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ORIGINAL ARTICLE

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Lewis enzyme (α1–3/4 fucosyltransferase) polymorphisms do not explain the Lewis phenotype in the gastric mucosa of a Portuguese population

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Abstract The human α-1,3/4 fucosyltransferase III (FucT III) catalyses the synthesis of Lewis antigens including Le^b antigen which is a ligand for *Helicobacter pylori* adhesion. Several polymorphisms have been described in the *FUT3* gene affecting both the transmembrane and catalytic domains, some of which affect the enzyme activity. The aim of the present work was to study the Lewis gene polymorphisms in a Caucasian Portuguese population, with a high rate of *H. pylori* infection, and to evaluate the implications of mutant enzymes in Le^b expression in the gastric mucosa. We studied 460 asymptomatic or dyspeptic individuals from northern Portugal. Screening for Lewis gene polymorphisms was performed by SSCP and direct sequencing. Lewis phenotype in gastric mucosa was determined by immunohistochemistry. In 47 individuals with a Lewis negative blood group, we found *FUT3* gene polymorphisms that were previously described in other populations: 59T > G, 202T > C, 314C > T, 508G > A and 1067T > A. Among the 47 Lewis negative individuals in blood, only nine were also negative in gastric mucosa, suggesting the existence of another α 1-4 fucosyltransferase that is responsible for Le^a and Le^b synthesis in gastric mucosa.

Keywords Lewis enzyme · Fucosyltransferase · Polymorphisms · Lewis antigens · *Helicobacter pylori*

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Introduction

The human α-1,3/4 fucosyltransferase III (FucT III) catalyses the transfer of fucose in α-1,4 and α-1,3 linkages onto type 1 and type 2 core structures, and uses both neutral and sialylated carbohydrates as acceptors (Kokowska-Latallo et al. 1990). FucT III is called the Lewis enzyme since it catalyses the synthesis of Lewis antigens: Le^a, sialyl-Le^a, Le^b, Le^x, sialyl-Le^x and Le^y (Kokowska-Latallo et al. 1990). The Lewis histo-blood group system comprises two major antigens, Le^a and Le^b, whose determinants can be found in large mucin structures or in cell membranes, associated to glycoproteins and glycolipids (Narimatsu 1994). These Le^a and Le^b fucosylated glycosphingolipids are synthesised by exocrine epithelial cells (Oriol et al. 1986) and then adsorbed on erythrocyte membranes in peripheral blood leading to the Lewis blood phenotype. The cell surface fucosylated oligosacharides participate in several biological processes such as embryogenesis, tissue differentiation, tumour metastasis, inflammation and bacterial adhesion (Schenkel-Brunner 1995).

A fundamental role was attributed to Le^b and H type 1 antigens in the adhesion of *Helicobacter pylori* to gastric mucosa mediated by blood group antigen-binding adhesin, Bab A (Boren et al. 1993; Gerhard et al. 1999; Ilver et al. 1998). A few groups have therefore explored the possibility that the host Lewis phenotype might be determinant for the frequency and characteristics of *H. pylori* infection. The results obtained from these studies gave conflicting results: Heneghan et al. (1998), Oberhuber et al. (1997), Clyne and Drumm (1997), Umlauft et al. (1996) and Niv et al. (1996) did not observe any significant relationship between the expression of Le^b

and *H. pylori* infection, whereas Klaamas et al. (1997) and Carneiro et al. (1996) observed a higher prevalence of *H. pylori* infection in Le^b positive individuals. A recent study, by Ikehara et al. (2001), brought again into focus the relevance of host Lewis status by demonstrating that polymorphisms of Lewis and Secretor genes affect the susceptibility to *H. pylori* infection.

Several polymorphisms have been described in the *FUT3* gene. The 59T > G polymorphism is localised in the transmembrane domain and does not affect the enzyme activity, though it can switch the FucT III cell localisation from the Golgi apparatus to the cytoplasm (Koda et al. 1993; Mollicone et al. 1994; Narimatsu et al. 1996). All the other polymorphisms described until now are located in the catalytic domain: 202T > C, 314C > T, 445C > A, 508G > A, 1067T > A (Elmgren et al. 1996; 1997; Liu et al. 1999; Narimatsu et al. 1996; Nishihara et al. 1999; Ørntoft et al. 1996), 304C > A, 370T > G, 848G > A, 667G > A and 808G > A (Pang et al. 1996, 1998). These Lewis gene variants codify enzymes with a lower activity (314C > T, 304C > A and 370T > G) or an inactive FucT III (202T > C, 508GaA, 1067T > A, 667G > A and 808G > A).

The aim of the present work was to study the Lewis gene polymorphisms in a Caucasian population from Northern Portugal, with a high rate of *Helicobacter pylori* infection, and to evaluate the implications of the mutant enzymes in the profile of Le^b expression in the gastric mucosa.

Materials and methods

Population

In the present study we evaluated 460 asymptomatic or dyspeptic Caucasian individuals from northern Portugal (Nogueira et al. 2001). From each patient biopsy specimens were taken from corpus ($n=1$) and antrum ($n=2$). Gastric biopsies for histology studies were fixed in 10% formalin and routinely embedded in paraffin wax. The histological classification of the biopsies was performed according to the revised version of the Sydney system (Dixon et al. 1996). The frequency of *H. pylori* infection and the characteristics of the infection were available from 360 individuals that were biopsied and part of this information was previously published (Nogueira et al. 2000, 2001). Forty-seven individuals from this population (10.2%) had a negative Lewis blood group phenotype (Le^{a-b-}) and were evaluated for *FUT3* gene polymorphisms and for the profile of Le^a and Le^b expression in the gastric mucosa.

Screening of polymorphisms of the FUT3 gene

The screening of *FUT3* polymorphisms in the 47 Lewis negative individuals was performed whenever possible by SSCP (single strand conformation pattern).

Polymerase chain reaction (PCR)

The primers used to amplify the *FUT3* fragments are presented in Table 1. Five fragments spanning most of the coding sequence of the *FUT3* gene were obtained. For the fifth fragment, two different sets of primers were used, *FUT3*.5F/*FUT3*.8R and *FUT3*.6F/*FUT3*.9R. The PCR was performed using DNA extracted from blood cells as a template. The 25 µl of final reaction mixture were composed of 2 µl DNA, 2.5 µl 10× Taq polymerase buffer, 25 pmol of each primer, 3 µl dNTPs (20 mM) and 1 µl Taq polymerase (Perkin Elmer). After 5 min of DNA denaturation at 95°C, PCR was performed for 35 cycles: DNA denaturation, 94°C, 30 s; annealing of primers at 70°C, 30 s, and elongation at 72°C, 1 min; with a final extension at 72°C for 10 min. The annealing temperature for the 3rd, 4th and 5th set of primers was 64°C. The PCR products were verified by electrophoresis in 1% agarose gel and then extracted using a Gel Extraction Kit (QIAGEN).

Single strand conformational pattern (SSCP)

Separation of single strand DNA was performed by electrophoresis in 0.8% MDE (BMA products) gel in Tris–Borate–EDTA (TBE) buffer (Sambrook et al. 1989). The electrophoresis was performed over 15 h under two different temperatures, 20°C and 6°C. The gels were stained by the $AgNO_3$ staining method.

Sequencing of FUT3 gene fragments

The *FUT3* gene fragments generated by PCR were purified and used as templates for the sequencing assays. Each sequencing reaction was performed in a final volume of 5 µl: 2 µl of DNA template, 3 pmol of primer and 2 µl of TRR mix (ABI Prism, Applied Biosystem). The sequencing PCR was performed for 30 cycles: DNA denaturation, 94°C, 30 s; annealing of primers at 50°C, 30 s, and elongation at 60°C, 4 min, with a final extension at 60°C for 20 min. The sequenced products were analysed in the ABI Prism 3100 Sequencer (Applied Biosystem).

Table 1 Sequences of the primers used to amplify *FUT3* gene fragments

	Primer sequence 5′-3′	FUT3 gene fragment	Annealing *T* (°C)
FUT3 1F	CCATGGCGCCGCTGTCTGGCCGCAC	33 bp–227 bp	70
FUT3 2R	CAGGGATGTGGAAAGGCCATGTCC		
FUT3 2F	GGACATGGCCTTTCCACATCCCTG	203 bp–474 bp	70
FUT3 4R	GGACATGGTCAGATTGAAGTATCTGTCC		
FUT3 3F	GGACAGATACTTCAATCTGACCATGTCC	446 bp–688 bp	64
FUT3 6R	GCAGGGGCTTGTGGGAGCGTC		
FUT3 4F	GACGCTCCCACAAGCCCCTGC	667 bp–880 bp	64
FUT3 7R	CCACGTGGATGAAGGCGTCGGG		
FUT3 5F	CCCGACGCCTTCATCCACGTGG	859 bp-1086–bp	64
FUT3 8R	TCAGGTGAACCAAGCCGCT		
FUT3 6F	CCCGACGCCTTCATCCACGT	859 bp–1157bp	64
FUT3 9R	CCACAAAGGACTCCAGCAGG		

Immunohistochemistry

Endoscopic gastric biopsies ($n=47$) were obtained from all the individuals after informed consent (Figueiredo et al. 2001; Silva et al. 2001). Tissues were embedded in paraffin and serial 4 μm sections were obtained for immunohistochemistry. Monoclonal antibodies were used to detect type 1 chain carbohydrate antigens, Ca3F4 (Young et al. 1981) to detect Le^a and BG6 (Signet Pathology Systems) to detect Le^b. Paraffin sections were deparaffinated and rehydrated. In all sections endogenous peroxidase was blocked using 0.5% hydrogen peroxide (H_2O_2) in methanol for 30 min, at room temperature. Sections were washed twice in TBS, incubated with rabbit normal serum, diluted 1:10 in bovine serum albumin (BSA) 10% for 20 min and then incubated overnight, at 4°C, with the primary antibodies (Ca3F4 and BG6). Sections were rinsed in water, incubated for 30 min with the secondary antibody, biotin-labelled rabbit anti-mouse serum, diluted 1:200 in BSA 5%, rinsed in TBS and incubated with avidin–biotin–peroxidase complex for 1 h. Slides were washed three times in TBS before staining with 1 mg/ml 3.3′-diaminobenzidine tetrahydrochloride, prepared in 0.05 M Tris-HCl, containing 0.02% H_2O_2. Sections were then stained with hematoxylin, dehydrated and mounted in Entellan.

Statistical analysis

Statistical analysis was performed using Fisher's exact test.

Results

Description of polymorphisms for each 5′ FUT3 fragment

Analysis of the first 5′ *FUT3* gene fragment (33 bp–227 bp) by SSCP showed six distinct migration patterns. Sequencing of PCR fragments obtained from representative samples from the six patterns, showed two polymorphisms (59T > G and 202T > C). The six SSCP patterns represent: normal genotype (1); Heterozygous 202T > C (2); Heterozygous 59T > G/202T > C (3); Homozygous 59T > G (4); Homozygous 202T > C (5), and Homozygous 59T > G/Heterozygous 202T > C (6) (Fig. 1A). The 59T > G polymorphism was found in 33 individuals, in 11 cases it was homozygous and in 22 cases it was heterozygous (Fig. 2), which represents an allele frequency of 0.47. The 202T > C polymorphism was found in 35 individuals, all except one were heterozygous (Fig. 2), which represents an allele frequency of 0.38.

The second 5′ *FUT3* gene fragment (203 bp–474 bp) showed three distinct patterns by SSCP. Sequencing of the PCR fragments from representative samples of the three patterns showed one polymorphism (314C > T). The three patterns represent: normal genotype (1); Homozygous 314C > T genotype (2), and Heterozygous 314C > T genotype (3) (Fig. 1B). The 314C > T polymorphism was found in 35 individuals, in 12 cases it was homozygous and in 23 cases it was heterozygous (Fig. 2), which represents an allele frequency of 0.50.

Analysis of fragments 3, 4 and 5 by SSCP did not show consistent patterns of migration under all the assayed conditions. We therefore analysed fragments 3, 4 and 5 by direct sequencing of all the samples.

For the third *FUT3* gene fragment (446 bp–688 bp), we found the 508G > A polymorphism in 13 individuals, 4 individuals were 508G > A homozygous and 9 were 508G > A heterozygous (Fig. 2), which represents an allele frequency of 0.18.

The fourth and the fifth 5′ *FUT3* gene fragments, 667 bp–880 bp and 859 bp–1086 bp, respectively, showed one polymorphism, 1067T > A. This alteration, 1067T > A, was found in 10 individuals, in 6 cases 1067T > A polymorphism was homozygous and in 4 cases it was heterozygous (Fig. 2), which represents an allele frequency of 0.17.

We have found 14 different profiles of *FUT3* gene polymorphisms that are described and depicted in Table 2. Profile (59T > G 202T > C 314C > T) was the most common and was observed in 28% of the cases.

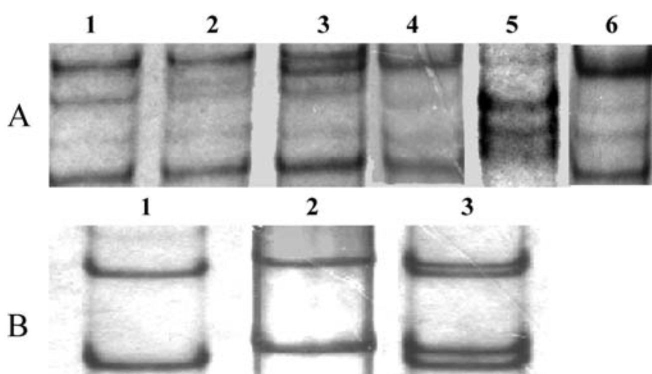

Fig. 1 A SSCP patterns obtained for the first 5′ FUT3 gene fragment (33 bp–227 bp) in individuals with normal genotype (*1*), Heterozygous 202T > C genotype (*2*), Heterozygous 59T > G/202T > C genotype (*3*), Homozygous 59T > G genotype (*4*) and Homozygous 202T > C genotype (*5*) Homozygous 59T > G/Heterozygous202T > C (*6*). **B** SSCP patterns obtained for the second 5′ FUT3 gene fragment (203 bp–474 bp) in individuals with normal genotype (*1*), Homozygous 314C > T genotype (*2*) and Heterozygous 314C > T genotype (*3*)

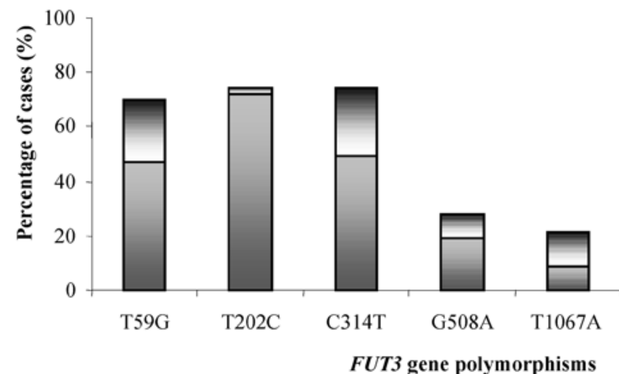

Fig. 2 Percentage of cases with each polymorphism, 59T > G, 202T > C, 314C > T, 508G > A and 1067T > A. The *bars* represent the percentage of homozygous individuals (*above line*) and heterozygous individuals for each polymorphism (*below line*)

Table 2 Patterns of *FUT3* gene polymorphisms and Lewis phenotype in gastric mucosa

Lewis in peripheral blood (Le^a and/or Le^b)	Lewis in gastric mucosa (Le^a and/or Le^b)	Profiles of FUT3 gene polymorphisms	No of individuals
Absent	Absent	Homozygous 202T > C/314C > T	1
		Heterozygous 59T > G/202T > C/314C > T	4
		Heterozygous 202T > C Homozygous 314C > T	1
		Heterozygous 202T > C Homozygous 314C > T/508G > A	1
		Homozygous 59T > G Heterozygous 202T > C/1067T > A	1
		Heterozygous 59T > G/202T > C Homozygous 314C > T	1
	Present	Homozygous 1067T > A	1
		Heterozygous 59T > G/202T > C/314C > T	6
		Homozygous 314C > T	2
		Heterozygous 202T > C/314C > T Homozygous1067T > A	1
		Heterozygous 59T > G/202T > C/314C > T/508G > A	5
		Heterozygous 59T > G/202T > C/314C > T/1067T > A	1
		Heterozygous 202T > C/314C > T	2
		Homozygous 59T > G Heterozygous 508G > A	1
		Heterozygous 59T > G/202T > C/314C > T Homozygous 508G > A	1
		Heterozygous 202T > C Homozygous 314C > T	4
		Homozygous 59T > G/508G > A Heterozygous 202T > C	1
		Heterozygous 59T > G/202T > C Homozygous 508G > A	1
		Homozygous 59T > G	1
		Homozygous 59T > G Heterozygous 1067T > A	3
		Heterozygous 59T > G/202T > C/508G > A Homozygous 314C > T	1
		Heterozygous 59T > G/202T > C Homozygous 314C > T	1
		Homozygous 59T > G Heterozygous 202T > C/314C > T	1
		Heterozygous 59T > G/202T > C/508G > A	1
		Homozygous 59T > G Heterozygous 314C > T/1067T > A	1
		Heterozygous 202T > C/314C > T/508G > A	1
		Homozygous 59T > G/ 1067T > A	2

Table 3 *FUT3* polymorphism profiles and Lewis phenotype in gastric mucosa

	Lewis negative *n* (%)	Lewis positive *n (%)*	Total
Homozygous for deleterious polymorphisms[a]	2 (22.2)	7 (77.8)	9
Homozygous 59T > G	1 (12.5)	7 (87.5)	8
Homozygous 314C > T	2 (20.0)	8 (80.0)	10
Heterozygous	4 (20.0)	16 (80.0)	20

[a]Homozygous for any of the deleterious polymorphisms, 202T > C, 508G > A and 1067T > A

FUT3 polymorphism patterns and Le phenotype in gastric mucosa

The study of gastric biopsies by immunohistochemistry showed that only 9 of the 47 individuals evaluated by immunohistochemistry (19%) were negative for Lewis antigen expression both in the tissues and in the red blood cells. The other 38 individuals showed expression of Lewis antigens in the tissues despite the blood Lewis negative phenotype (Table 2). Due to the extensive number and complexity of the polymorphism profiles observed for each individual (Table 2), we decided to group the cases in the following categories: homozygous for deleterious polymorphisms (202T > C, 508G > A and 1067T > A); homozygous for 59T > G; homozygous for 314C > T, and cases that do not have homozygosity for any of the polymorphisms. Table 3 shows the Lewis expression in the gastric mucosa according to the recoded polymorphism profiles. Seven of the nine cases with homozygosity for deleterious polymorphisms showed expression of Lewis antigens in the gastric mucosa, in contrast to theoretical expectations. One of the cases homozygous for 59T > G, two of the cases homozygous for 314C > T and four of the cases heterozygous for all the polymorphisms did not show Lewis antigen expression in the gastric mucosa. Two identical polymorphism profiles were observed both in Lewis negative and Lewis positive cases in the group of homozygous for 314C > T polymorphism (two cases) and in the group of the heterozygous (ten cases) (Table 2 and Fig. 3). The polymorphism profiles of these cases (Tables 2 and 3) are complex and possible interpretations will be addressed in the discussion.

H. pylori infection and Lewis expression/polymorphism

In the present series most of the individuals were infected by *H. pylori* (95.0%) (Nogueira et al. 2001). No significant differences were observed between the presence and severity of *H. pylori* infection (Table 4) as well as the presence of atrophic gastritis and intestinal metaplasia and the Lewis positive/negative phenotype (data not shown).

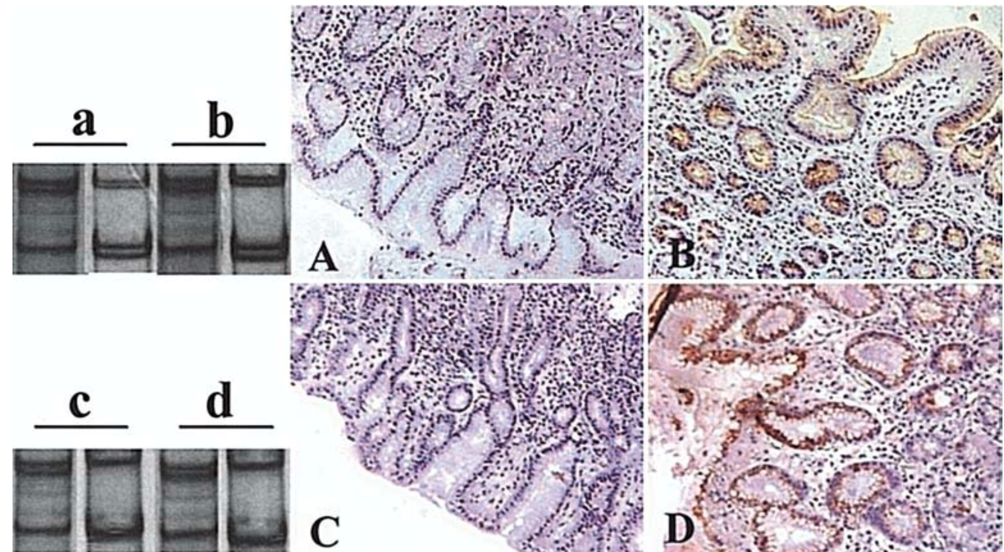

Fig. 3A–D FUT3 polymorphism profiles with both Lewis negative and Lewis positive individuals in gastric mucosa, *a*/*b*: SSCP patterns for cases 1 and 2 (Heterozygous 59T > G/202T > C/314C > T); **A** Lewis negative gastric mucosa for case 1; **B** Lewis positive gastric mucosa for case 2; *c*/*d*: SSCP patterns for cases 3 and 4 (Heterozygous 59T > G/ 202T > C and Homozygous 314C > T); **C** Lewis negative gastric mucosa for case 3, and **D** Lewis positive gastric mucosa for case 4

Discussion

In the present study we identified five polymorphisms of the *FUT3* gene in 47 Portuguese individuals that have a Lewis negative blood group phenotype (10.2% of 460 individuals evaluated). All the polymorphisms we have identified were previously reported in other populations (Elmgren et al. 1993, 1996; Koda et al. 1993; Nishihara et al. 1994; Ørntoft et al. 1996; Pang et al. 1996).

The frequency of Lewis negative blood group phenotype we have observed (10.2%) is very similar to the frequency (10%) reported by Watkins (1980) in Caucasians. This indicates that polymorphisms inactivating the FucT III enzyme have a similar and relatively low prevalence in the populations evaluated so far.

Three of the *FUT3* polymorphisms were identified at a high frequency within the Lewis negative population: 59T > G (0.47), 202T > C (0.38) and 314C > T (0.50). The three polymorphisms are described in all the populations previously studied (Elmgren et al. 1993, 1996; Koda et al. 1993; Liu et al. 1999; Mollicone et al. 1994; Nishihara et al. 1994; Ørntoft et al. 1996) and their frequency, whenever it was evaluated, was similar to the one we observed in the present study: 59T > G was observed with an allele frequency of 0.43 in 15 cases; 314C > T was observed with an allele frequency of 0.57 in 15 cases of individuals from Denmark (Elmgren et al. 1996). Polymorphism 202T > C has a higher allele frequency in Denmark (0.60) (Elmgren et al. 1996).

Individuals homozygous for 59T > G polymorphism were observed both in Lewis negative and in Lewis positive cases at the tissue expression level (see Table 2). This is partly in agreement with the evidence showing that the mutant enzyme has the same affinity (K_m) as the wild type enzyme (Mollicone et al. 1994). The reduced enzyme activity in vivo can be due to an inappropriate localisation of the enzyme in the Golgi since it affects the transmembrane domain (Ørntoft et al. 1996). The presence of homozygous 59T > G individuals in the Lewis negative cases can be explained by the coexistence in all these cases of other polymorphisms that abrogate enzyme activity.

Table 4 *H. pylori* infection and Lewis expression in gastric mucosa

	H. pylori colonisation			
	–	+	+ +	+ + +
Lewis positive[a]	18 (5.1%)	32 (9.1%)	140 (39.9%)	161 (45.9%)
Lewis negative[b]	0 (0.0%)	0 (0.0%)	4 (44.4%)	5 (55.6%)

[a]Includes all cases with Lewis blood positive phenotype as well as all cases that despite having a Lewis negative blood phenotype have Lewis expression in the gastric mucosa
[b]Includes nine Lewis negative cases in blood and in gastric mucosa

The other two polymorphisms that we identified (202T > C and 314C > T) are localised in the catalytic domain and are associated with a negative Lewis phenotype in Sweden (De Vries et al. 1995; Elmgren et al. 1996) and in Denmark (Ørntoft et al. 1996). Curiously, De Vries et al. (1995) showed that the 314C > T polymorphism has enzymatic activity similar to wild type enzyme when it catalyses the Le antigens synthesis on glycoprotein acceptors. Both in Sweden and in Denmark 202T > C and 314C > T polymorphisms co-localised in the same allele (Elmgren et al. 1996; Ørntoft et al. 1996). Although we did not study polymorphism profiles of individual alleles, we have identified individuals that carry the 202T > C polymorphism alone (four individuals – Table 2) or the 314C > T polymorphism alone (three individuals – Table 2).

The two other polymorphisms, 508G > A and 1067T > A, we have identified have already been described in Indonesian (Mollicone et al. 1994), Japanese (Koda et al. 1993; Nishihara et al. 1994), Swedish (Elmgren et al. 1996) and Danish (Ørntoft et al. 1996)

populations. Both 508G > A and 1067T > A polymorphisms were observed with a low frequency in our series (0.18 and 0.17, respectively); in the series of Elmgren et al. (1996) these two polymorphisms were found with a frequency of 0.07 and 0.30, respectively. Both 508G > A and 1067T > A polymorphisms affect the sequence of the catalytic domain and are deleterious for enzyme activity (Ørntoft et al. 1996).

The high homology between *FUT3* and *FUT6* genes may be responsible for the technical problems found in SSCP to separate the fourth and the fifth *FUT3* gene fragments. We observed the 1007A > C and 1029A > G alterations (data not shown), previously reported by Nishihara et al. (1993) as polymorphisms and after that called by the same authors as Taq polymerase artefacts (Nishihara et al. 1994). By comparing the sequence of the *FUT3* and *FUT6* genes, we verified that 1007A > C alteration corresponds to the 1004 nucleotide from *FUT6*. Other alterations were also found in the present study that correspond to the sequence of *FUT6* (data not shown).

The comparison we have performed between *FUT3* polymorphism profiles in Lewis negative blood phenotype individuals and the gastric tissue Lewis expression profile has not, to the best of our knowledge, been previously performed. The first interesting finding is that most cases (38 of 47 cases) have gastric tissue expression of Lewis antigens despite a Lewis negative blood phenotype. The expression of Lewis antigens in 81% of cases shows that although our population has a frequency of blood Lewis negative individuals expected for Caucasians (10%), just 2% of these individuals are Lewis negative in gastric mucosa. This fits with previous observations (Kobayashi et al. 1993) and probably reflects different FucT III enzyme activities towards different acceptor substrates (e.g. glycolipids vs glycoproteins) (De Vries et al. 1995). Seven of the cases with a Lewis positive expression profile in the gastric mucosa are homozygous for one of the deleterious polymorphisms (202T > C, 508G > A or 1067T > A) (Table 3). This observation suggests that fucosyltransferases other than FucT III can catalyse, in gastric tissues, the α 1,4 fucosyltransferase activity necessary to build Le^a and Le^b structures. A candidate gene product capable, in vitro, to build Le^a and Le^b antigens is FucT V (Kokowska-Latallo et al. 1990). Further studies should test the FucT V capability, in vivo, to build the Le^a and Le^b antigens. The observation that individuals homozygous for deleterious polymorphisms are either Lewis negative or Lewis positive in the gastric mucosa, indicates that the hypothetical alternative α 1,4 fucosyltransferase activity is also subject to inactivating polymorphisms in some individuals.

The nine cases that have a Lewis negative phenotype in the gastric mucosa as well as in the peripheral blood correspond to two cases homozygous for deleterious polymorphisms and seven cases homozygous for non-inactivating polymorphisms or heterozygous (Table 3). The two cases with homozygosity for deleterious polymorphisms have, as discussed in the previous paragraph, a good reason for the negative Lewis phenotype in the mucosa. In one of the other seven cases (Table 2), the inactivation of Lewis enzyme can be explained by allelic combination, since this individual is heterozygous for two deleterious polymorphisms (202T > C and 1067T > A). The other six cases do not have more than one inactivating polymorphism either in homo or in heterozygosity. In these six cases the evidence from in vitro experiments would predict the maintenance of enzyme activity and the expression of Lewis antigens. Our data clearly show that either the in vitro studies are not accurate to predict the in vivo situation or that we are not taking in account aspects of regulation of gene expression that may affect enzyme expression/activity in normal and pathological conditions.

The absence of relationship we have observed between the Lewis polymorphisms and the presence of *H. pylori* infection is in disagreement with the recent study by Ikehara et al. (2001), and suggests that, at least in a population with a high rate of *H. pylori* infection, the Lewis polymorphism does not affect the presence or absence of infection.

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