Jpn J Human Genet 42, 489-498, 1997

# PCR-BASED GENOTYPING OF MNSs BLOOD GROUP: SUBTYPING OF M ALLELE TO $M^G$ AND $M^T$

Atsushi AKANE,* Tetsuya KOBAYASHI, Zhi-Xiang LI,
Sumitaka YOSHIMURA, Yutaka OKII, Manabu YOSHIDA,
Takuma TOKIYASU, and Toshimitsu WATABIKI

*Department of Legal Medicine, Kansai Medical University, Moriguchi 570, Japan*

*Summary* PCR-based genotyping of MNSs blood group system was investigated in combination with restriction fragment length polymorphism (RFLP), single-strand conformation polymorphism (SSCP) and allele-specific PCR amplification (ASPA) techniques. M and N alleles are based on three nucleotide substitutions in exon 2 and one base change (G or T) in an intron of glycophorin A locus. The latter single base change was also found among M alleles analyzed in this study, so that M allele appeared to be subdivided into $M^G$ and $M^T$. All three alleles, $M^G$, $M^T$ and N were identified clearly by RFLP or SSCP analysis following a single amplification. S and s alleles are based on one nucleotide substitution in exon 3 of glycophorin B gene. Genotyping of Ss blood group system was also explored by PCR-SSCP or ASPA analysis, and problems in the methods were discussed.

*Key Words* MNSs blood group system, PCR, restriction fragment length polymorphism, single-strand conformation polymorphism

## INTRODUCTION

MN and Ss blood group systems are based on polymorphic red cell antigens. Glycophorin A (GPA), a sialoglycoprotein on the erythrocyte membrane, carries M and N antigens, based on two substitutions at residues 1 and 5 of the amino acid sequence (M, serine and glycine; N, leucine and glutamic acid) (Blumenfeld and Adamany, 1978). Glycophorin B (GPB) carries S and s antigens, based on a single amino acid substitution at position 29 of the glycopeptide (S, methionine; s, threonine) (Dahr *et al.*, 1980a, b). Genes encoding GPA, GPB and glycophorin E (GPE) were more than 95% homologous, and closely linked, presenting MNSs haplotype polymorphism (Kudo and Fukuda, 1989, 1990; Vignal *et al.*, 1990; Rearden *et al.*, 1990).

Since the M and N alleles of GPA gene are attributed to three nucleotide

*Received July 31, 1997; Revised version accepted October 3, 1997.*
*To whom correspondence should be addressed.

substitutions in exon 2 (Fig. 1) (Huang and Blumenfeld, 1991), MN blood group can be genotyped by allele-specific PCR amplification (ASPA) (Corfield *et al.*, 1993; Nakayashiki and Sasaki, 1996) or reverse dot blot hybridization (Herrin *et al.*, 1994); The M- and N-specific sequences are also observed in exon 2 of GPE and GPB genes, respectively (Fig. 1) (Kudo and Fukuda, 1989, 1990, 1994; Vignal *et al.*, 1990; Rearden *et al.*, 1990; Huang and Blumenfeld, 1991), thus GPA-specific primers should be designed to avoid co-amplification of GPB and GPE segments. The present study evaluated MN genotyping by restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) analyses following a single PCR amplification, and M allele was found to be divided into two alleles, $M^G$ and $M^T$, as described below.

S and s alleles of GPB gene are based on a single nucleotide substitution in exon 3 (Fig. 1) (Kudo and Fukuda, 1990). However, it had been reported that Ss blood groups could not be typed by ASPA (Corfield *et al.*, 1993) or by Southern blot analysis (Huang *et al.*, 1991). Thus, we also explored Ss genotyping by ASPA and PCR-SSCP techniques, and discuss problems in the methods.

## MATERIALS AND METHODS

*Genotyping of MN blood group.* DNA was isolated from peripheral leukocytes. MN phenotypes of the samples were identified serologically. Two forward primers, M ($M^G$)-specific MN-GF (5′-TTA ATC CCT TTC TCA ACT TCT ATG-3′) and N- (and $M^T$-) specific MN-TF (5′-TTC TCA ACT TCT ATT TTA TAC AGC-3′), and one reverse primer, MN-R (5′-GAC AGG TCC CCT AAA ATA GGG TTA-3′), were used for PCR. Lengths of PCR products using primers MN-GF and MN-R and primers MN-TF and MN-R are 208 and 199 base-pair (bp), respectively. The PCR reaction mixture (50 $\mu$l) consisted of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each deoxyribonucleoside-5′-triphosphates (dNTP), appropriate concentrations of the primers, 2.5 units of *Taq* polymerase (Boehringer Mannheim, Mannheim, Germany), and 150 ng/reaction of template DNA. Following a preheating at 95°C for 2 min, 30 PCR cycles (94°C for 40 sec, 55–60°C for 40 sec and 72°C for 40 sec) were carried out using a thermal cycler, GeneAmp® PCR system 2400 (Perkin Elmer, Foster City, CA, USA) or Takara PCR Thermal Cycler MP (Takara, Kyoto, Japan), followed by post-incubation at 72°C for 7 min.

The effect of the annealing temperature on the genotyping was studied. Moreover, for PCR using all three primers, MN-GF, MN-TF and MN-R, in one tube, the effect of the ratio of the primer concentrations in the reaction mixture (1 : 1 : 1, 1 : 3 : 1 or 1 : 5 : 1) was investigated, because two forward primers MN-GF and MN-TF overlap for 15 bp. PCR products were purified using microconcentrator Microcon® 100 (Amicon, Danvers, CT, USA); briefly, PCR mixtures were transferred to the devices, which were then centrifuged at 3,000 rpm for 5 min.

Following the addition of 400 $\mu$l of pure water into the devices, the centrifugation was performed again for 20 min. The 'washed' PCR products were recovered in appropriate amounts of pure water, and analyzed by RFLP or SSCP technique.

For RFLP analysis, the PCR products were digested by the restriction endonuclease *Mse*I (New England Biolabs, Beverly, MA, USA) at 37°C for more than 2 hr, and electrophoresed in a 12% native polyacrylamide gel at 300 V for 1 hr, then stained with ethidium bromide. By *Mse*I digestion, $M^G$ allele was cut to 151, 56 and 1 bp fragment, $M^T$ allele to 143 and 56 bp fragments, and N allele to 99, 56 and 44 bp fragments. SSCP analysis was performed using the automated electrophoresis system PhastSystem™ (Pharmacia Biotech, Piscataway, NJ, USA) with PhastGel® Homogeneous 20 (20% polyacrylamide gel) and PhastGel® Native Buffer Strips as reported previously (Akane *et al.*, 1996). Briefly, the 'washed' PCR products mixed with an equal volume of 98% formamide–2% glycerol were boiled for 5 min and chilled rapidly in ice-water. following pre-electrophoresis at 400 V, 10 mA, 2.5 W, and 15°C for 50 Vhr (run time), 1 $\mu$l each of the samples was applied and electrophoresed for 300 Vhr. Then the gel was stained using PhastGel® DNA Silver Staining Kit (Pharmacia).

*Genotyping of Ss blood group.* Ss phenotypes of the samples were identified serologically. Three forward primers, S-specific SS-LF (5′-GAA ATT TTG CTT TAT AGG AGA AAT-3′), s-specific SS-SF (5′-GAA ATT TTG CTT TAT AGG AGA AAC-3′) and non-specific SS-1F (5′-ATT TTT TTC TTT GCA CAT GTC TTT-3′), and one reverse primer, SS-2R (5′-TAA CAA CAT ATG CTC TTC TGT TTT-3′), were prepared, and PCR was carried out as described above. Lengths of PCR products using primers SS-1F, SS-LF and SS-SF in combination with SS-2R are 211, 169 and 169 bp, respectively. To check whether the PCR products were sequences of GPB gene or not, the products were digested using a restriction enzyme *Rsa*I following the 'wash' using Microcon 100. *Rsa*I does not cut GPE sequence but cuts both GPA and GPB genes at different positions (Fig. 1). For example, if GPA, GPB and GPE sequences were co-amplified by primers SS-LF and SS-2R, the sequences would be identified as 137, 118 and 164 bp fragments following *Rsa*I digestion, respectively.

ASPA was carried out using 0.5 $\mu$M each of primers SS-LF and SS-2R or primers SS-SF and SS-2R for detection of S or s allele, with the annealing temperature at 58–62°C; the effect of the annealing temperature on the genotyping was studied. The PCR products were electrophoresed in 2% NuSieve® GTG® agarose gel (FMC, Rockland, ME, USA) and stained with ethidium bromide.

PCR products of 211 bp were amplified using 0.5 $\mu$M each of primers SS-1F and SS-2R (annealing at 55°C) for SSCP analysis of Ss genotypes. Amplification of 169 bp fragments using 0.5 $\mu$M each of primers SS-LF, SS-SF and SS-2R (1 : 1 : 1) in one tube (annealing at 60°C) was also performed for the SSCP analysis. Conditions for SSCP analysis were as described above.

RFLP analysis of Ss genotypes was not carried out because no restriction

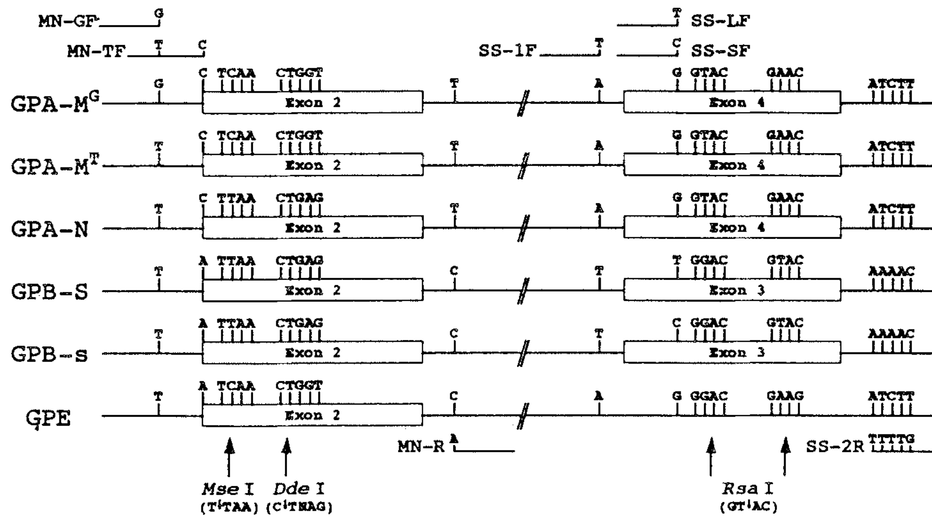


Fig. 1. Comparative nucleotide sequences of alleles of GPA, GPB and GPE genes and primers. Boxes and horizontal lines represent exons and introns, respectively. The sequence of GPB gene corresponding to exon 3 of GPA gene does not act as an exon, so that the sequence homologous to exon 4 of GPA gene acts as the third exon in GPB gene. Neither of these sequences act as exons in GPE gene.

enzyme detects S or s allele.

## RESULTS

### *Genotyping of MN blood group*

PCR using a set of primers MN-GF and MN-R with the annealing temperature at 55-60°C resulted in the amplification of 208 bp fragments (Table 1), all of which were typed as M alleles by *Mse*I digestion (151 bp fragments, Table 1, Fig. 2). However, some M alleles, typed by the serological method, were not amplified by the primers, suggesting that M alleles should be divided into two alleles, $M^G$ and $M^T$ with nucleotides G and T, respectively, at the position corresponding to

Table 1. MN genotyping by PCR using primers MN-GF, MN-TF and MN-R in combination with RFLP or ASPA analysis.

| Phenotype | Genotype | Fragment length (bp) | | | |
|---|---|---|---|---|---|
| | | PCR product | *Mse*I-RFLP | *Dde*I-RFLP | ASPA* |
| M | $M^GM^G$ | 208 | 151 | 208 | 255 |
| | $M^GM^T$ | 208+199 | 151+143 | 208+199 | 255 |
| | $M^TM^T$ | 199 | 143 | 199 | 255 |
| MN | $M^GN$ | 208+199 | 151+99 | 208+145 | 270+255 |
| | $M^TN$ | 199 | 143+99 | 199+145 | 270+255 |
| N | NN | 199 | 99 | 145 | 270 |

*According to Nakayashiki and Sasaki (1996).

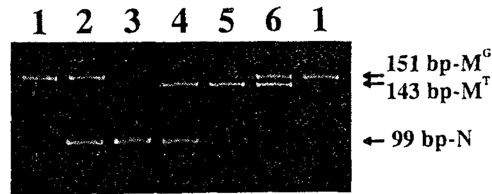


Fig. 2. PCR-RFLP pattern of MN genotypes using primers MN-GF, MN-TF and MN-R (1 : 3 : 1). 1, Genotype $M^GM^G$; 2, $M^GN$; 3, NN; 4, $M^TN$; 5, $M^TM^T$; and 6, $M^GM^T$.

the 3′-terminus of the primer MN-GF (Fig. 1). N allele with the nucleotide G was not found in the samples analyzed in this study. When PCR was performed using another set of primers, MN-TF and MN-R, with the annealing temperature at 60°C, 199 bp fragments were amplified from $M^T$ and N alleles; by *Mse*I digestion, $M^T$ and N alleles were identified as 143 bp and 99 bp fragments, respectively (Table 1, Fig. 2). With the annealing temperature at 55–58°C, $M^G$ allele was also amplified using primers MN-TF and MN-R, and typed as $M^T$ allele incorrectly by *Mse*I digestion. Thus the annealing temperature for amplification of MN alleles was set at 60°C. Under the PCR conditions, MN blood group including $M^G$ and $M^T$ alleles was genotyped correctly, suggesting that GPB and GPE genes, which encode N and M allele-homologous sequences, respectively, were not co-amplified by the primer sets. These results were reproducible when GeneAmp PCR System 2400 or Takara PCR Thermal Cycler MP was used.

For MN genotyping following a single PCR amplification, all three primers, MN-GF, MN-TF and MN-R, were added into the same reaction mixture. When concentrations of primers MN-GF, MN-TF and MN-R in the mixture were 0.5, 0.5 and 0.5 $\mu$M (1 : 1 : 1), respectively, PCR using $M^GN$ template resulted in amplification of a 208 bp $M^G$ fragment only. When the concentrations were 0.5, 1.5 and 0.5 $\mu$M (1 : 3 : 1), respectively, M and N alleles were amplified equally. When the concentrations were 0.5, 2.5 and 0.5 $\mu$M (1 : 5 : 1), respectively, more N fragment was amplified than $M^G$ segment.

Following *Mse*I digestion, 151 bp $M^G$, 143 bp $M^T$ and 99 bp N fragments could be identified by polyacrylamide gel electrophoresis as shown in Fig. 2. The three alleles could also be identified clearly by SSCP analysis (Fig. 3).

Allele frequencies of $M^G$, $M^T$ and N in 150 unrelated Japanese individuals were 0.547, 0.040 and 0.413, respectively. The heterozygosity, the polymorphism information content (PIC) and the probability of paternity exclusion of this improved MN system were 0.529, 0.425 and 0.232, whereas those of conventional MN system were 0.485, 0.367 and 0.184, respectively.

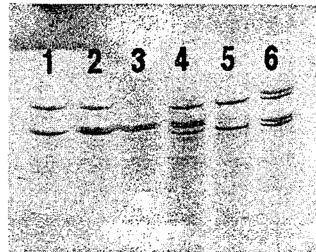


Fig. 3. PCR-SSCP pattern of MN genotypes using primers MN-GF, MN-TF and MN-R (1 : 3 : 1). 1, Genotype $M^GM^G$; 2, $M^GN$; 3, NN; 4, $M^TN$; 5, $M^TM^T$; and 6, $M^GM^T$.

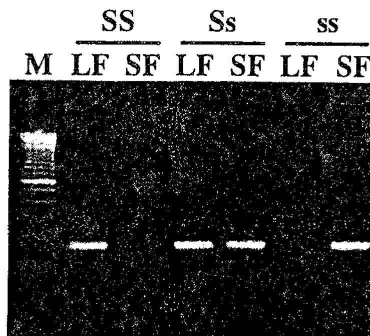


Fig. 4. ASPA pattern of Ss genotypes using an allele-specific primer SS-LF or SS-SF. M: a 100 bp ladder molecular weight standard marker (GIBCO BRL, Gaithersburg, MD, USA).

Fig. 5. PCR-SSCP pattern of Ss phenotypes using primers MN-1F and SS-2R (left), or SS-LF, SS-SF and SS-2R (right).

*Genotyping of Ss blood group*

PCR using the primer SS-LF or SS-SF in combination with SS-2R with the annealing temperature at 60°C resulted in ASPA of S or s allele (Fig. 4). The pattern of *Rsa*I digestion revealed that neither GPA nor GPE sequence was

co-amplified. However, when the annealing temperature was lowered to 58°C, mismatched amplification of S allele by SS-SF and s allele by SS-LF was observed. When annealed at 62°C, no product was detected in the gel even following PCR using matched primers. These results were reproducible when GeneAmp PCR System 2400 or Takara PCR Thermal Cycler MP was used.

The *Rsa*I digestion pattern also confirmed that the GPB sequences were amplified specifically by primers SS-1F and SS-2R. However, the PCR products did not show a polymorphic pattern in the SSCP gel (Fig. 5). In contrast, S and s alleles could be identified clearly by SSCP analysis of the products using primers SS-LF, SS-SF and SS-2R. S and s alleles could not be subtyped further in this study.

## DISCUSSION

MN and Ss red cell antigens are frequently typed for forensic purposes, such as personal identification and parentage testing. Since red blood cells cannot be stored for a long time, it is important to establish the MN and Ss typing using storable DNA samples. PCR-based methods were thus investigated as described below, and we found that conventional M allele of MN system can be divided into two alleles, $M^G$ and $M^T$. These alleles are based on the single base change in the intron, which does not affect the antigen structure. However, this nucleotide substitution and other base changes between M and N alleles can be detected by a single PCR amplification followed by RFLP or SSCP analysis, and the subtyping of M alleles improves the heterozygosity, PIC and probability of paternity exclusion of MN system. Although the frequency of $M^T$ allele is much lower than those of $M^G$ and N, about 8 in 100 individuals have $M^T$ allele(s) in their genotypes ($M^GM^T$, $M^TM^T$ or $M^TN$); it's not so rare. Subtyping of M alleles is thus significant for personal identification and linkage analysis.

Since GPA, GPB and GPE genes are highly homologous, specificity of primers for PCR was important to avoid co-amplification of non-target sequences. The 3′-terminal G in the primer MN-GF is specific to $M^G$ allele. The 3′-end C in the primer MN-TF is specific to GPA gene, and the specificity to $M^T$ and N alleles was attributed to the 15th T in the 24 bp oligonucleotide. Mismatched annealing of MN-TF to $M^G$ allele occurred at 55–58°C, probably because lowering of annealing temperature generally lessens the specificity of primers. Repeated experiments guaranteed appropriate annealing of the primer at 60°C, and neither GPB nor GPE sequence was co-amplified.

Specificity of allele-specific primers SS-LF and SS-SF were based on their 3′-terminal nucleotides, T and C, respectively. When annealed at 60°C, each allele was amplified specifically as had been expected. However, mismatched annealing occurred at 58°C, thus the specificity of the primers depends strictly on the temperature. According to Kwok *et al.* (1990), the only primer/template mis-

matches that can significantly reduce the overall PCR product yields are A/G, G/A, C/C and A/A at the 3′-terminus of a primer, and all other 3′-end mismatches (*e.g.*, T/G and C/A) will allow amplification as efficient as that of the perfectly matched primer/template. In their experiment, 30 bp primers were used and the annealing temperature was set at 55°C. Changes in the PCR conditions such as shortening primer length and raising annealing temperature may improve the specificity of a primer. Since the mismatch of MN-GF against $M^T$ and N alleles is G/A, MN-GF anneals only to $M^G$ allele even at 55°C. In contrast, the 3′-end mismatches of SS-LF/s allele and SS-SF/S allele were T/G and C/A, respectively, so strict setting of the annealing temperature was required to ensure sufficient specificity. The difficulty in Ss genotyping by ASPA reported previously (Corfield *et al.*, 1993) was likely due to the primer specificity. When annealed at 60°C, co-amplification of GPA and GPE genes was not observed.

The MN and Ss genotyping described above was carried out using two thermal cyclers, GeneAmp PCR System 2400 and Takara PCR Thermal Cycler MP. No difference was found in the results between the instruments, although the annealing temperature was extremely strict. This suggested that the results were reproducible at other laboratories when these instruments were used for the typing. Even if other thermal cycler was used, appropriate annealing temperature would be determined around 60°C.

In a simple and rapid ASPA method reported by Nakayashiki and Sasaki (1996), conventional M and N alleles are identified as fragments of different lengths, by shifting the annealing sites of M- and N-specific primers based on three nucleotide substitutions between M and N alleles. Since S and s alleles were attributed to one base change, fragments of the same length were amplified from the alleles in this study. Use of primers differing in length (Watanabe *et al.*, 1997) or shifting the annealing sites may permit the identification of the alleles as different fragments, but it would be difficult to guarantee the specificity of the modified primers in Ss genotyping, as described above. It was also impossible to identify $M^G$, $M^T$ and N alleles by ASPA method. Although $M^G$-specific primer (MN-GF) and N-specific primer (Nakayashiki and Sasaki, 1996) could be designed, a primer specific only to $M^T$ allele could not. $M^T$ allele is characterized by a nucleotide T in the intron and three nucleotides in exon 2, all of which are also observed in GPE gene (Fig. 1). To amplify $M^T$ allele alone, one primer should be specific to GPA gene. Although MN-R acts as a GPA-specific reverse primer, the former nucleotide T is located too far (30–43 bp upstream) from the latter three bases to design an $M^T$-specific forward primer. Thus both $M^T$ and N alleles were amplified using primers MN-TF and MN-R, and further analysis by RFLP or SSCP technique was required to discriminate $M^T$ from N.

Instead of *Mse*I, a restriction enzyme *Dde*I can be used for the RFLP analysis; *Dde*I cuts 199 bp N allele but not 208 bp $M^G$ and 199 bp $M^T$ alleles (Table 1). However, incomplete *Dde*I digestion of N fragment would lead to genotyping NN

as $M^TN$. *Mse*I digests both M and N alleles, and detection of uncut 208 or 199 bp fragment only indicates incomplete digestion of the PCR products. *Mse*I digestion was thus more reliable for the genotyping.

The three alleles, $M^G$, $M^T$ and N, could be detected clearly by SSCP analysis (Fig. 3). Not only to a single base substitution of G to T but also a difference in the fragment length of 9 bp enhanced the difference in electrophoretic mobility in the SSCP gel between $M^G$ and $M^T$ alleles. S and s fragments amplified using primers SS-LF, SS-SF and SS-2R could be separated clearly. However, the sequences amplified using allele-non-specific primers SS-1F and SS-2R did not show a polymorphic pattern (Fig. 5). Although SSCP analysis is known to detect single nucleotide substitutions of the PCR products (Akane *et al.*, 1996; Orita *et al.*, 1989), the SSCP electrophoretic pattern is attributed to the folded conformation of the single-strand fragments stabilized by intrastrand interactions, which may be affected by several factors such as fragment length, number and position of base changes, electrophoresis temperature (Sugano *et al.*, 1995), and gel components. In this study, the effect of electrophoresis temperature was investigated, but no polymorphic pattern could be detected for the SS-1F/SS-2R products (data not shown). The length of the SS-LF/SS-SF/SS-2R products and the distance of the base substitution (T to C) between S and s alleles from the 5′-end of the fragments were shorter than those of the SS-1F/SS-2R products. The base change was likely to affect the folded conformation in the former products but not in the latter. This suggested that designing of primers is important not only in ASPA but also in PCR-SSCP analysis.

*Acknowledgments* The authors wish to thank Dr. N. Nakayashiki for his guidance and valuable criticism. This work was supported in part by a grant from the Ministry of Education, Science, Sports and Culture of Japan.

## REFERENCES

Akane A, Yoshimura S, Yoshida M, Okii Y, Watabiki T, Matsubara K, Kimura K (1996): ABO genotyping following a single PCR amplification. J Forensic Sci **41**: 272-274

Blumenfeld OO, Adamany AM (1978): Structural polymorphism within the amino-terminal region of MM, NN, and MN glycoproteins (glycophorins) of the human erythrocyte membrane. Proc Natl Acad Sci USA **75**: 2727-2731

Corfield VA, Moolman JC, Martell R, Brink PA (1993): Polymerase chain reaction-based detection of MN blood group-specific sequences in the human genome. Transfusion **33**: 119-124

Dahr W, Gielen W, Beyreuther K, Krüger J (1980a): Structure of the Ss blood group antigens. I. Isolation of Ss-active glycopeptides and differentiation of the antigens by modification of methionine. Hoppe-Seyler's Z Physiol Chem **361**: 145-152

Dahr W, Beyreuther K, Steinbach H, Gielen W, Krüger J (1980b): Structure of the Ss blood group antigens. II. A methionine-threonine polymorphism within the N-terminal sequence of the Ss glycoprotein. Hoppe-Seyler's Z Physiol Chem **361**: 895-906

Herrin G Jr, Fildes N, Reynolds R (1994): Evaluation of the AmpliType® PM DNA test system on forensic case samples. J Forensic Sci **39**: 1247-1253

Huang C-H, Blumenfeld OO (1991): Molecular genetics of human erythrocyte MiIII and MiVI glycophorins. J Biol Chem **266**: 7248-7255
Huang C-H, Guizzo ML, McCreary J, Leigh EM, Blumenfeld OO (1991): Typing of MNSs blood group specific sequences in the human genome and characterization of a restriction fragment tightly linked to S-s- alleles. Blood **77**: 381-386
Kudo S, Fukuda M (1989): Structural organization of glycophorin A and B genes: Glycophorin B gene evolved by homologous recombination at Alu repeat sequences. Proc Natl Acad Sci USA **86**: 4619-4623
Kudo S, Fukuda M (1990): Identification of a novel human glycoprotein, glycophorin E, by isolation of genomic clones and complementary DNA clones utilizing polymerase chain reaction. J Biol Chem **265**: 1102-1110
Kudo S, Fukuda M (1994): Contribution of gene conversion to the retention of the sequence for M blood group type determinant in glycophorin E gene. J Biol Chem **269**: 22969-22974
Kwok S, Kellogg DE, McKinney N, Spasic D, Goda L, Levenson C, Sninsky JJ (1990): Effects of primer-template mismatches on the polymerase chain reaction: Human immunodeficiency virus type 1 model studies. Nucleic Acids Res **18**: 999-1005
Nakayashiki N, Sasaki Y (1996): An improved method for MN genotyping by the polymerase chain reaction. Int J Leg Med **109**: 216-217
Orita M, Suzuki Y, Sekiya T, Hayashi K (1989): Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics **5**: 874-879
Rearden A, Phan H, Dubnicoff T, Kudo S, Fukuda M (1990): Identification of the crossing-over point of a hybrid gene encoding human glycophorin variant St$^a$. J Biol Chem **265**: 9259-9263
Sugano K, Fukayama N, Ohkura H, Shimosato Y, Yamada Y, Inoue T, Sekiya T, Hayashi K (1995): Single-strand conformation polymorphism analysis by perpendicular temperature-gradient gel electrophoresis. Electrophoresis **16**: 8-10
Vignal A, Rahuel C, London J, Cherif Zahar B, Schaff S, Hattab C, Okubo Y, Cartron J-P (1990): A novel gene member of the human glycophorin A and B gene family. Eur J Biochem **191**: 619-625
Watanabe G, Umetsu K, Yuasa I, Suzuki T (1997): Amplified product length polymorphism (APLP): a novel strategy for genotyping the ABO blood group. Hum Genet **99**: 34-37