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SHORT COMMUNICATION

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Lewis genotyping by the PCR-RFLP method in a Japanese population and its evaluation in forensic analysis

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Abstract Lewis phenotyping of red blood cells has many problems such as the influence of many biological conditions, the change during the period from newborn to early childhood and mistyping by non-specific anti-Lewis antibodies. Therefore, it would be useful to determine the Lewis genotype. Recently a method of Le-genotyping by PCR-RFLP was established. We determined the frequencies of Lewis genotypes in a Japanese population and discuss the applicability to paternity tests and other forensic applications. The gene frequencies of Le, le1 and le2 in the Japanese population studied were 0.7032, 0.2358 and 0.0610 respectively. Out of 12 paternity cases where paternity was excluded by other markers, 3 alleged fathers could also be excluded by Lewis genotyping. The genotype from organs of a fetus from a 3-month pregnancy was Le/Le. The determination of Lewis genotypes could play a useful role as a genetic marker in paternity tests and forensic analyses.

Key words Lewis genotyping · PCR-RFLP · Japanese population · Forensic analysis

Introduction

Lewis phenotypes of red blood cells (RBC) are used in forensic investigation to predict salivary ABH secretor status. The Lewis phenotyping is influenced by many factors such as pregnancy [2], alcoholic pancreatitis and liver cirrhosis [12], hydatid cysts [8] and cancer [4, 11, 13]. Hauser has indicated that there could be two independent mechanisms for the expression of Lewis^a and Lewis^b substances on the erythrocyte membranes [3]. It is also well known that Lewis phenotypes change during the first few years of life. In addition, Lewis phenotypes are often mistyped. With different commercial Lewis antisera, we have found the same individuals to be phenotyped as Le(a+b+) and Le(a+b–), Le(a+b+) and Le(a–b–), or Le(a–b–) and Le(a–b+). Thus, the actual Lewis blood types could not always be correctly determined by serological tests.

Recently, it has been reported that the expression of Lewis antigens is determined by Le gene-encoded α(1, 3/1, 4) fucosyltransferase (Fuc-TIII) and the le allele has three point mutations, T59G, G508A, and T1067A [9, 10]. Nishihara et al. [10], Yazawa et al. [14] and Liu et al. [7] have developed PCR-RFLP methods to determine the Le genotype.

In this study, the frequencies of Lewis genotypes in a Japanese population were determined using PCR-RFLP methods. The applicability to paternity tests and Lewis typing in infants is also discussed.

Materials and methods

Blood was obtained from 123 unrelated healthy individuals living in Kagawa Prefecture, Japan and from individuals involved in 23 paternity cases. Various organs and tissues were also obtained from a 3-month-old fetus after a miscarriage.

Genomic DNA from blood and organs were isolated by phenol/chloroform extraction. The conditions of amplification and digestion by restriction enzymes were carried out according to Yazawa et al. [14]. Genomic DNA (500 ng) was amplified to obtain the full-length open reading frame of the Fuc-TIII gene. To

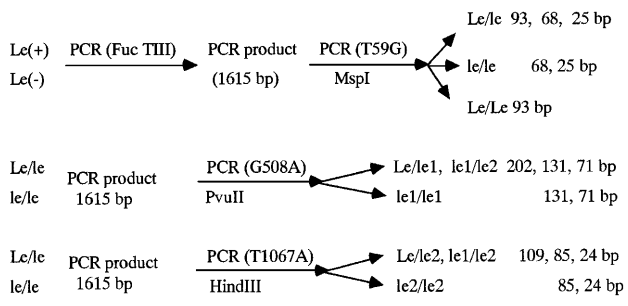

Fig. 1 The strategy of Lewis genotyping

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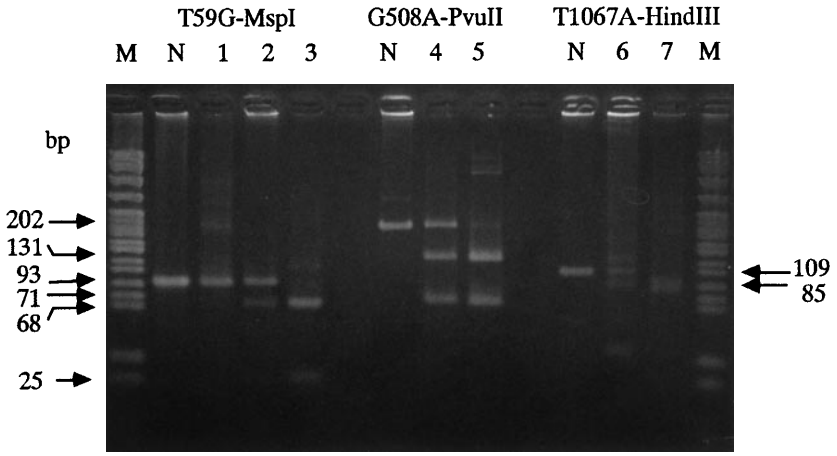

Fig. 2 PCR-RFLPs of Le, le1 and le2 genes. The PCR products for detection of T59G, G508A and T1067A mutations were digested by MspI, PvuII and HindIII for 2 h, respectively. M: pBR322 DNA-MspI digest, N: no digest, 1: Le/Le, 2: Le/le, 3: le/le (1–3; MspI digest), 4: le1 heterozygote, 5: le1 homozygote (4 and 5; PvuII digest), 6: le2 heterozygote, 7: le2 homozygote (6 and 7; HindIII digest). Genomic DNA of the genotype with le2/le2 was supplied by Yazawa

detect the T59G, the G508A, and the T1067A mutations, the first PCR products were amplified with each primer, respectively. The second PCR products for detecting T59G, G508A, and T1067A mutations were digested by MspI, PvuII, and HindIII, respectively (Fig. 1). Separation was performed by 4% agarose (Nusieve GTG: Sea Kem GTG = 3:1; FMC Bioproducts, USA) gel electrophoresis.

Lewis phenotypes were determined on red blood cells by the hemagglutination test. Anti-Le^a and anti-Le^b monoclonal antibodies produced by three companies, Ortho (New Jersey, USA), Biotest (Dreieich, Germany) and Behring (Marburg, Germany), were used.

Results and discussion

All le alleles had the T59 to G mutation. In addition, le1 had a G508 to A mutation and le2 had a T1067 to A mutation [10]. Koda et al. [6] have also reported the T59G point mutation of the le allele. Liu et al. [7] have reported one rare Lewis-negative allele of le3 with the T1067A mutation only.

The strategy of Lewis genotyping is shown in Fig. 1. Since the Le/Le genotype does not have a MspI site, only the 93-bp fragment needs to be detected to determine the T59G mutation. The second PCR products from the heterozygote (Le/le) and homozygote (le/le) forms of the le gene are cleaved into 68 and 25 bp fragments by MspI (Le/le: 93, 68, and 25 bp, le/le: 68 and 25 bp). To detect the G508A mutation, the 202-bp PCR product of le1 gene is digested by PvuII. The products from the heterozygote (Le/le1 and le1/le2) and homozygote (le1/le1) forms of le1 are cleaved into 131 and 71 bp fragments (Le/le1 or le1/le2: 202, 131, and 71 bp, le1/le1: 131 and 71 bp). The 109-bp PCR products from the heterozygote (Le/le2 and le1/le2) and homozygote (le2/le2) forms of le2 are cleaved into 85 and 24 bp by HindIII (Le/le2 or le1/le2: 109, 85, and 24 bp, le2/le2: 85 and 24 bp). For amplification a minimum of 50 ng template DNA was needed. PCR-RFLPs of the Le, le1, and le2 genes from blood are shown in Fig. 2. The five genotypes, Le/Le, Le/le1, Le/le2, le1/le1 and le1/le2, were detected in our examinations but le2/le2 has not yet been found. The DNA with the le2/le2 genotype was supplied by Yazawa (Gunma University School of Medicine).

Table 1 Distribution of Lewis genotypes and allele frequencies in Japanese (sample: 123 individuals from Kagawa Pref.)

Genotypes	No. observed (%)	No. expected (%)	Gene frequency
Le/Le	64 (52.0)	60.9 (49.5)	Le = 0.7032
Le/le1	33 (26.8)	40.8 (33.2)	le1 = 0.2358
Le/le2	12 (9.8)	10.6 (8.6)	le2 = 0.0610
le1/le1	11 (9.0)	6.9 (5.6)	
le1/le2	3 (2.4)	3.6 (2.9)	
le2/le2	0 (0)	0.5 (0.37)	

$\chi^2 = 4.924$; df = 5; $0.25 < P < 0.5$

The distributions of Lewis genotypes and allele frequencies of Le, le1 and le2 in the Japanese population are shown in Table 1 and five genotypes were observed in the samples tested. Of the 109 Lewis-positive phenotype individuals [Le(+), Le(a+b–) and Le(a–b+)] 64 were Le/Le genotype. The numbers of heterozygous genotypes with Le/le1 and Le/le2 were 33 and 12, respectively. Of the 14 Lewis-negative phenotype individuals [Le(–), Le(a–b–)] 11 were le1/le1, the remaining 3 were le1/le2. Our data were compared with those reported by Nishihara et al. [10] and Liu et al. [7] using a RXC contigency table test and the results indicated that the genotype frequencies are similar. ($\chi^2 = 13.6263$, df = 12, $0.25 < P < 0.500$). The power of the discrimination in Lewis genotype was calculated to be 0.63 and the probability of exclusion 0.22.

To evaluate the applicability of this Lewis genotyping to paternity testing, 23 cases were examined. In 12 cases in which paternity was excluded by other genetic markers, 3 alleged fathers could also be excluded by the Lewis genotyping as shown in Table 2. It appears that the infant in case 2 became Le(a+b+) at the age of 10 months. The Lewis phenotypes of the individuals in cases 1 and 3 did not contribute to the paternity test. In 11 cases where paternity was practically proven, the Lewis genotypes supported the familiar relationship. These results demonstrate that Lewis genotyping is useful in paternity testing cases.

There are some reports about the Lewis phenotype of fetuses. Jordal [5] reported that 50 fetuses 3–6 months of age and 152 cord samples examined were Le(–) only, but

Table 2 Three cases of non-paternity proven by Lewis genotyping

Case no.		Lewis phenotype	Lewis genotype
1	M	Le(a–b+)	Le/Le
	C	Le(a–b+)	Le/le2
	AF	Le(a–b+)	Le/le1
2	M	Le(a–b+)	Le/Le
	C	Le(a+b+)	Le/le2
	AF	Le(a–b+)	Le/Le
3	M	Le(a–b+)	Le/le1
	C	Le(a–b+)	Le/Le
	AF	Le(a–b–)	le1/le1

M: mother, C: child, AF: alleged father

Cutbush et al. [1] reported that 13 out of 22 samples of cord blood revealed the Le^a antigen. We determined the Lewis genotype of each of the main organs in a miscarried fetus after a 3-month pregnancy. The heart, lung, liver, kidney and digestive organs from the fetus were all Le/Le genotype. Although we could not obtain the fetus blood, the Lewis phenotype in our single fetus was estimated to be Le(+) from these results. Further investigation about the Lewis genotypes of fetus samples in order to confirm the discrepancy in reports between Jordal [5] and Cutbush et al. [1] is needed. Lewis genotyping is more useful than Lewis phenotyping which changes during the period from newborn to early childhood.

In our study, the phenotypes of 4 out of 15 Le(–) individuals did not conform with their genotypes which were Le/le1. It is necessary to select anti-Le^a or anti-Le^b monoclonal antibodies which can precisely distinguish Lewis phenotypes.

The determination of Lewis genotypes could play a more useful role as a genetic marker in paternity tests and forensic analyses in comparison with Lewis phenotyping which is only used to predict salivary ABH secretor status.

References

1. Cutbush M, Giblett ER, Mollison PL (1956) Demonstration of the phenotype Le(a+b+) in infants and adults. Br J Haematol 2:210–220
2. Hammer L, Mansson S, Rohr T, Chester MA, Ginsburg V, Lundblad A, Zopt D (1981) Lewis phenotype of erythrocytes and Le^b actine glycolipid in serum of pregnant women. Vox Sang 40:27–33
3. Hauser R (1995) Observations on the $Lewis^a$ and $Lewis^b$ activity of erythrocytes. Int J Legal Med 108:50–52
4. Hirano K, Kawa S, Oguchi H, Kobayashi T, Yonekura H, Ogata H, Homma T (1984) Loss of Lewis antigen expression on erythrocytes in some cancer patients with high serum CA19-9 levels. J Natl Cancer Inst 79:1261–1268
5. Jordal K (1956) Lewis blood groups in children. APMIS 39: 399–406
6. Koda K, Soejima M, Kimura H (1995) Detection of T to G mutation at position 59 in the Lewis gene by mismatch polymerase chain reactions. Int J Legal Med 107:280–282
7. Liu Y, Koda Y, Soejima M, Uchida N, Kimura H (1996) PCR analysis of Lewis-negative gene mutations and the distribution of Lewis alleles in a Japanese population. J Forensic Sci 41: 1018–1021
8. Makni S, Dalix AM, Caillard T, Compagnon B, Pendu JL, Ayed K, Oriol R (1987) Discordance between red cell and saliva Lewis phenotypes in patients with hydatid cysts. Exp Clin Immunogenet 4:136–143
9. Nishihara S, Yazawa S, Iwasaki H, Nakazato M, Kudo T, Ando T, Narimatsu H (1993) α (1,3/1,4) Fucosyl transferase (FucTIII) gene is inactivated by a single amino acids substitution in Lewis histo-blood type negative individuals. Biochem Biophys Res Commun 196:624–631
10. Nishihara S, Narimatsu H, Iwasaki H, Yazawa S, Akamatsu S, Ando T, Seno T, Narimatsu I (1994) Molecular genetic analysis of the human Lewis histo-blood group system. J Biol Chem 269:29271–29278
11. Ørntoft TF, Holmes EH, Johnson P, Hakomori S, Clausen H (1991) Differential tissue expression of the Lewis blood antigens: enzymatic, immunohistologic, and immunochemical evidence for Lewis a and b antigen expression in Le(a–b–) individuals. Blood 77:1389–1396
12. Stigendal L, Olsson R, Rydberg L, Samuelsson BE (1984) Blood group Lewis phenotype on erythrocytes and in saliva in alcoholic pancreatitis and chronic liver disease. J Clin Pathol 37:778–786
13. Yazawa S, Asao T, Izawa H, Miyamoto Y, Matta KL (1988) The presence of CA19-9 in serum and saliva from Lewis blood-group negative cancer patients. Jpn J Cancer Res 79: 538–543
14. Yazawa S, Oh-kawara H, Nakajima T, Hosomi O, Akamatsu S, Kishi K (1996) Histo-blood group Lewis genotyping from human hairs and blood. Jpn J Hum Genet 41:177–188