BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **222,** 390–394 (1996)
ARTICLE NO. 0754

Heterogeneity of the Human *Secretor* α(1,2)Fucosyltransferase Gene among Lewis(a+b−) Non-secretors

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Received March 20, 1996

The human *Secretor* α(1,2)fucosyltransferase gene determines the ABH secretor status and influences the Lewis phenotype of an individual. Two different *se* alleles with point mutations, C^{571} to T and G^{849} to A respectively, in the coding region were identified in Le(a+b−) non-secretors from one of the Taiwanese indigenous groups. The base substitutions predict the alteration of Arg^{191} and Trp^{283} to stop codons respectively, resulting in deletion of the Secretor enzyme's C-terminal segment. Both alleles of the *Secretor* locus in all Le(a+b−) non-secretors, but not in Le(a−b+) secretors, were further demonstrated to be either one of these two *se* alleles with nonsense mutations. These results suggest two new molecular bases for the null *se* allele responsible for the formation of the non-secretor phenotype.

The human Lewis and ABH histo-blood group systems belong to a family of structurally related oligosaccharides, which are synthesized by the sequential action of glycosyltransferases. These molecules were first identified as red blood cell (RBC) antigens, but later were also discovered in the exocrine secretions, including saliva (for reviews, see Ref. 1–3). The expression of the two main Lewis antigens, Le^a (4) and Le^b (5), is controlled by the interaction of the products of two genetically independent loci, the *Secretor* (*Se* or FUT2) (6–8) and the *Lewis* (*Le* or FUT3) (9) loci, encoding the *Secretor* α(1,2)fucosyltransferase and the *Lewis* α(1,3/1,4)fucosyltransferase, respectively. The *Se* locus is also responsible for the formation of salivary ABH substances, and thus correlation of the Lewis phenotype with the ABH secretor status of an individual has long been observed (10). In Lewis negative individuals (*le/le* genotype) (11,12), the *Se* genotype dose not affect the Lewis negative phenotype, Le(a−b−), but different *Se* genotypes divide Lewis positive individuals (*Le/Le* or *Le/le* genotypes) into different Lewis-secretor phenotypes: Le(a−b+) secretors having *Se/Se* or *Se/se* genotypes, Le(a+b−) non-secretors having the *se/se* genotype (for a review, see Ref. 13) and Le(a+b+) partial secretors having homozygosity for the weak *Secretor* allele (Se^w/Se^w)(14–16).

The expression of the Lewis-secretor phenotypes varies considerably among different population (15,17–21). In Caucasians, three Lewis phenotypes are present: Le(a+b−), Le(a−b+) and Le(a−b−) (20,21), whereas in Taiwanese Chinese (17,18) and some of the Taiwanese indigenous groups (19), the Le(a+b−) phenotype is replaced by the Le(a+b+) phenotype so that the three Lewis phenotypes present in these groups are Le(a−b+), Le(a+b+) and Le(a−b−). However, the remaining indigenous groups express all the four different Lewis phenotypes: Le(a+b−), Le(a−b+), Le(a+b+) and Le(a−b−) (19). Recently, the *Secretor* α(1,2)fucosyltransferase gene was cloned and the molecular basis for a null *se* allele resulting in the non-secretor phenotype among the U.S. population was also demonstrated (7,8). An enzyme-inactivating nonsense mutation (G^{428} to A), altering the Trp^{143} to a stop codon in the *Se* gene, was demonstrated to be responsible for this non-secretor phenotype.

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0006-291X/96 $18.00

The non-secretor phenotype appears to be absent (or rare) among Taiwanese Chinese, but recently was found in some of the Taiwanese indigenous groups (19). Nevertheless, the nonsense mutation found commonly in the *se* alleles of non-secretors reported previously (8) is not present in the *se* alleles of the Taiwanese indigenous groups with the Le(a+b−) non-secretor phenotype. In this paper, two new *se* alleles with different nonsense mutations identified in the Taiwanese indigenous group are reported and correlation of these *se* alleles with the Le(a+b−) non-secretor phenotype is also demonstrated.

MATERIALS AND METHODS

Sample preparation. Blood and saliva samples from healthy Taiwanese indigenous Paiwan group (19) were collected. The RBC Lewis phenotype was determined by a microplate method (19) using mouse monoclonal anti-Le^a (LM112/161, batch 041/01/90) (22) and anti-Le^b 073 (LM129/181 anti-Le^{bL}, batch 010/01/90) (23) antibodies, which were a gift from Dr. R.H. Fraser of Glasgow and the West Scotland Blood Transfusion Service at Law Hospital, U.K.. Salivary ABH and Lewis substances were determined by a salivary inhibition study (19,24). Blood samples from individuals determined as Le(a+b−) non-secretors and Le(a−b+) secretors were used in this study. Genomic DNAs were prepared from leucocytes by a proteinase K-SDS method (25).

Molecular cloning of Se allele. The polymerase chain reaction (PCR) and a pair of specific primers for the Sec2 DNA segment (8) encoding the *Secretor* α(1,2)fucosyltransferase gene were used to amplify the coding region of the *Se* gene. The sense primer locates at nucleotides −79 through −51 of the *Se* gene (CCTCCATCTCCCAGCTAACGTGTCCCGTT), 5′ to the first initiation codon, and the antisense primer (GCTTCTCATGCCCGGGCACTCATCTTGAG) is complementary to nucleotides 1042 through 1070 within the 3′ untranslated region. 200 ng of genomic DNA and 0.3 μM of each primer were combined in 50 μl of PCR buffer containing low concentrations of dNTP (15 μM each) and *Taq* polymerase (0.4 unit) (Promega, Madison, WI, U.S.A.) to minimize the PCR-mediated DNA sequence alteration. The PCR program consisted of 5 min at 94°C followed by 30 cycles of 1.5 min at 94°C and 3 min at 72°C. The 1149 bp PCR products were cloned into pGEM-T vectors (Promega). DNA sequences were determined by the dideoxy chain termination method (26) using an AmpliCycle Sequencing Kit (Perkin Elmer, Foster City, CA, U.S.A.). Multiple clones from two batches of PCR products were sequenced to distinguish PCR errors from actual sequence polymorphisms.

PCR-RFLP analysis. Restriction fragment length polymorphism (RFLP) analyses were designed to detect the sequence polymorphisms in the *Se* allele. PCR was used to sample the nucleotide positions 571 and 849. The sense primer anneals to positions −15 through 15 (CCTTTCTCCTTTCCCATGGCCCACTTCATC) of the *Se* gene and the antisense primer was the same as that used in the cloning of the *Se* gene (see above). 200 ng of genomic DNA, 0.6 μM of each primer, 200 μM of each dNTP and 1 unit of *Taq* polymerase were combined in 50 μl of PCR buffer, and then subjected to the same PCR program described above. The PCR products of 1085 bp were digested with *Dde*I restriction enzyme (Premega) and analyzed by 2.5%-agarose-gel electrophoresis.

ASO hybridization analysis. Another set of PCR primers (sense, CAGGTGAACGGGAGCCGGCCGGGCA, nucleotides 523–547; antisense, GGAGAAAAGGTCTCAAAGAATGGGCCAGCA, complementary to nucleotides 1000–1029) was used to sample the nucleotide positions 571 and 849 in the *Se* gene for allele-specific oligonucleotide (ASO) hybridization analysis. The same PCR conditions as those for the PCR-RFLP analysis described above were used. The PCR products of 507 bp were fixed to nylon membranes by a dot filtration manifold (Gibco BRL, Grand Island, NY, U.S.A.) and probed with ^{32}P-labelled (27) allele-specific oligonucleotides (C^{571} wild type probe, GTTCGCCGAGGGGA; T^{571} mutant probe, GTTC-GCTGAGGGGA; G^{849} wild type probe, GGATCTGGGCCGCA; A^{849} mutant probe, GGATCTGAGCCGCA). After denaturing and UV crosslinking procedures, membranes were hybridized at 37°C in 5×SSC (1×SSC consists of 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 5×Denhardt's solution, 0.5% SDS and 0.1 mg/ml of sheared salmon sperm DNA. The membranes were washed once at room temperature, and three times at 44°C (wild type probes) or 43°C (mutant probes) in 2×SSC and 0.1% SDS for 20 min at each interval, and subjected to autoradiography.

RESULTS AND DISCUSSION

Since the nonsense mutation, G^{428} to A, in the *se* allele reported recently (8) creates an *Mae*I restriction site (CTAG), a PCR-RFLP analysis for *Mae*I was designed to examine the *Se* alleles of the Taiwanese indigenous group with the Le(a+b−) non-secretor phenotype. The results demonstrated that none of the seven Le(a+b−) non-secretors analyzed has the mutation in each *Se* allele (results not shown). Thus the *Se* alleles of these Le(a+b−) non-secretors were further analyzed through molecular cloning and DNA sequencing.

The coding region of the *Se* gene of one Le(a+b−) non-secretor was cloned. The DNA sequence of 1063 bp, from nucleotide −50 to 1013 of the Sec2 DNA segment encompassing the whole coding

region of the longer isoform of the *Se* gene (8) was analyzed. The inserts of five recombinant plasmids from two batches of PCR products were sequenced. Two different alleles, designated as *se3* and *se4* respectively as shown in Fig. 1, were identified. The *se3* and *se4* alleles comprise the C^{571} to T and G^{849} to A mutations respectively, which yield translation termination codons within the open reading frame of the *Secretor* α(1,2)fucosyltransferase gene. The two DNA sequence polymorphisms correspond to the alteration of the Arg^{191} and Trp^{283} residues to stop codons and are predicted to truncate 142 and 50 amino acid residues of the Secretor enzyme's C-terminal segment, respectively. The silent mutation at position 357 (C to T) in the *se3* allele and the mutations within the 3′ untranslated region (G^{1009} to A and C^{1011} to T) in both alleles are also present in the wild type *Sel* and a potential Se^{w} allele identified in Taiwanese Chinese (16). Two of the five clones analyzed demonstrated the four mutations of the *se3* allele, and the remaining three clones demonstrated the three mutations of the *se4* allele, suggesting that the Le(a+b−) non-secretor propositus is a heterozygote with the *se3/se4* genotype, which was further ascertained by PCR-RFLP and ASO hybridization analyses.

To further inspect whether the Le(a+b−) non-secretor phenotype is accompanied by the two *se* alleles newly identified, PCR-RFLP and ASO hybridization analyses for detection of the sequence polymorphism at positions 571 and 849 of the *Se* allele were used to analyze the *Se* genotype of Le(a+b−) nonsecretors and Le(a−b+) secretors. Both of the mutations create a *Dde*I restriction site (CTNAG) (Fig. 1), and thus a PCR-RFLP analysis for *Dde*I was designed. Two DNA fragments, 161 and 924 bp, were generated from the PCR product of 1085 bp amplified from a wild type *Se* allele by *Dde*I enzyme digestion, whereas the PCR products amplified from the *se3* and *se4* alleles were further cleaved into 161, 425, 499 bp and 161, 223, 701 bp DNA fragments respectively. As shown in Fig. 2, each *Se* allele of the seven unrelated Le(a+b−) non-secretors analyzed, including the propositus, presented either the C^{571} to T or G^{849} to A mutations, and the three different *Se* genotypes resulting from the *se3* and *se4* alleles (*se3/se3, se3/se4* and *se4/se4*) were all found among these Le(a+b−) non-secretors. The *Se* genotypes determined by the PCR-RFLP analysis (Fig. 2A) agreed with the results of the ASO hybridization analysis (Fig. 2B). On the other hand, at least one wild type *Se* allele at positions 571 and 849 was obtained in each of the thirteen unrelated Le(a−b+) secretors analyzed (Fig. 3) and heterozygotes with *Se/se3* (lane 9) or *Se/se4* (lanes 2,3,5,6,8) genotypes still demonstrated the Le(a−b+) secretor phenotype. The coding region of the *Se* allele of one of the Le(a−b+) secretors was also cloned and analyzed, and demonstrated

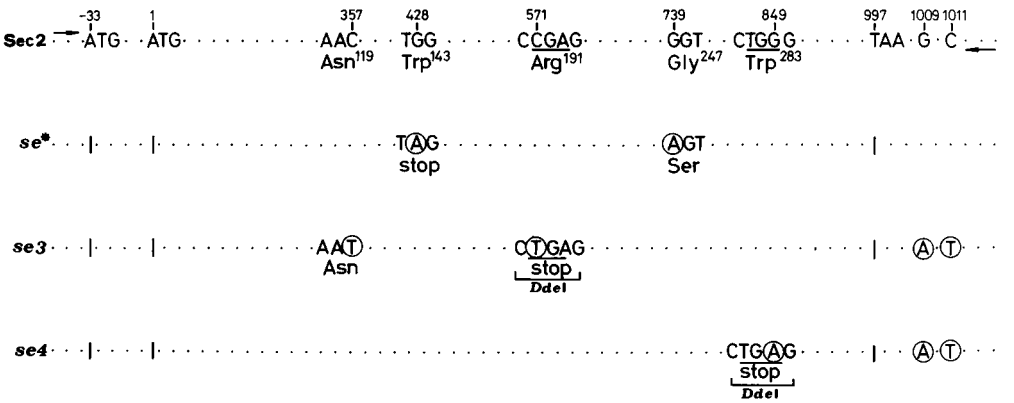

FIG. 1. Location of mutations within the *Se* allele. Sec2 indicates the coding region of the *Secretor* α(1,2)fucosyltransferase gene reported by Kelly *et al.* (8). The two ATG and the TAA codons correspond to the enzyme's initiation and termination codons, respectively. PCR primers used to amplify the coding region are indicated by arrows. *se** represents the null *se* allele identified earlier (8). *se3* and *se4* represent the variants of the *Se* allele reported in this paper. Point mutations found in these variants are circled.

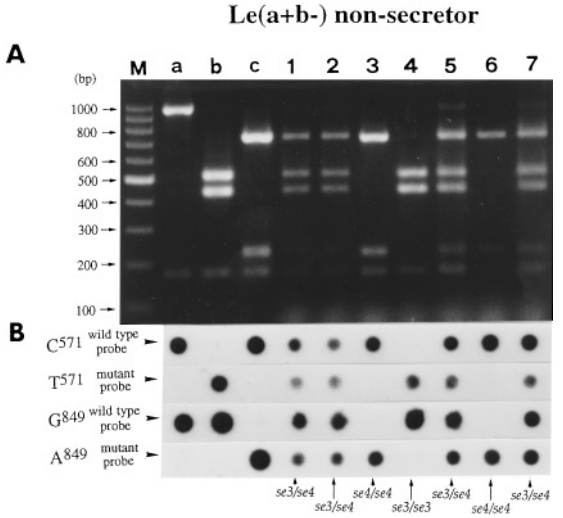

FIG. 2. Analysis of the C^{571} to T and G^{849} to A sequence polymorphisms of the *Se* alleles in Le(a+b−) non-secretors. (A) PCR-RFLP analysis. Both of the two mutations create a *Dde*I restriction site. The PCR products of 1085 bp encompassing the polymorphic sites were generated from genomic DNA obtained from each of seven unrelated Le(a+b−) non-secretors (lane 1–7) and were digested with *Dde*I, followed by 2.5%-agarose-gel-electrophoretic analysis. *Dde*I cleavage of the PCR product from a wild type *Se* allele yields two segments of 161 and 924 bp, whereas the products from *se3* and *se4* alleles were further cleaved into 161, 425, 499 bp and 161, 223, 701 bp DNA fragments respectively. Results obtained with control samples derived from the cloned wild type, *se3* and *se4* alleles are displayed in lanes a, b and c, respectively. Molecular size standards (bp) are in lane M. (B) ASO hybridization analysis. The PCR products of 507 bp encompassing the polymorphic sites were generated from control templates of the cloned wild type, *se3* and *se4* alleles and also for each individual and probed with ^{32}P-labelled wild type and mutant oligonucleotides, as described in Materials and Methods. Results obtained from the Le(a+b−) non-secretor propositus for the molecular cloning of the *Se* allele are in lane 1. *Se* locus genotype of each individual denoted under each lane is inferred from the results of PCR-RFLP and ASO hybridization analyses.

a wild type *Se1* allele bearing the silent mutation C^{357} to T, identified in Taiwanese Chinese previously (16).

These results demonstrate a strong correlation of the two nonsense mutations in the *Se* allele with the formation of the Le(a+b−) non-secretor phenotype in one of our Taiwanese indigenous groups.

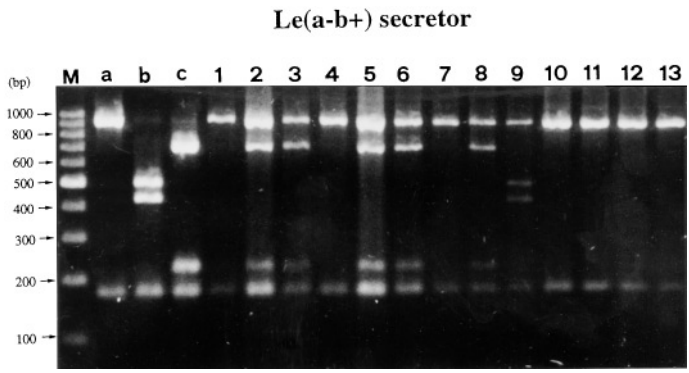

FIG. 3. PCR-RFLP analysis of the C^{571} to T and G^{849} to A sequence polymorphisms of the *Se* allele in Le(a−b+) secretors. The PCR products of 1085 bp generated from control templates of the cloned wild type, *se3* and *se4* alleles (lanes a, b and c respectively) and from genomic DNA obtained from each of thirteen unrelated Le(a−b+) secretors (lanes 1–13) were digested with *Dde*I, followed by 2.5%-agarose-gel-electrophoretic analysis, as described in Fig. 2A and Materials and Methods. Molecular size standards (bp) are in lane M.

Obviously, the deletion of the C-terminal end of the Secretor enzyme resulting from the two nonsense mutations will abolish, or greatly reduce at least, the activity of the corresponding α(1,2)fucosyltransferases. Confirmation of this awaits further research through functional analysis of the *se3* and *se4* alleles.

The frequencies of different Lewis-secretor phenotypes vary markedly among different populations (15,17–21). Three variants of the *Se* allele affecting the Lewis-secretor phenotype have been identified since the successful clonging of the *Secretor* locus (8). Previously, we proposed a variant of the *Se* allele, *Sw* having a missense mutation (A^{385} to T) (16), to be the candidate for the weak *Secretor* allele (14,15) responsible for the Le(a+b+) phenotype, which has a relatively high incidence among Taiwanese Chinese (17,18) but is virtually absent in Caucasians (20,21). Now, two new *se* alleles, different to the common null *se* allele in Caucasian non-secretors (8), appear to account for the non-secretor phenotype in one of the Taiwanese indigenous groups. Other variants of the *Se* allele may await identification. A systematic study of the molecular basis for the *Se* alleles responsible for the different Lewis-secretor phenotypes in different ethnic populations should be both interesting and significant.

ACKNOWLEDGMENTS

This work was partially supported by Grant DOH-84-HR-223 from the National Health Research Institute and Grant NSC 85-2331-B-195-009 from the National Science Council, Taiwan.

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