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Molecular basis for erythrocyte Le(a+b+) and salivary ABH partial-secretor phenotypes: expression of a FUT2 secretor allele with an A→T mutation at nucleotide 385 correlates with reduced α(1,2)fucosyltransferase activity

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The Se^{wA385T} mutation of the FUT2 gene was found to correlate with both the erthrocyte Le(a+b+) and/or salivary ABH partial-secretor phenotypes of Polynesians. Constructs with FUT1 and FUT2 wild type genes, and the FUT2 Se^{wA385T}, se^{G428A} and se^{C571T} mutated alleles, were cloned into pcDNAI, and expressed in COS-7 cells. COS-7 cells transfected with the Se^{wA385T} allele had weak, but detectable, α(1,2)fucosyltransferase activity, with an acceptor substrate pattern similar to the wild type FUT2 gene. Comparative kinetic studies from cell extracts with mutated Se^{wA385T} and wild type FUT2 alleles gave similar K_m values, but less enzyme activity was present in cells transfected with Se^{wA385T} (V_{max} 230 pmol h^{-1} mg^{-1}), as compared to those transfected with FUT2 (V_{max} 1030 pmol h^{-1} mg^{-1}), suggesting that the mutated enzyme is more unstable. These results confirm that the molecular basis for the erythrocyte Le(a+b+), and the associated ABH salivary partial-secretor phenotype, is an amino acid change of Ile129 → Phe in the secretor α(1,2)fucosyltransferase.

Keywords: secretor, fucosyltransferase, Lewis a, Lewis b, saliva ABH, blood group, FUT2, ABO, Polynesia

Abbreviations: α(1,3/1,4)fucosyltransferase, GDP-L-fucose:β-D-*N*-acetylglucosaminide 3/4-α-L-fucosyltransferase; α(1,2)fucosyltransferase, GDP-L-fucose: β-D-galactoside-2-α-L-fucosyltransferase; bp, base pairs; FUT1, *H* gene; FUT2, *Se* gene; FUT3, *Lewis* gene or Fuc-TIII gene; FUT4, Fuc-TIV gene; FUT5, Fuc-TV gene; FUT6, Fuc-TVI gene; MAb, monoclonal antibody; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; se^{G428A}, FUT2 nonsecretor G → A mutation at nucleotide 428; se^{C571T}, FUT2 nonsecretor C → T mutation at nucleotide 571; Se^{wA385T}, FUT2 secretor weak A → T mutation at nucleotide 385; SSP, sequence specific primer.

Introduction

The Lewis histo-blood group system is constituted of two major serologically defined antigens, Le^a and Le^b (as reviewed in [1]). The two fucosyltransferase genes, FUT2 and FUT3, responsible for the synthesis of Le^a and Le^b glycoconjugates have been cloned, sequenced and expressed [2, 3].

As a consequence of polymorphism of the genetically independent Lewis and secretor systems and interaction of their transferases, four major red cell and salivary ABH secretor phenotypes are common in Europeans. These are: Lewis-negative secretors and nonsecretors, both red cell phenotyping as Le(a−b−); Lewis-positive nonsecretors whose red cell phenotype will be Le(a+b−); and Lewis-positive secretors whose red cell phenotype will be Le(a−b+). There is also another red cell phenotype known as Le(a+b+). This red cell phenotype

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is rare (or absent) in Caucasians [4]. It is however common in Polynesians [5], Asians [6, 7], Indonesians [8], and Australian aborigines [9, 10]. By immunochemistry it has been established that the Le(a+b+) phenotype represents a unique glycoconjugate profile in plasma, on red cells and in the intestinal epithelium (reviewed in [1]). The Le(a+b+) phenotype is believed to be caused by an inefficient transferase, encoded by a weak secretor gene (Se^{w}) [6], because poor expression of salivary ABH substances associate with this red cell phenotype. The poor expression of salivary ABH substances is known as the partial-secretor phenotype and is defined by more salivary ABH substances than expected in nonsecretors and less than is present in secretors [11].

An A→T mutation at nucleotide 385 of the FUT2 gene, termed Se^{wA385T}, has recently been reported to associate with the Le(a+b+) phenotype in family studies from Taiwan [12] and Indonesia [13]. We report here the expression of this mutation and its correlation with the Le(a+b+) and partial secretor phenotypes in Polynesians.

Materials and methods

Polynesian Le(a+b+) and partial-secretor individuals

Thirty-one Polynesian samples were obtained from the Auckland Regional Blood Centre, New Zealand. Where known, the Polynesian ethnic group of the samples is given, otherwise the sample is given the generic term Polynesian. The erythrocyte Lewis phenotypes of most of these samples were determined in previous studies [5, 11, 14, 15]. For some of the Polynesian samples glycolipids have also been immunochemically profiled [14, 16, 17]. The phenotypes reported here are based on red cell serology with polyclonal goat anti-Lewis reagents, however the Polynesian Le(a+b−) samples 33, 57 [14] and 114 (unpublished) have glycolipid profiles similar to those of Le(a+b+) individuals.

Forty-four European samples, predominantly of Swedish origin (38 of 44), were tested in parallel [18]. Erythrocyte phenotypes were determined with monoclonal anti-Lewis reagents and salivary phenotypes by standard serology [19].

PCR for sequencing, RFLP and PCR-SSP analysis

Genomic DNA was prepared essentially according to Miller *et al.* [20]. Primers were purchased from Scandinavian Synthesis AB, Köping, Sweden, and diluted in sterile water to 25 μM for PCR amplification, and to 0.5 μM for nucleotide sequencing. The primers used are listed in Table 1. Unless stated otherwise, PCR amplifications were run for 30 cycles with denaturation at 94 °C for 1.5 min, annealing/extension 2.5 min at 72 °C, and stopped by cooling to 8 °C.

Nucleotide sequencing of genomic DNA

The FUT2 coding sequence of ten Polynesians and three Europeans were sequenced, both strands, from nucleotides −170 to 999 using methods as described in [21].

Screening for the se^{G428A}, se^{G571T} and Se^{wA385T} alleles

Restriction enzyme cleavage for the se^{G428A} and se^{C571T} nonsecretor alleles was done as described [2, 21]. Sequence specific oligonucleotide primers were constructed for screening of the Se^{wA385T} mutation [13]

Table 1. PCR primers for sequencing, RFLP and PCR-SSP analysis

*Primer**	*Position*		*Sequence (5′–3′)*	*Reference*
II-10-s	−187	−216	TATAAACACACTTGAGATACATGCGTGTGC	[2]
II-3s	−6	−35	CCATGCTGGTCGTTCAGATGCCTTTCTCCT	[2]
II-19s	−15	14	gcgcgaattcCCTTTCTCCTTTCCCATGGCCCACTTCAT	[2]
II-18as	−15	10	AGTGGGCCATGGGAAAGGAGAAAGG	
II-11s	271	300	ACCCTGGCCCCCATCTTCAGAATCACCCTG	
II-16s	359	385	ACTGGATGGAGGAGGAATACCGCCACA	[13]
II-17s	359	385	ACTGGATGGAGGAGGAATACCGCCACT	[13]
II-1s	366	399	GGAGGAGGAATACCGCCACATCCCGGGGGAGTAC	[2]
II-12as	366	399	GTACTCCCCCGGGATGTGGCGGTATTCCTCCTCC	
II-13s	535	564	AGCCGGCCGGGCACCTTTGTAGGGGTCCAT	
II-2as	535	564	ATGGACCCCTACAAAGGTGCCCGGCCGGCT	[2]
II-15s	808	837	TGTAACCACACCATCATGACCATTGGGACG	
II-14as	808	837	CGTCCCAATGGTCATGATGGTGTGGTTACA	[21]
II-4as	999	1030	AGGAGAAAAGGTCTCAAAGGACGGGCCAGCA	
II-20as	999	1030	gcgctctagaGGAGAAAAGGTCTCAAAGGACGGGCCAGCA	[2]
FUT1s	−23	7	gcgcgaattcTTCGCCTTTCCTCCCCTGCAGCCATGTGGC	[35]
3938as	1136	1166	gcgctctagaCAGGCCTCTGAAGCCACGTACTGCTGGCTC	[35]

FUT1s and 3938as are FUT1 gene PCR primers, all the others belong to the FUT2 gene. Primers with underlined restriction sites (EcoRI and XbaI) were used for amplification and cloning of FUT1 and FUT2 genes. *the s and as at the end of the primer name designates sense and antisense respectively

(Fig. 1). PCR-SSP was also used in conjunction with restriction enzyme cleavage (PCR-SSP-RFLP), using the antisense primer II-14as to determine if nonsecretor mutations, when present, were on the same allele.

Molecular cloning of FUT2 alleles and corresponding FUT2 expression vectors with wild and mutated alleles

The FUT2 coding sequence of an individual (55, Table 2) who was heterozygous for the se^{C571T} and Se^{WA385T} mutations was selected for cloning. For expression we used the vector pcDNAI (Clontech) and PCR primers II-19s and II-20as (Table 1) as described in [2]. PCR conditions that minimize sequence alterations were used [22, 23]. The sequences of the PCR primers used for the amplification of the FUT2 gene of individual 55 have additional bases at their 5′ end, including *Eco*RI (II-19s) and *Xba*I (II-20as) restriction sites, which facilitate subcloning of the PCR product into the pcDNAI vector. The product from this amplification was restricted with *Eco*RI and *Xba*I, gel purified, and ligated into *Eco*RI-*Xba*I-doubly digested pcDNAI. Twenty clones with the correct insert were sequenced. Two plasmids devoid of PCR errors within the full coding sequence were selected, one with the A385 → T mutation and the second with the C571 → T mutation. Using these two constructs, we made a wild type FUT2 Polynesian expression vector, via restriction fragment interchange procedures. The 5′ mutated region of the A385 → T mutated clone was replaced with the same 5′ unmutated region of the C571 → T clone. This was facilitated by a single *Pst*I restriction site located at position 524 in the coding region of the FUT2 gene. The integrity of this FUT2 wild type construct was verified again by sequencing on the restriction sites.

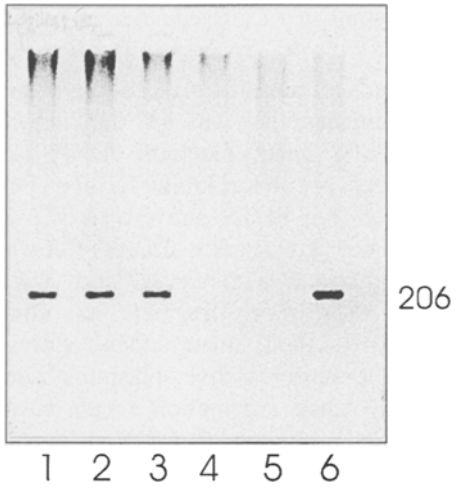

Figure 1. PCR-SSP screening for the Se^{wA385T} mutation. A 206 bp product is obtained with the SSP primers. Lanes 1, 3 and 5 reflect the result obtained using the unmutated primer (II-16s), while lanes 2, 4, and 6 reflect the result of the mutated primer (II-17s). In this example, the sample in lanes 1–2 is heterozygous, while the sample in lanes 3–4 is homozygous wild type and the sample in lanes 5–6 is homozygous for the Se^{wA385T} mutation.

In addition, we used for comparison, the Caucasian nonsecretor mutated G428 → A allele (se^{G428A}) [2], generously donated by Dr J.B. Lowe, Ann Arbor, MI, USA.

Table 2. Summary of the Polynesian and European samples sequenced, their red cell and salivary phenotypes, and genetic mutations/variations found.

		Phenotypes			*Nucleotide variations detected at positions*								*References to glycolipid profiles*
		Red cell		*Saliva*									
Code	*Ethnic group*	*ABO*	*Lewis*	*ABH*	*171*	*216*	*357*	*385*	*428*	*571*	*739*	*960*	
46	Polynesian	O	a−b−	P	AA	CC	TT	TT	GG	CC	GG	AA	[14, 43]
29	Niuean	O	a+b+	P	AA	CC	TT	TT	GG	CC	GG	AA	[14, 17]
78	Maori	O	a+b+	P	AA	CC	TT	TT	GG	CC	GG	AA	[17]
79	Samoan	O	a+b+	P	AA	CC	TT	TT	GG	CC	GG	AA	
116	Polynesian	O	a+b+	S	AA	CC	TT	TT	GG	CC	GG	AA	[17]
120	Polynesian	O	a+b+	S	AA	CC	TT	TT	GG	CC	GG	AA	[17]
Jk2	Niuean	O	a+b+	U	AA	CC	TT	TT	GG	CC	GG	AA	
55	Samoan	O	a+b+	P	AA	CC	TT	AT	GG	CT	GG	AA	
Jk5	Samoan	O	a+b+	U	AA	CC	TT	AT	GG	CT	GG	AA	
57	Polynesian	O	a+b−	NS	AA	CC	TC	AA	GG	CT	GG	AA	[14, 17]
Hen	European	O	a+b−	NS	GG	TT	CC	AA	AA	CC	AA	GG	[43]
B	European	A	a+b−	U	GG	TT	CC	AA	AA	CC	AA	GG	
26	European	A	a−b−	S	AG	TC	TC	AA	GA	CC	AG	AG	

Red cell phenotypes are those defined by polyclonal goat anti-Lewis reagents. Salivary ABH phenotypes are defined as S = secretor, NS = nonsecretor, P = partial secretor and U = saliva unavailable. The effect of the nucleotide changes are summarized in Table 3.

Molecular cloning of FUT1 wild type gene from genomic DNA

PCR was used to amplify the coding region and the immediately adjacent 5′- and 3′- flanking regions of a wild type FUT1 gene. Genomic DNA (200 ng) was amplified using primers (Table 1), the PCR program previously used for FUT5 and FUT6 [22–24] and the Gene Amp PCR kit (Perkin Elmer Cetus). The PCR product was digested with *Eco*RI and *Xba*I restriction enzymes, and the 1161 bp fragment was cloned into the restricted (*Eco*RI-*Xba*I) mammalian expression vector pcDNAI. Eight representative plasmids containing the insert in the sense orientation, with respect to the cytomegalovirus promoter of pcDNAI, were sequenced, both strands, and one, free of PCR errors, was used as a positive control for the FUT1 wild type allele.

Transfection in COS-7 cells

Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% inactivated foetal calf serum and transfected with expression vectors using the DEAE-dextran method [25]. A control plasmid, containing the coding region of the bacterial chloramphenicol acetyl-transferase (pcDM7-CAT) [26], was co-transfected to allow for normalization of transfection efficiency. The cells were transfected with pcDNAI without insert and with the different constructs, containing the FUT1 and FUT2 wild type alleles, and the se^{G428A}, se^{C571T} and Se^{wA385T} mutated alleles. Transfected COS-7 cells were harvested after 48 h. One aliquot of the cells was processed for indirect immunofluorescence and the remainder was used for α(1,2)fucosyltransferase and chloramphenicol acetyltransferase activity assays [26].

α(1,2)fucosyltransferase assay

Protein concentrations of transfected COS-7 cell extracts containing 1% Triton X-100 were determined by the micro-BCA assay reagent (Pierce Chemicals Co.). The α(1,2)fucosyltransferase assays were performed with GDP-[^{14}C]fucose (Amersham Corp. 300 mCi mmol^{-1}) as donor substrates and phenyl-β-D-galactoside (Sigma), or type 1 to type 6 synthetic disaccharides (Table 6), coupled to a methoxycarbonyloctyl ($-(CH_2)_8COOCH_3$) (Chembiomed, Alberta Research Council, Edmonton, Canada) as acceptor substrates. The hydrophobic properties of these acceptors allow them to separate easily by the Sep-Pak C_{18} isolation procedure [27].

Assays were performed in a volume of 50 μL and contained 25 mM sodium cacodylate (pH 6.2), 5 mM ATP, 10 mM L-fucose, 20 mM $MnCl_2$, 3 μM GDP-[^{14}C]fucose, 50 μg of cell extract proteins, and 10 μg of the different methoxycarbonyloctyl-glycosides or 25 mM of phenyl-β-D-galactoside as acceptor substrates. Reactions were incubated at 37 °C for 4 h. The reaction was stopped by addition of 5 ml of water, centrifuged and the supernatant was applied to a conditioned Sep-Pak C_{18} cartridge (Waters, Milford, USA) attached to a 10 ml syringe. The unreacted GDP-[^{14}C]fucose and its hydrolysis products were washed out with 20 ml of water for the synthetic methoxycarbonyloctyl-glycoside reactions, and only with 10 ml of water for the phenyl-β-D-galactoside reactions. Radiolabelled reaction products were eluted with two 5 ml portions of methanol and were collected directly into scintillation vials. The vials were counted with one volume of Instagel (Packard, Illinois, USA) in a liquid scintillation counter (counting efficiency 86%) [28].

Apparent Michaelis-Menten constants (K_m) and maximum velocity (V_{max})

Kinetic parameters of the Se^{wA385T} and the wild type FUT2 enzymes were determined for GDP-fucose donor and phenyl-β-D-galactoside acceptor substrates. All assays were performed in duplicate, along with parallel controls without acceptors. Reactions were incubated at 37 °C for 2 h and then treated as above.

To determine the apparent K_m for GDP-fucose, 3 μM GDP-[^{14}C]fucose was supplemented with different amounts of unlabelled GDP-fucose to achieve final concentrations that ranged from 3 μM to 400 μM. The K_m determination was completed in a volume of 50 μl in reactions containing 25 mM sodium cacodylate buffer pH 6, 25 mM phenyl-β-D-galactoside, 20 mM $MnCl_2$, 5 mM ATP and 10 mM L-fucose.

For the FUT2 wild type enzyme, the apparent K_m for phenyl-β-D-galactoside was determined with a range of acceptor concentrations from 0 mM to 150 mM, with a saturation concentration of GDP-fucose of 400 μM.

For the FUT2 Se^{wA385T} enzyme, the determination was done with a range of 0–100 mM of phenyl-β-D-galactoside and a saturating concentration of GDP-fucose of 120 μM. All other ingredients of the reaction were the same as above. K_m and V_{max} were derived from Lineweaver-Burk plots of substrate concentration-rate determinations.

Immunofluorescent expression of H ligands on the surface of transfected COS-7 cells

Transfected COS-7 cells were released from the culture dishes by trypsinization, washed and labelled with different reagents. The reagents used were two anti-H type 1: rabbit polyclonal, affinity purified antibodies [29] and mouse monoclonal antibody (MAb) B5 (N. Bovin, Shemyakin Institute of Bioorganic Chemistry, Moscow Russia). Five anti-H type 2 reagents: the commercial MAb from Dako, three MAbs 058, 060, 064 [30] and FITC labelled *Ulex europaeus* lectin I (Vector Laboratories, Burlingame, Cal, USA). A MAb anti-Le^x (82H5, Chembiomed, Alberta Research Council, Edmonton, Canada) was used as a negative control. After 30 min incubation with the first antibody, the cells were washed with PBS

and stained with fluorescein isothiocyanate (FITC) labelled anti-mouse immunoglobulins (Pasteur Diagnostics, Marnes la Coquette, France). After the direct lectin or the indirect antibody labelling, the cells were washed, fixed with 2% paraformaldehyde, mounted under coverslips with Mowiol 4-88 and observed with an epifluorescence microscope.

Results

Identification of a candidate Se^w allele

The sequence analysis of nine Le(a+b+) and/or partial-secretor samples (Table 2) revealed a common mutation of A → T at nucleotide 385. It was the only consistent mutation found which correlated with the Le(a+b+) and/or partial-secretor phenotypes [12, 13].

Other genetic polymorphisms corresponding with those previously reported were also found at nucleotides 171, 216, 357, 739 and 960 [2, 12]. A summary of the effects of FUT2 variations on amino acid and fucosyltransferase activity is shown in Table 3.

Screening for the Se^{wA385T} mutation

Samples from 31 Polynesians including those sequenced, and 44 Europeans were screened (Table 4). All saliva partial-secretors and red cell Le(a+b+) individuals (including two with a secretor phenotype) had either the Se^{wA385T} mutation in a homozygous state, or present in a heterozygous state with a nonsecretor allele (Table 4). The Se^{wA385T} mutation was not found in any of the 44 European samples tested.

Expression of se^{G428A} and se^{C571T} nonsecretor alleles confirms the inactivating nature of these mutations

The se^{G428A} had previously been shown to code for an inactivating nonsense mutation of FUT2 by transfection expression into COS-7 cells [2]. The se^{C571T} mutation had also been defined as a nonsecretor allele, on the basis that it also codes for a nonsense mutation, which prematurely results in the termination of the protein, and correlates with the nonsecretor phenotype [21]. Here we confirm that both G428 → A and C571 → T mutations result in a lack of fucosyltransferase activity, by the absence of H type 1 and H type 2 epitopes at the surface of transfected cells (Table 5), and the lack of α(1,2)fucosyltransferase activity in the extracts of these cells (Table 6).

Table 3. Nucleotide substitutions found in the coding region of the FUT2 secretor gene and their effect on fucosyltransferase activity

Nucleotide change	*Effect on amino acid*	*Effect on expressed fucosyltransferase activity*
171 A → G	Ala57 → Ala	No effect [2]
216 C → T	Tyr72 → Tyr	No effect [2]
357 C → T	Asn119 → Asn	No effect (this paper)
385 A → T	Ile129 → Phe	Reduced fucosyltransferase activity (this paper)
428 G → A	Trp143 → stop	Inactivating [2]
571 C → T	Arg191 → stop	Inactivating (this paper)
739 G → A	Gly247 → Ser	No apparent effect [2]
960 A → G	Thr320 → Thr	No effect [2]

Expression of the Se^{wA385T} allele induces weak α(1,2)fucosyltransferase activity

Both H type 1 and H type 2 epitopes were found at the surface of a small percentage of COS-7 cells transfected with the Se^{wA385T} construct. The percentage of positive cells and the intensity of the fluorescence were lower than those obtained with the corresponding wild type FUT2 gene (Table 5). In accordance with these results, some α(1,2)fucosyltransferase was detected in the extracts of COS-7 cells transfected with the Se^{wA385T} construct, with phenyl-β-D-galactoside, type 1, 3 and 4 acceptors. No activity was detected with the other acceptors (Table 6). The loss of activity could not be explained by shedding of enzyme to the cell culture medium, as no activity was found in the supernatant with any of the acceptors.

Expression of the FUT1 and FUT2 wild type alleles

The two known α(1,2)fucosyltransferase genes, FUT1 and FUT2, were transfected into COS-7 cells and expressed in parallel. Stronger expression of the H type 2 epitope at the surface of cells was observed with FUT1 as compared to FUT2, while the reverse was observed with H type 1 epitopes (Table 5). Unlike this, all the acceptors tested (types 1 to 6 and phenyl-β-D-galactoside) gave strong α(1,2)fucosyltransferase activity with the extracts of FUT1 transfected COS-7 cells, and a larger range of enzyme activities with the extracts of cells transfected with the FUT2 wild type allele. These last extracts had higher enzyme activities with phenyl-β-D-galactoside and types 1, 3 and 4, as compared to types 2, and 6 acceptors (Table 6).

Comparative kinetic parameters of the wild type FUT2 and the Se^{wA385T} allele

The available amounts of the synthetic types 1 to 6 disaccharide acceptors, coupled to the hydrophobic $-(CH_2)_8COOCH_3$ chain, were not enough to determine the K_m of the enzyme coded by the Se^{wA385T} allele. Therefore, enzyme kinetics could only be determined with the phenyl-β-D-galactoside acceptor and GDP-fucose donor substrates, which are commercially available.

Comparative kinetic studies for both the mutated Se^{wA385T} and the wild type FUT2 alleles gave similar K_m values for both acceptors, GDP-fucose (120–125 μM) and phenyl-β-D-galactoside (10.0–10.5 mM), but the V_{max} was five times lower for the extracts of cells transfected

Table 4. Summary of the Polynesian and European samples screened for the presence of Se^{wA385T}, se^{G428A} and se^{C571T} mutations

		Phenotype				*Genotype*		
Identity or number (n) tested	*Ethnic group*	*Red cell*			*Saliva*	*Nucleotide position*		
		ABO	*Le^a^*	*Le^b^*	*ABH secretion*	*385*	*428*	*571*
Expected partial-secretor phenotype								
46	Polynesian	O	–	–	P	TT	GG	CC
Jk1	Niuean	O	–	–	U	TT	GG	CC
114	Polynesian	O	+	–	U	TT	GG	CC
29	Niuean	O	+	+	P	TT	GG	CC
78	Maori	O	+	+	P	TT	GG	CC
79	Samoan	O	+	+	P	TT	GG	CC
116	Polynesian	O	+	+	S	TT	GG	CC
120	Polynesian	O	+	+	S	TT	GG	CC
Jk2	Niuean	O	+	+	U	TT	GG	CC
55	Samoan	O	+	+	P	AT	GG	CT
Jk5	Samoan	O	+	+	U	AT	GG	CT
33	Polynesian	O	+	–	P	AT	AG	CC
Expected secretor phenotype								
121	Maori	O	–	–	S	AT	GG	CC
Jk6	Niuean	O	–	+	U	AT	GG	CC
Jk7	Samoan	O	–	+	U	AT	GG	CC
Jk4	Niuean	O	–	+	U	AT	GG	CC
c408	Cook Is.	A_1	–	+	U	AT	GG	CC
124	Polynesian	A_1	–	+	S	AT	GG	CC
127	Polynesian	A_1	–	+	S	AT	GG	CC
115	Polynesian	A_2	–	+	S	AA	AG	CC
113	Polynesian	O	–	+	S	AA	AG	CC
($n = 8$)	Europeans	O = 3, A = 3, B = 2	–	–	S = 5, U = 3	AA	AG	CC
($n = 13$)	Europeans	O = 6, A = 7	–	+	S = 3, U = 10	AA	AG	CC
122	Tongan	O	–	+	S	AA	GG	CT
57	Polynesian	O	+	–	NS	AA	GG	CT
117	Polynesian	O	–	–	S	AA	GG	CC
119	Maori	A_1	–	–	U	AA	GG	CC
c363	Samoan	A_1	–	–	U	AA	GG	CC
126	Polynesian	A_1	–	+	S	AA	GG	CC
c070	Polynesian	O	–	+	U	AA	GG	CC
c409	Tongan	O	–	+	U	AA	GG	CC
(n=4)	Europeans	O = 2, A = 2	–	–	S = 4	AA	GG	CC
(n=6)	Europeans	O = 3, A = 3	–	+	S = 2, U = 4	AA	GG	CC
Expected nonsecretor phenotype								
118	Polynesian	O	–	–	NS	AA	AA	CC
($n = 7$)	Europeans	O = 6, B = 1	–	–	NS = 6, U = 1	AA	AA	CC
($n = 6$)	Europeans	O = 3, A = 3	+	–	NS = 3, U = 3	AA	AA	CC
125	Polynesian	O	+	–	NS	AA	GG	TT

Polynesian red cell phenotypes are those defined by polyclonal goat antiserum. Salivary ABH phenotypes are defined as S = secretor, NS = nonsecretor, P = partial-secretor and U = saliva unavailable. By PCR-SSP-RFLP it was established for samples 33, 55, and Jk5 that the Se^{wA385T} mutation and the nonsecretor mutations (either se^{G428A} and se^{C571T}), were not on the same allele.

with Se^{wA385T} (230 pmol h^{-1} mg^{-1}), as compared to the extracts of cells transfected with the wild type FUT2 allele (1030 pmol h^{-1} mg^{-1}) (Table 7). The K_m values of the product of our wild type FUT2 Polynesian construct were similar to those previously reported for a Caucasian wild type allele [2].

Discussion

In Polynesians of the Le(a+b+) and/or salivary partial-secretor phenotype a point mutation, A → T at nucleotide 385, which causes a change of Ile129 to Phe, was present in a homozygous state, or heterozygously with a

Table 5. Expression of H type 1 and H type 2 epitopes on the membrane of COS-7 cells transfected with the pcDNAI vector alone the Se^{wA385T}, se^{G428A} and se^{C571T} mutated FUT2 constructs, and the FUT2 and FUT1 wild type constructs. Results are expressed as the percentage of positive cells and the relative intensity of fluorescence (– to +++) of the positive cells

	Constructions					
Antigen detecting reagents	*pcDNAI no insert*	*428 G → A*	*571 C → T*	*385 A → T*	*FUT2 wild*	*FUT1 wild*
Anti-H type 1						
Rabbit	0 –	0 –	0 –	3 +	3 ++	0 –
B5	0 –	0 –	0 –	4 +	10 +	0 –
Anti-H type 2						
Dako	0 –	0 –	0 –	5 +	9 +++	36 +++
058	0 –	0 –	0 –	3 ++	17 +++	69 +++
060	0 –	0 –	0 –	3 +	10 ++	36 +++
064	0 –	0 –	0 –	4 +	9 ++	25 +++
Ulex europaeus	0 –	0 –	0 –	20 +	20 ++	67 +++
Anti-Le^x						
82H5	0 –	0 –	0 –	0 –	0 –	0 –

Table 6. Comparative enzyme activities (c.p.m.) of the extracts of COS-7 cells transfected with pcDNAI vector alone, se^{G428A}, se^{C571T} and Se^{wA385T} FUT2 mutations and FUT2 and FUT1 wild type constructs with phenyl-β-D-galactoside and type 1 to type 6 precursor disaccharide acceptor substrates, coupled to the hydrophobic aliphatic chain, R = $-(CH_2)_8COOCH_3$

Acceptor substrate	*pcDNAI no insert*	*428 G → A*	*571 C → T*	*385 A → T*	*FUT2 wild*	*FUT1 wild*
Phenyl-β-D-galactoside	0	0	0	1560	26 000	57 400
Type 1 Galβ1-3GlcNAcβ-R	0	0	0	180	9550	9900
Type 2 Galβ1-4GlcNAcβ-R	0	0	0	0	2720	12 930
Type 3 Galβ1-3GalNAcα-R	0	0	0	460	10 300	12 300
Type 4 Galβ1-3GalNAcβ-R	0	0	0	375	11 500	10 700
Type 5 Galβ1-3Galβ-R	0	0	0	0	3520	10 900
Type 6 Galβ1-4Glcβ-R	0	0	0	0	1280	13 340

Table 7. Kinetic parameters of the enzyme activities measured in extracts of COS-7 cells transfected with the wild type FUT2 and the Se^{wA385T} FUT2 mutated constructs

Construction	*Acceptor*	K_m	V_{max}
FUT2	GDP-fucose	125 μM	1020 pmol h^{-1} mg^{-1}
A385 → T	GDP-fucose	120 μM	230 pmol h^{-1} mg^{-1}
FUT2	phenyl-β-D-galactoside	10.5 mM	1040 pmol h^{-1} mg^{-1}
A385 → T	phenyl-β-D-galactoside	10.0 mM	230 pmol h^{-1} mg^{-1}

nonsecretor allele. This Se^{wA385T} mutation has recently been reported to associate with the Le(a+b+) red cell phenotype in Asians [12] and Indonesians [13]. The Se^{wA385T} mutation could not be found in any of the 44 Europeans tested. Although this statistically represents a small number, it suggests that the Se^{wA385T} mutation is uncommon in Europeans.

Transfection expression experiments on COS-7 cells confirmed that the product of the Se^{wA385T} mutated allele is able to make H type 1 and H type 2 epitopes, which can be detected at the surface of transfected cells, but the amounts of antigen detected are lower than the amounts of antigen on cells transfected with the wild type FUT2 allele.

The α(1,2)fucosyltransferase activities of cell extracts transfected with the FUT2 wild type allele were stronger with disaccharide acceptors of type 1, 3, and 4, as compared to type 2, and 6 acceptors. The disaccharide

acceptors of types 1, 3, 4, and 5 have a $\beta 1 \rightarrow 3$ linkage, making their exposed surface different from the disaccharide acceptors of types 2 and 6, which have the $\beta 1 \rightarrow 4$ linkage (see Table 6 and Mollicone *et al.* [30]). The enzyme activities of the cells transfected with the Se^{wA385T} mutated allele were overall much weaker, but had a similar pattern of acceptor specificity, with weak, but detectable enzyme activity for type 1, 3 and 4, and no activity for type 2 and 6 acceptors. No enzyme activity could be detected at 4 h with the type 5 acceptor, which has the same $\beta 1 \rightarrow 3$ linkage as types 1, 3 and 4, but the lack of the *N*-acetyl group in this type 5 disaccharide, also makes it a poor acceptor for the wild type FUT2 enzyme.

Altogether, these results suggest that the A385 → T mutation generates a Se^{w} allele, which causes lower enzyme activity than the normal secretor FUT2 wild type, and higher enzyme activity than the nonsecretor mutated alleles (se^{G428A} or se^{C571T}). However, the K_m values of the enzyme coded by this Se^{wA385T} allele and those of the wild type FUT2 allele were very similar for donor (GDP-fucose) and acceptor (phenyl-β-D-galactoside) substrate, suggesting that the two enzymes have the same affinities for these substrates. Therefore, the difference between these two alleles seems to be supported by a fivefold decrease in the amount of enzyme activity (V_{max}) in the Se^{wA385T}, as compared to the FUT2 wild type allele. These kinetic results and the similar acceptor substrate specificity pattern suggest that both alleles end up with an enzyme with similar catalytic function. Therefore, it is possible that the enzyme made by the Se^{wA385T} allele is more susceptible to proteolysis and its catabolism is accelerated.

It was originally postulated that expression of ABH antigens in saliva was under the control of the secretor gene (*Se*), and was independent of the expression of the same ABH antigens on red cells. A genetic model was proposed postulating the existence of one *H* structural gene coding for one α-2-fucosyltransferase whose expression was modulated by two regulatory genes, the *Se* gene in saliva and the *Z* gene on red cells [31]. Over the years this model has been strongly refuted [32, 33], and later disproved by the finding of two separate genes, one for *H* [34, 35] and one for *Se* [2, 36].

Two pieces of evidence reported here, suggest that there may also be some regulation of expression of the *Se* gene in different tissues/individuals: Firstly, one individual in this study (57) (Tables 2 and 4) was of the Le(a+b−) red cell phenotype, with no detectable salivary ABH substances, but detectable Lewis salivary substances, and a glycolipid profile identical to that of Le(a+b+) individuals [14]. This individual, as revealed by sequencing, had only a single se^{C571T} nonsecretor allele (Table 2). No other genetic mutations could be found in the coding sequence, or up-stream to base pair − 170. Therefore, the genotype of this individual should result in the Le(a−b+) salivary ABH secretor phenotype. It is probable that some form of transcriptional control, post-transcriptional or post-translational modification is influencing the expression or activity of the secretor transferase in this individual, since the phenotype of this individual can not be explained by the mutations found to-date.

Secondly, in populations which express the Le(a+b+) phenotype there is a wide range of expression of red cell and salivary phenotypes. In some individuals the amount of Le^b antigen has fallen to such a low level that it becomes very difficult to detect by serology. However, ABH and Le^b reactive substances are usually present in saliva in these Lewis-positive secretors whose red cells phenotype as Le(a+b−). Two individuals in this study (33, 114; Table 4) had genotypes which were suggestive of the Le(a+b+) phenotype, but red cell phenotyped as Le(a+b−). Both of these individuals have glycolipid profiles identical to Le(a+b+) samples. Others of the red cell Le(a+b+) phenotype have salivary ABH substance levels similar to those expected in secretors [11]. Two individuals, 116 and 120 (Tables 2 and 4), were of the Le(a+b+) red cell phenotype, but had salivary secretor phenotypes. This is, however, not unexpected as the partial-secretor phenotype is very difficult to distinguish from the small percentage of secretors who secrete lower levels of ABH substances. As a consequence some partial-secretors may secrete levels of ABH substances which overlap with the range seen for secretors. Despite the two above groups having similar secretor genotypes, the differences in salivary ABH substances and expression of the red cell phenotype is suggestive of differential regulation of the *Se* transferase in different tissues/individuals. Tissue specific post-transcriptional modifications are known to be associated with expression patterns of FUT3, FUT5, and FUT6 [37].

Establishing an accurate Lewis and/or secretor phenotype can be difficult. In Lewis-positive diseased, pregnant or transplanted individuals, red cell Lewis phenotypes are frequently changed from the original Lewis positive phenotypes of the individual, to an apparent Lewis negative red cell phenotype [38–41]. In populations where the Le(a+b+) phenotype exists, accurate Lewis and secretor phenotyping is technically very difficult, and underestimated [1]. It is very likely that in non-European populations many apparent Le(a+b−) individuals are in fact Le(a+b+), and have been incorrectly phenotyped.

Molecular genotyping now offers the potential to genotype for three FUT2 alleles which modify or negate the expression of the secretor transferase. Typing for these three alleles should be done to confirm phenotype and to identify individuals with potentially new alleles. Other FUT2 alleles are suspected [13].

The high incidence of the erythrocyte Le(a+b+) and

salivary ABH partial-secretor phenotypes, and the unique glycoconjugate profiles in these individuals suggests that some common, but as yet not identified biological pressure exists in the South Pacific [42].

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