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A Second Nonsecretor Allele of the Blood Group α(1,2)Fucosyltransferase Gene (FUT2)

Abstract

While screening Le(a+b+) Polynesian DNA samples for a candidate *Se*^{w} allele, a point mutation ($C^{571}\rightarrow T$) resulting in a new stop codon ($Arg^{191}\rightarrow$stop) in the α(1,2)fucosyltransferase gene (FUT2) was identified. This point mutation resulted in the gaining of a new restriction enzyme cleavage site (*Dde*I), which allowed restriction enzyme cleavage screening of 40 selected Polynesians and 42 random Caucasians. The nonsecretor phenotype in two of the three nonsecretor Polynesians analyzed was due to homozygosity for the 'new' mutation, whereas the third Polynesian nonsecretor (with Caucasian ancestors) was due to homozygosity of the 'old' ($Trp^{143}\rightarrow$stop) mutation. The nonsecretor phenotype in all Caucasians analyzed was a consequence of homozygosity for the 'old' mutation. Both the new and the old nonsecretor mutations were identified in the heterozygous state in some secretor-positive Polynesians, while only the old mutation was found in the heterozygous state in Caucasians of the same phenotype.

Introduction

Expression of type 1 ABH and Le^{b} blood group antigens in human exocrine secretions is determined by the presence of a secretor (*Se*) gene [reviewed in ref 1]. The term 'secretor' strictly refers to the ability to make large amounts of soluble A, B and H determinants, usually detected in saliva, with the ABH substances found being dependent on the ABO blood group of the individual [2]. The relative expression of the Le^{a} or Le^{b} antigens is dependent not only on the Lewis genotype but also on the individual's genetically independent secretor genotype. Fucosyltransferases coded by Lewis and secretor genes thus compete with each other and with other glycosyltransferases to determine the Lewis and secretor phenotypes of an individual. In Lewis-negative individuals, the secretor genotype does not affect the Lewis phenotype, but in Lewis-positive individuals, the nonsector genotype generates the Le(a+b–) red cell phenotype, the secretor genotype causes the Le(a–b+) red cell phenotype, and the partial-secretor genotype gives rise to the Le(a+b+) red cell phenotype [reviewed in ref 3].

Genetic, biochemical and molecular analyses indicate that the *Se* gene, termed FUT2 in the Genome Data Base, corresponds to an α(1,2)fucosyltransferase gene distinct from the H blood group α(1,2)fucosyltransferase [4–8]. The recent identification and sequencing of the *Se* gene predicts a 332-amino-acid-long polypeptide sharing 68% sequence identity with the COOH-terminal 292 residues of the human H blood group α(1,2)fucosyltransferase. A nonsecretor allele, causing the Le(a+b–) phenotype in Lewis-positive individuals, corresponded with the homozygous presence of an enzyme-inactivating nonsense point mutation ($Trp^{143}\rightarrow$stop) at this locus [9].

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Determination of secretory phenotypes is usually done serologically by inhibiting ABH antiserum with saliva. In Lewis-positive individuals, it is sometimes deduced from the direct red cell agglutination test, because Le(a–b+) and Le(a+b–) red cell phenotypes correlate with secretor and nonsecretor salivary phenotypes, respectively. These techniques, however, may be inaccurate, particularly in certain circumstances (e.g. pregnancy, disease, transfusion, transplantation) and in some populations (e.g. Polynesians [3], Asians [10], Australian aborigines [11]), and so may not necessarily accurately reflect the genotype of an individual [reviewed in ref 3]. Genotyping now offers the opportunity to determine the secretor status of an individual without the requirement to phenotype. However, before this can happen, all alleles should be identified.

In this paper we report the presence of a second FUT2 nonsecretor allele (Arg^{191}→stop) which in homozygous form correlates with the salivary ABH nonsecretor and red cell Le(a+b–) phenotype in two Polynesian Lewis-positive individuals.

Materials and Methods

Samples and Phenotypes

The 40 Polynesian samples were mostly from previous studies where they were selected on the basis of unusual red cell phenotypes. These samples have been extensively Lewis and secretory phenotyped and in some instances glycolipids were immunochemically profiled [12–15]. Lewis phenotypes reported are those based on red cell serology; however, for 3 samples, the Le(a+b–) phenotype was not supported by glycolipid analysis of plasma [15, unpubl. obs.]. These 3 samples (033, 057 and 114) are examples of the Le(a+b+) phenotype in which the Le^b reaction on red cells cannot be serologically demonstrated routinely, and these samples have been classified here as Le(a+b+).

The Caucasian samples were predominantly from another study of Swedish blood donors [16], and although they had been selected on the basis of being predominantly Lewis negative (16/42), no selection was made for secretor phenotypes. The Swedish Caucasian samples were Lewis phenotyped on red blood cells with monoclonal Lewis reagents (Dominion Biologicals, Dartmouth, Canada) and the Lewis-negative samples were salivary ABH phenotyped as described elsewhere [17].

DNA Preparation

The DNA preparation technique was essentially according to Miller et al. [18]. After lysis of the erythrocytes, the remaining leukocytes/nuclei were treated with proteinase K in EDTA buffer with SDS. After addition of 5 *M* NaCl, DNA was extracted from the supernatants by precipitation, washing and drying, and finally dissolved to a concentration of approx 500 μg/ml in TE buffer (10 m*M* Tris-HCl, 1 m*M* Na_2-EDTA, pH 7.5).

PCR Amplification

PCR amplification was performed on a PTC-100 Programmable Thermal Controller (MJ Research, Watertown, USA). Half a microgram of genomic DNA was used as template for each amplification reaction. The primers were purchased from Scandinavian Synthesis, Köping, Sweden, and diluted in sterile water to 25 μ*M* for PCR amplification, and to 0.5 μ*M* for nucleotide sequencing.

All PCR amplifications were done with heating to 85°C for 5 min before adding 1 U Taq DNA polymerase (5 U/μl, No. 1435 094, Boehringer Mannheim, Mannheim, Germany). PCR amplification was run for 30 cycles with denaturation at 94°C for 1.5 min, annealing/extension for 2.5 min at 72°C and stopped by cooling to 8°C [9].

All PCR products and fragments after restriction enzyme cleavage were analyzed by agarose gel electrophoresis in TBE buffer (45 m*M* Tris borate, 1 m*M* Na_2-EDTA, pH 8.0) with 5 μl ethidium bromide and run at 125 V. The molecular-weight marker used was the *Msp*I digest of pBR322 DNA (No. 73318, United States Biochemical, Cleveland, Ohio, USA).

Nucleotide Sequencing

PCR-amplified FUT2 gene segments were separated on a 2% NuSieve® GTG® agarose gel (No. 50082, FMC BioProducts, Rockland, Me., USA). The bands were excised, melted and extracted with TE buffer (20 m*M* Tris-HCl, 1 m*M* Na_2-EDTA, pH 7.8), partitioned with phenol and the DNA was extracted and precipitated with ethanol. A DNA sequencer model 373A (Applied Biosystems, Calif., USA) and the DyeDeoxy™ terminator cycle sequencing technique (No. 401095, Perkin Elmer-Cetus, Calif., USA) were used according to the manufacturer's protocol with Taq DNA and the cyclic amplification reaction run for 15 s at 96°C, 1 s at 50°C, 4 min at 60°C and stopped at 4°C after 25 cycles. The products were purified by repeated phenol-chloroform extractions, chloroform extraction, precipitation, centrifugation, washing in ethanol and drying. Finally, the samples were dissolved in 6 μl of deionized formamide (No. 72020, IBI, New Haven, Conn., USA) containing 50 m*M* Na_2-EDTA 5:1 (vol/vol) and 3% blue dextran (no. D-5751, Sigma, St. Louis, Mo., USA), heated for 2 min at 95°C, cooled on ice and loaded onto 6% polyacrylamide sequencing gels.

Mutation Screening with Restriction Enzyme Cleavage

Restriction enzyme *Bst*NI (10 U/μl, No. 168 S, New England Biolabs, Beverly, Mass., USA) cleavage identification of the Trp^{143}→stop (nucleotide G^{428}→A) mutation was used with PCR products obtained from the primers as described elsewhere [9]. Restriction cleavage products were separated on a 4% NuSieve GTG agarose gel.

Restriction enzyme *Dde*I (10 U/μl, No. 835293, Boehringer Mannheim) cleavage for the Arg^{191}→stop (nucleotide C^{571}→T) mutation was used with PCR products obtained from the *Bst*NI sense primer (as above) and a new antisense primer 5'-CGTCCCAATGGTCATGATGGTGTGGTTACA-3'. *Dde*I cleavage reactions were performed at 37°C for 2 h and the reactions were stopped with gel-loading solution (G 2526, Sigma). Restriction cleavage products were separated on a 2% agarose gel (No. 15510–027, Gibco-BRL, Paisley, UK).

Results

The coding sequence of the FUT2 gene of 13 individuals [10 Polynesians: 9 Le(a+b+) and 1 Le(a–b–) partial secretor; 3 Caucasians: 2 Le(a+b–) and 1 Le(a–b–) secretor] were completely sequenced (both strands). Heterozygosity (C and T) of the nucleotide sequence at position 571 (wild type corresponding to Arg^{191}) was found in 3 Polynesian sam-

Table 1. Presence and zygosity of the *Bst*NI-determined se^{428} allele and the *Dde*I-determined se^{571} allele in each Lewis and salivary phenotype for Polynesians and Caucasians

	Red cell Lewis phenotype	n	se^{428}		se^{571}		Unmutated
			homo	hetero	homo	hetero	
Polynesians							
Secretors	Le(a–b+)	18	0	2	0	3	13
	Le(a+b+)[a]	14	0	1	0	3	10
	Le(a–b–)	5	0	0	0	0	5
Nonsecretors	Le(a+b–)	2	0	0	2	0	0
	Le(a–b–)	1	1	0	0	0	0
Caucasians							
Secretors	Le(a–b+)	20	0	13	0	0	7
	Le(a–b–)	10	0	6	0	0	4
Nonsecretors	Le(a+b–)	6	6	0	0	0	0
	Le(a–b–)	6	6	0	0	0	0

Secretors include both normal and partial secretors. homo = Homozygous state of mutated allele; hetero = heterozygous state of mutated allele.

[a] Includes 3 individuals whose red cells phenotype as Le(a+b–) but by glycolipid analyses are Le(a+b+).

ples. Translation of this ($C^{571}\rightarrow T$) mutation revealed the codon would read as a stop. The mutation also resulted in the gain of a new restriction enzyme cleavage site for *Dde*I.

All available samples were screened with the appropriate restriction enzymes for both the se^{428} ($TRP^{143}\rightarrow$stop) and se^{571} ($Arg^{191}\rightarrow$ stop) mutations (table 1). The *Bst*NI restriction enzyme cuts a 195-base pair (corresponding to nucleotides 370 to 564) PCR product of the secretor allele (*Se*) into four fragments (101, 57, 25 and 12), while the nonsecretor allele (se^{428}) is identified by the loss of a restriction site and results in three fragments (101, 82 and 12; fig. 1). The *Dde*I restriction enzyme does not cut a 468-base pair (corresponding to nucleotides 370 to 837) PCR product of the secretor allele while the nonsecretor allele (se^{571}) is identified by a restriction site resulting in two fragments (267 and 201; fig. 1).

The se^{428} mutation was found in a homozygous state in all 6 Le(a+b–) and all 6 Le(a–b–) nonsecretor Caucasians and in a heterozygous state in 10 of 30 secretor individuals. The remaining 11 secretor individuals were all wild type. The se^{428} mutation was also found in Polynesians but only in 3 of 37 secretors and homozygously in 1 Le(a–b–) nonsecretor. The se^{428} mutation was not present in the two Le(a+b–) nonsecretor individuals.

In contrast the se^{571} mutation was absent from the Caucasians but present among Polynesians. This mutation was found in a homozygous state only in the two Le(a+b–) nonsecretor Polynesians (and confirmed by sequencing), and was present in a heterozygous state in 3 of 14 Le(a+b+) partial secretors, and in 3 of 18 Le(a–b+) secretor individuals. No individual had both the *Dde*I- and *Bst*NI-determined *se* alleles.

Discussion

The common distinction between secretors and nonsecretors of ABH and/or Lewis substances is made by inhibiting an antiserum agglutinin reaction with saliva. Many variables influence the detection of salivary substances. These include the ethnic group of the donor, ABO and Lewis genotype, saliva collection method and antiserum used. Determination of the salivary ABH secretor phenotype, especially in some populations, can be difficult, if not impossible with routine serological techniques [reviewed in ref. 3]. Furthermore, the inconvenience and reluctance of donors to produce a suitable saliva sample adds to the difficulty of secretor phenotyping. With the recent identification of the coding sequence for the secretor gene [9], restriction enzyme cleavage assays for nonsecretor allele genotyping offer an attractive alternative to salivary and red cell phenotyping. However, as seen here, an alternative nonsecretor allele exists among Polynesians which would not have been detected with the method used for detecting the common se^{428} ($Trp^{143}\rightarrow$stop) Caucasian nonsecretor mutation.

In this paper, the second nonsecretor allele, se^{571} ($Arg^{191}\rightarrow$stop), was found while screening for a candidate Se^{w} allele. Subsequent restriction enzyme analysis revealed

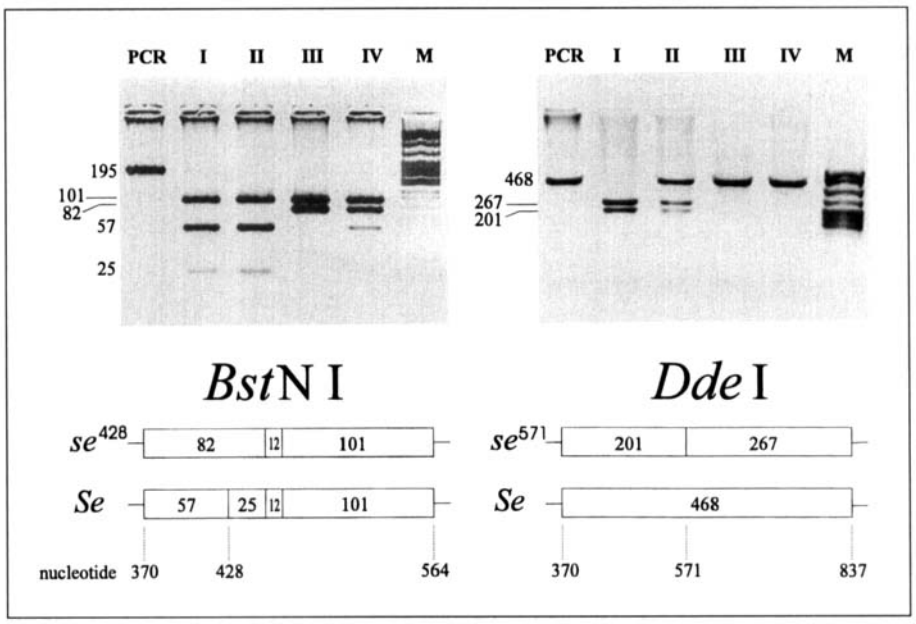

Fig. 1. Examples of the PCR-restriction enzyme cleavage products for the *Bst*NI-determined se^{428} allele and the *Dde*I-determined se^{571} allele. The se^{428} allele is characterized by the *absence* of enzyme cleavage of the 82-base pair fragment into 57- and 25-base pair fragments. The se^{571} allele is characterized by enzyme cleavage of a 468-base pair fragment into 201- and 267-base pair fragments. PCR = The uncleaved PCR product of sample 125; M = *Msp*I digest of pBR332 molecular-mass marker.

Lane	Individual	Serological phenotype (Lewis)	Secretor status[a]	Restriction enzyme cleavage[b]		Genotype
				*Bst*NI	*Dde*I	
I	125	Le(a+b–)	NS	–/–	+/+	se^{571} se^{571}
II	128	Le(a–b+)	S	–/–	–/+	*Se* se^{571}
III	Hen	Le(a+b–)	NS	+/+	–/–	se^{428} se^{428}
IV	113	Le(a–b+)	S	–/+	–/–	*Se* se^{428}

[a] S = Secretor; NS = nonsecretor.
[b] –/– = Homozygous negative; +/+ = homozygous positive; +/– = heterozygous.

that this new mutation was present only in the Polynesian samples and when in a homozygous state correlated with the red cell Le(a+b–) salivary nonsecretor phenotype. In Polynesians, most of the apparently phenotypically Le(a+b–) individuals are in fact Le(a+b+). Detection of the true Le (a+b–) nonsecretor phenotype requires testing with antiserum known to detect weak Le^b expression and carefully standardized salivary inhibition assays [12]. The two Le (a+b–) Polynesian samples in this study were determined by thorough serological analyses.

The Caucasian se^{428} nonsecretor allele [9] was present with a frequency equal to the expected gene frequency of this allele in the predominantly Swedish sample. This allele was also found in Polynesians but much less frequently. Furthermore, each of the four Polynesians who had the se^{428} nonsecretor allele also had a Caucasian ancestor within the last two generations. Because of the selected nature of the Polynesian samples in this study, it is not adequate to estimate gene frequency, but the results suggest that the se^{428} nonsecretor allele is an infrequent allele in Polynesians compared to Caucasians.

The new point mutation (C^{571}→T) identified in the FUT2 gene resulted in Arg^{191}→ stop (numbered from the putative initiator methionine [9]). This mutation is predicted to trun-

cate a large part (141 amino acid residues) of the enzyme catalytic domain on the COOH terminal segment. The evidence that this mutation yields an inactive allele comes from the fact that this mutation was found in a homozygous state in two known Polynesian red cell Le(a+b–) nonsecretor individuals who did not have the Caucasian se^{428} mutation. Furthermore, the new nonsense Polynesian mutation was not found in homozygous form in any of the secretor or partial-secretor individuals. No Caucasian individual had the se^{571} mutation.

To date, at least one coding secretor allele *Se*, and two noncoding alleles (termed se^{428} and se^{571}) are known. Further mutations are expected as we have found partial secretors with the red cell Le(a+b+) phenotype who cannot be explained on the basis of these alleles. Molecular genotyping for secretor should now be done in parallel with routine red cell and saliva phenotyping in order to establish the reliability of genotyping, before it can replace saliva phenotyping.

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