genomic DNA. The samples were amplified with 32 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 2 min. The PCR product (256 bp) mixed with SSCP solution was denatured at 90 °C for 3 min, chilled rapidly on ice, and subjected to polyacrylamide gel (12% T, 1% C, 3.5% glycerol; 200 \times 300 \times 0.5 mm) electrophoresis in a cold chamber (4 °C) at 400 V for 15 h. SSCP bands were visualized by silver staining.

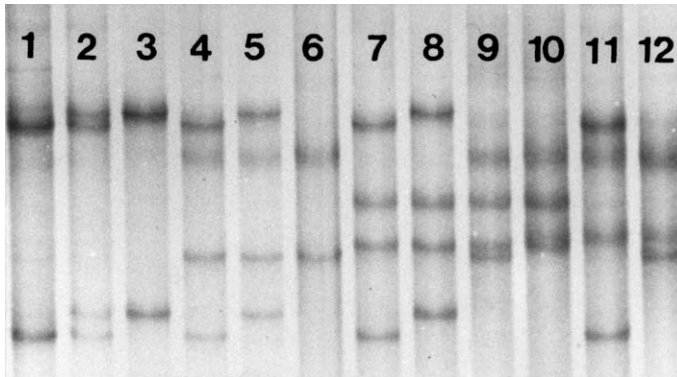


Fig. 1. Electrophoretic band patterns of MN genotypes by PCR-SSCP. Lanes 1–12 are 12 different samples with genotypes $M^G M^G$, $M^G M^T$, $M^T M^T$, $M^G N^1$, $M^T N^1$, $N^1 N^1$, $M^G N^2$, $M^T N^2$, $N^1 N^2$, $N^2 N^V$, $M^G N^V$, and $N^1 N^V$, respectively.

3. Results and discussion

Fig. 1 shows the 12 MN genotypes observed in this study. Table 1 demonstrates the frequency distribution of each MN allele and statistical data of tested populations. Four common MN alleles (M^G , M^T , N^1 and N^2) were detected from most populations with the exception of the German, which lacked N^2 . The M^G and N^1 alleles were dominant in all populations, and followed by M^T and N^2 in order of decreasing frequency. The rare N^V allele was detected in one of each sample from the Aomori, Miyazaki, Okinawa, and Shenyang populations.

On the distribution of N^2 , Aomori and Miyazaki showed higher frequencies than Iwate, Kagoshima and Okinawa. A distinct distribution diversity of N^2 was also found between Chinese populations (Shenyang: 0.066, Nanjing: 0.004). These results might indicate that there is a regional or racial difference for allele distribution of N^2 .

There was a tendency that the allele M was more frequently observed than N. However, Japanese in Aomori, Kagoshima and Okinawa, and both Chinese populations showed contrary results. This might be caused by the small size of each population sample, since there were no inconsistencies between the results of genotypes by PCR-SSCP and routine hemagglutination test [5].

Table 1
Allelic distribution of GPA (MN) locus in several populations

	<i>n</i>	$GPA * M^G$	$GPA * M^T$	$GPA * N^1$	$GPA * N^2$	$GPA * N^V$	χ^2 (<i>df</i> = 3) ^a	HET	PIC	PE	
Iwate	409	0.445	0.098	0.430	0.027	0	2.5, <i>P</i> >0.25	0.607	0.53	0.322	[5]
Iwate	140	0.446	0.125	0.414	0.014	0	1.1, <i>P</i> >0.75	0.613	0.53	0.326	This study
combined	549	0.445	0.105	0.426	0.024	0	2.9, <i>P</i> >0.25	0.608	0.53	0.323	
JAPANESE Aomori	106	0.382	0.094	0.458	0.061	0.005	1.3, <i>P</i> >0.5	0.632	0.56	0.360	This study
Miyazaki	99	0.399	0.126	0.414	0.056	0.005	3.7, <i>P</i> >0.25	0.650	0.58	0.379	This study
Kagoshima	53	0.406	0.066	0.500	0.028	0	6.1, <i>P</i> >0.1	0.580	0.49	0.295	This study
Okinawa	100	0.370	0.075	0.530	0.020	0.005	2.1, <i>P</i> >0.5	0.576	0.49	0.296	This study
CHINESE Shenyang	99	0.318	0.131	0.480	0.066	0.005	6.3, <i>P</i> >0.05	0.647	0.59	0.385	This study
Nanjing	113	0.354	0.093	0.549	0.004	0	5.8, <i>P</i> >0.1	0.565	0.48	0.283	This study
GERMAN	71	0.444	0.092	0.465	0	0	10.2, <i>P</i> >0.01	0.579	0.49	0.284	This study

HET, expected heterozygosity; PIC, polymorphism information content; PE, probability of paternity exclusion.

^a Three alleles model: $GPA * M^G$, $GPA * N^1$, ($GPA * M^T + GPA * N^2 + GPA * N^V$).

Classification of MN alleles throughout the GPA gene has been investigated and a number of mutation sites were demonstrated [6]. Analysis for a more extensive region of the GPA gene could give more detailed information for the MN blood group system. However, even the restricted region of the GPA targeted in this study can classify the five alleles and has intensive information related to the MN phenotypes. Therefore, it should be one of the useful tools for forensic identification or anthropological investigation.

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