European Journal of Human Genetics (2001) 9, 548–552

www.nature.com/ejhg

ARTICLE

MUC1 gene polymorphism in the gastric carcinogenesis pathway

Filipe Silva*[,1,5], Filipa Carvalho[1,2,5], António Peixoto[1,5], Mário Seixas[1,2], Raquel Almeida[1], Fátima Carneiro[1,2], Patricia Mesquita[1], Céu Figueiredo[1], Cristina Nogueira[1], Dallas M Swallow[3], António Amorim[1,4] and Leonor David[1,2]

[1]*Institute of Molecular Pathology and Immunology of the University of Porto, IPATIMUP, Porto, Portugal;* [2]*Medical Faculty of Porto, Porto, Portugal;* [3]*MRC Human Biochemical Genetics Unit, London, UK;* [4]*Science Faculty of Porto, Porto, Portugal*

***MUC1* like most mucin genes shows extensive length polymorphism in the central core region. In a previous study it was shown that individuals with small *MUC1* alleles/genotypes have an increased risk for development of gastric carcinoma. Our aim was to see if *MUC1* gene polymorphism was involved in susceptibility for the development of conditions that precede gastric carcinoma: chronic atrophic gastritis (CAG) and intestinal metaplasia (IM). We evaluated *MUC1* polymorphism in a population of 174 individuals with chronic gastritis (CG) displaying (CAG) and/or intestinal metaplasia (IM). The population of patients with CG shows *MUC1* allele frequencies significantly different from the gastric carcinoma patients and blood donors population. A significantly lower frequency of CAG and IM was observed in *MUC1* VNTR heterozygotic patients. Within the group of patients with IM, *MUC1* large VNTR homozygotes show a significantly higher frequency of complete IM while small VNTR homozygotes show a significantly higher frequency of incomplete IM. These findings show that *MUC1* polymorphism may define different susceptibility backgrounds for the development of conditions that precede gastric carcinoma: chronic atrophic gastritis (CAG) and intestinal metaplasia (IM).** *European Journal of Human Genetics* (2001) **9,** 548–552.

Keywords: mucin; polymorphism; gastric cancer; *MUC1*; susceptibility; gastritis; intestinal metaplasia

Introduction

Mucins are the main component of the mucous layer that protects most epithelial interfaces.[1] These high molecular weight glycoproteins characteristically contain many serine and threonine residues that are potentially glycosylated. The protein backbone of most epithelial mucins shows a highly polymorphic central core with a variable number of repetitive units. To date 12 *MUC* genes have been described (http://www.gene.ucl.ac.uk/nomenclature) that encode secreted and/or membrane associated epithelial mucins, and their expression is variable in different tissues.[1] The complete sequence of *MUC1* including the central repetitive domain with 60 bp units has been reported.[2–4] The membrane associated mucin *MUC1* shows extensive allelic variation ranging from 25–125 repeat units.[2,5–7]

A multitude of interactions between genetic and environmental factors remain elusive in the aetiopathogenic model of gastric carcinoma. We have previously shown significantly different *MUC1* allele frequencies between a control population and gastric carcinoma patients and have observed that individuals with small VNTRs at *MUC1* core have an increased risk (OR 4.3 × –95% CI: 1.8–10.5) to develop gastric carcinoma.[6] Based on that data we hypothesised that such susceptibility trait might also be present in precursor lesions of the gastric carcinogenesis pathway. In the present study we explored the possibility that *MUC1* gene polymorphism may define different susceptibility backgrounds for the development of conditions that precede gastric

*Correspondence: F Silva, IPATIMUP, Rua Dr Roberto Frias s/n, 4200 Porto, Portugal. Phone: +351 22 5570700; Fax: +351 22 5570799; E-mail: fsilva@ipatimup.pt
[5]These authors contributed equally to this work
Received 18 January 2001; revised 17 April 2001; accepted 2 May 2001

carcinoma: chronic atrophic gastritis (CAG) and intestinal metaplasia (IM). To test this hypothesis, individuals with these conditions were evaluated for the *MUC1* VNTR polymorphism.

Material and methods

We evaluated the *MUC1* gene polymorphism in a series of 174 patients (age 43.8 ± 6.9; male : female ratio 9.2 : 1) with chronic gastritis, with (n=49) or without (n=125) glandular atrophy and with (n=48) or without (n=126) IM. The patients included in the series belong to a prospective study group from a population of Northern Portugal with a high incidence of gastric carcinoma. All cases in the study are from Viana do Castelo, and the enrolment was based on volunteer participation after informed consent of individuals presenting symptoms of dyspepsia. Both blood samples and gastric biopsies were analysed in all cases. Only patients with gastric lesions were included in the present study due to the small number of individuals showing normal gastric biopsies (n=6). There were no cases with peptic ulcer, dysplasia or gastric carcinoma. The data were compared with our previous data from blood donors (controls) (age: 39.7 ± 11.0; male : female ratio 3.6 : 1) and patients with gastric cancer (age: 61.0 ± 12.3; male : female ratio 1.6 : 1).[6] All individuals from the previous series and from the present work were from Northern Portugal. Differences in age and sex distributions between the three populations were not taken into account for comparative purposes since *MUC1* gene polymorphism is not affected by age or sex in the control population of blood donors.[6]

MUC1 polymorphism analysis

DNA was isolated from blood samples (10 ml), using a salt-chloroform extraction method, as previously described by Mullenbach *et al*.[8] DNA samples were digested with *Eco*RI, that recognises restriction sites in the regions flanking the tandem repeats of the *MUC1* gene. Electrophoretic separation and Southern blotting were performed using the conditions previously described.[6]

All the alleles identified in controls and patients with chronic gastritis were ranked and numbered from 1 to 15 according to their size and by comparison with a ladder of alleles previously characterised. Allele 1 represents the longest allele and allele 15 represents the shortest allele. Allele frequencies of patients with gastritis were estimated by gene counting. The high polymorphism of *MUC1* locus prompted us to group the genotypes according to Carvalho *et al*.[6] Alleles 1–5 were grouped into the category of Large *MUC1* alleles (L) and alleles 6–15 were grouped into the category of Small *MUC1* alleles (S). The partition of alleles into Large and Small categories was performed using the median value of the distribution of the alleles in the control population of blood donors.[6] Genotypes were thereafter recoded as: LL (*MUC1* large VNTR homozygotes), SS (*MUC1* small VNTR homozygotes) and Het (all heterozygotes).

Endoscopy and histological characterisation of the gastric lesions

From each patient biopsy specimens were taken from corpus (n=1) and antrum (n=2) and immediately frozen at -80°C until use for further processing. Endoscopes were reprocessed after each procedure with adequate clearing and disinfection in a 2% glutaraldehyde solution according to the ESGE guidelines.[9]

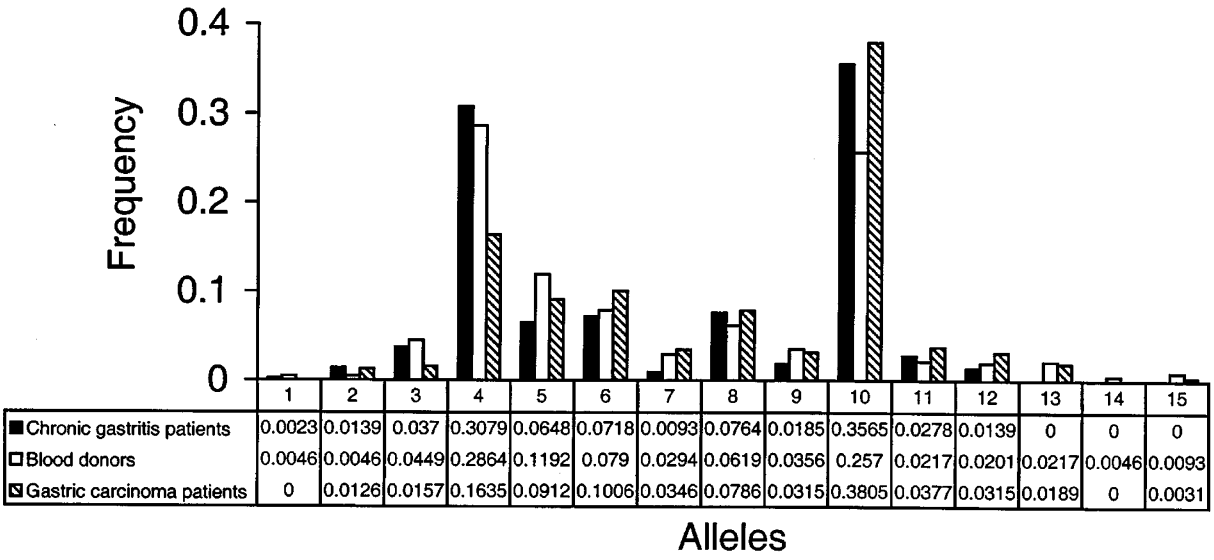
The gastric biopsies for histology were fixed in 10% formalin and routinely embedded in paraffin wax. The histological study of the 174 biopsies included evaluation of glandular atrophy and of IM. Chronic gastritis was subdivided in two groups: nonatrophic gastritis and chronic atrophic gastritis (CAG).[10] IM was classified into complete and incomplete subtypes according to the pattern of mucin expression.[11,12] Complete IM is characterised by a terminal intestinal differentiation pattern and incomplete IM is characterised by a mixed intestinal/gastric phenotype.[12]

Statistical analysis

The frequencies of *MUC1* alleles in the population of patients with chronic gastritis were compared with frequencies in blood donors (n=323) and gastric carcinoma patients (n=159).[6] Heterogeneity analysis between populations was based on allelic contingency tables using the STRUC program from software package GENEPOP.[13] Associations between polymorphism and the presence of CAG or IM were evaluated using STATVIEW 4.02 software (SAS Institute, Carry NC, USA). Monte Carlo test was also applied (10 000 iterations) whenever expected values were less than 5 as described by Sham and Curtis[14] using the computer program CLUMP. Odds ratios and 95% confidence limits were determined using the BMDP 4F computer program (Statistical Package program BMDP; Los Angeles, CA, USA).

Results

We analysed *MUC1* polymorphism in a population of patients with CG from the northern region of Portugal. The population showed 12 alleles combined in 36 distinct genotypes with a heterozygosity of 71.3%. All the alleles had been previously described in blood donors, and 11 were also present in patients with gastric carcinoma[6] (Figure 1). Comparison of overall *MUC1* allele frequencies between patients with CG and blood donors shows a significant difference ($P<0.0001$): alleles 5, 7 and 13 are more frequent in blood donors, while allele 10 is more prevalent in patients with CG (Figure 1). A significant difference ($P<0.0001$) was also observed between patients with CG and patients with gastric carcinoma: allele 4 is more frequent in patients with CG, whereas alleles 7 and 13 are more prevalent among patients with CG (Figure 1). A more focused analysis on modal alleles (4 and 10) shows that patients with CG have a frequency of allele 10 which is similar to carcinoma patients and significantly higher than that observed in blood donors,



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Chronic gastritis patients	0.0023	0.0139	0.037	0.3079	0.0648	0.0718	0.0093	0.0764	0.0185	0.3565	0.0278	0.0139	0	0	0
Blood donors	0.0046	0.0046	0.0449	0.2864	0.1192	0.079	0.0294	0.0619	0.0356	0.257	0.0217	0.0201	0.0217	0.0046	0.0093
Gastric carcinoma patients	0	0.0126	0.0157	0.1635	0.0912	0.1006	0.0346	0.0786	0.0315	0.3805	0.0377	0.0315	0.0189	0	0.0031

Figure 1 Distribution of allele frequencies of *MUC1* gene in patients with CG (*n*=174), blood donors (*n*=323) and patients with gastric carcinoma (*n*=159). Alleles are numbered according to their size by decreasing order of molecular weight (allele 1 is the largest).

while allele 4 displays similar frequencies in patients with CG and blood donors, which are significantly higher than that observed in gastric carcinoma patients.

Chronic atrophic gastritis (CAG) was observed in 28.2% of the patients with CG. Comparison between the frequency of glandular atrophy for different *MUC1* genotypes showed a significant difference (*P*=0.004) (Table 1). A lower prevalence of CAG was observed in heterozygotes (21%), when compared to homozygotes for large VNTR (47%) or small VNTR (45%). Significant differences were also present considering only the genotypes involving modal alleles (4 and 10) (data not shown).

Intestinal metaplasia (IM) was observed in 27.0% of patients with chronic gastritis. A detailed evaluation of IM complete and incomplete sub-types and *MUC1* genotypes revealed a significant association (*P*=0.01) (Table 2): large VNTR homozygotes are associated with complete IM (28.6%, in contrast to 14.7% in incomplete IM and 7.9% in individuals with no IM), while small VNTR homozygotes are associated with incomplete IM (32.4%, in contrast to 7.1% in complete IM and 15.2% in individuals with no IM) (Table 2).

Considering the aforementioned associations we calculated the odds ratio for the development of atrophy and IM in patients with different *MUC1* VNTR genotypes (Table 3). Comparisons were performed within the population with CG and all odds ratios were calculated for the morphologic lesions (CAG; complete IM; incomplete IM) according to the MUC1 genotypes (LL; Het; SS). Significant odds ratios, despite the large 95% CI, were found for the development of CAG in *MUC1* VNTR homozygotes: LL 2.6 (95% CI: 1.0–6.8) and SS 2.5 (95% CI: 1.1–5.7). The odds ratio for the development of complete IM in patients with CG is 3.9 (95% CI: 1.1–13.9) for homozygotes with large VNTR. In contrast, the odds ratio for the development of incomplete IM is 2.9 (95% CI: 1.2–6.0) for homozygotes with small VNTR (Table 3).

Table 1 Relationship between chronic atrophic gastritis (CAG) and *MUC1* gene polymorphism in patients with chronic gastritis

MUC1 *recoded genotypes* n=*174*	*CAG*	
	Present n (%)	*Absent* (n (%)
LL*	9 (47.0)	10 (53.0)
Het*	26 (21.0)	98 (79.0)
SS*	14 (45.0)	17 (55.0)

*Allele were recoded to large (L) and small (S) categories according to the median allele of the control population (allele 5). LL and SS genotypes are notations for homozygous individuals for L and S VNTR alleles and Het is the notation for all hereozygotes. *P* value=0.004

Table 2 Relationship between intestinal metaplasia (IM) and *MUC1* gene polymorphism in patients with chronic gastritis

MUC1 *recoded genotypes* n=*174*	*No IM* n (%)	*Complete IM* n (%)	*Incomplete IM* n (%)
LL	10 (7.9)	4 (28.6)	5 (14.7)
Het	97 (76.9)	9 (64.3)	18 (52.9)
SS	19 (15.2)	1 (7.1)	11 (32.4)

P value=0.01

Discussion

In the present study we have shown that patients with chronic gastritis have *MUC1* allele frequencies that are

significantly different both from the allele frequencies previously described in blood donors and from those in gastric carcinoma patients.[6] Analysis of the modal alleles, shows that patients with chronic gastritis have a frequency of allele 10 which is similar to carcinoma patients and significantly higher than that observed in blood donors, while allele 4 displays similar frequencies in patients with chronic gastritis and blood donors, which are significantly higher than that observed in gastric carcinoma patients. These data suggest that the population of patients with chronic gastritis includes two sub-groups: one that is genetically closer to blood donors, based on a similar frequency of *MUC1* allele 4, and the other that is closer to gastric carcinoma patients, based on a similar frequency of *MUC1* allele 10. This observation suggests the idea that the population of patients with chronic gastritis may be heterogeneous and fits with the concept that only a small proportion of patients with CG carries an increased risk for gastric carcinoma development.[11,15]

Our study further showed that CAG is a frequent lesion (28.2%) in patients with chronic gastritis. The frequency of CAG also varies according to *MUC1* genotype with heterozygotes having a significantly lower prevalence of CAG. These observations suggest that CG in individuals with *MUC1* heterozygosity tends to maintain a non-atrophic phenotype while homozygosity constitutes a susceptible genetic background for the development of CAG. The putative biological significance of the protective effect conferred by heterozygosity should be addressed in future biochemical studies aiming to explore the possibility that imbalance of mucin size (VNTR alleles) might affect mucin oligomerisation.

Table 3 Odds ratios (95% confidence limits) for the development of chronic atrophic gastritis (CAG) and complete and incomplete intestinal metaplasia (IM) in patients with chronic gastritis according to *MUC1* polymorphism

MUC1 *recorded genotypes*	*CAG*	*Complete IM*	*Incomplete IM*
LL	2.6 (1.0–6.8)*	3.9 (1.1–13.9)*	1.6 (0.5–4.7)
Het	0.3 (0.2–0.6)	0.7 (0.2–2.2)	0.4 (0.2–0.8)
SS	2.5 (1.1–5.7)*	0.3 (0.5–4.5)	2.9 (1.2–6.0)*

*Significant OR.

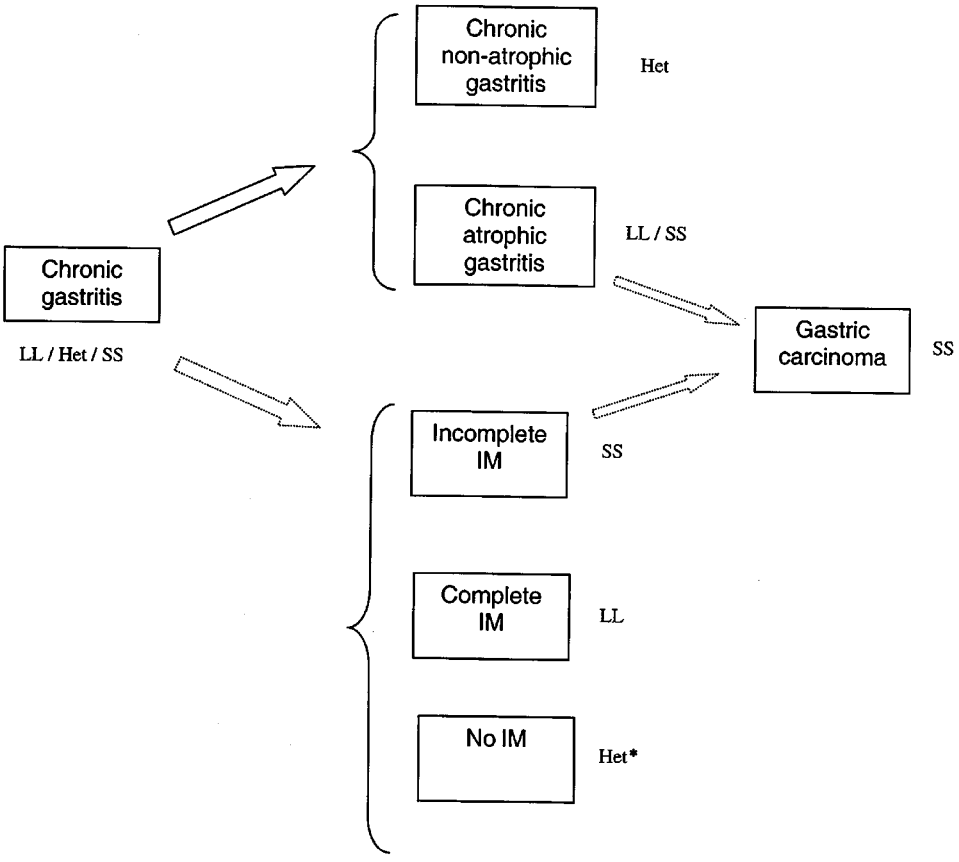


Figure 2 Schematic representation of the association between *MUC1* VNTR polymorphism and the different lesions in the gastric carcinogenesis pathway. * Results from a previous publication of our group.[6]

Several reports have shown that only incomplete IM carries an increased risk of malignant transformation.[16–19] Thus we detailed the evaluation of *MUC1* polymorphism in patients with IM taking into account the two main types of IM – complete and incomplete types. The significant associations of large VNTR homozygotes with complete IM and small VNTR homozygotes with incomplete IM suggest that *MUC1* genotypes are relevant for intestinal metaplasia outcome.

Summing up, our results show that *MUC1* genotypes are significantly associated with susceptibility for the development of non-atrophic chronic gastritis on one hand and of CAG on the other. Furthermore, our study showed that homozygosity for large VNTRs is significantly associated with complete IM, whereas homozygosity for small VNTRs is significantly associated with incomplete IM. The interaction between *MUC1* gene polymorphism and the different lesions in the gastric carcinogenesis pathway is outlined in Figure 2. Noteworthy is that the homozygotes for small *MUC1* VNTR alleles (SS) are significantly associated with gastric carcinoma[6] as well as with CAG and incomplete IM, the two well established precursor lesions of gastric carcinoma (Figure 2).

Acknowledgments

We thank Professor Manuel Sobrinho-Simões for many helpful suggestions and critical reading of the manuscript. The authors are grateful to the following departments where patient's data and samples were collected: Estaleiros Navais de Viana do Castelo, Departments of Surgery 3 and Gastroenterology of Hospital S. João, Porto, Portugal. This work was supported by grants from: Fundação para a Ciência e a Tecnologia (PRAXIS XXI, Project SAU/14111/1998), EU Concerted Action BMH4-CT98-3222 and Administração Regional de Saúde do Norte.

References

1 Gendler SJ, Spicer AP: Epithelial mucin genes. *Annu Rev Physiol* 1995; **57**: 607–634.
2 Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, *et al*: Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem* 1990; **265**: 15286–15293.
3 Lan MS, Batra SK, Qi WN, Metzgar RS, Hollingsworth MA: Cloning and sequencing of a human pancreatic tumor mucin cDNA. *J Biol Chem* 1990; **265**: 15294–15299.
4 Lancaster CA, Peat N, Duhig T, Wilson D, Taylor-Papadimitriou J, Gendler SJ: Structure and expression of the human polymorphic epithelial mucin gene: an expressed VNTR unit. *Biochem Biophys Res Commun* 1990; **173**: 1019–1029.
5 Swallow DM, Gendler S, Griffiths B, Corney G, Taylor-Papadimitriou J, Bramwell ME: The human tumour-associated epithelial mucins are coded by an expressed hypervariable gene locus PUM. *Nature* 1987; **328**: 82–84.
6 Carvalho F, Seruca R, David L *et al*: MUC1 gene polymorphism and gastric cancer–an epidemiological study. *Glycoconj J* 1997; **14**: 107–111.
7 Carvalho F, Peixoto A, Steffensen R, Amorim A, David L, Sobrinho-Simões M: MUC1 gene polymorphism does not explain the different incidence of gastric cancer in Portugal and Denmark. *Ann Hum Genet* 1999; **63**: 187–191.
8 Mullenbach R, Lagoda PJ, Welter C: An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends Genet* 1989; **5**: 391.
9 The European Society of Gastrointestinal Endoscopy: Guidelines on cleaning and desinfection in GI endoscopy. Update 1999. *Endoscopy* 2000; **32**: 77–80.
10 Dixon MF, Genta RM, Yardley JH, Correa P: Classification and grading of gastritis. The updated Sydney sistem. *Am J Surg Pathol* 1996; **20**: 1161–1181.
11 Filipe MI, Jass JR: Intestinal metaplasia subtypes and cancer risk. In: Filipe MI, Jass JR (eds): *Gastric Carcinoma*. Edinburgh: Churchill Livingstone, 1986, pp. 87–115.
12 Reis CA, David L, Correa P *et al*: Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression. *Cancer Res* 1999; **59**: 1003–1007.
13 Raymond M, Rousset F: GENEPOP (version 1.2): A population genetics software for exact tests and ecumenicism. *J Heredity* 1995; **86**: 248–249.
14 Sham PC, Curtis D: Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet* 1995; **59**: 97–105.
15 Correa P: Human gastric carcinogenesis: a multistep and multifactorial process–First American cancer society award lecture on cancer epidemiology and prevention. *Cancer Res* 1992; **52**: 6735–6740.
16 Craanen ME, Dekker W, Ferwerda J, Blok P, Tytgat GN: Early gastric cancer: a clinicopathologic study. *J Clin Gastroenterol* 1991; **13**: 274–283.
17 Rokkas T, Filipe MI, Sladen GE: Detection of an increased incidence of early gastric cancer in patients with intestinal metaplasia type III who are closely followed up. *Gut* 1991; **32**: 1110–1113.
18 Silva S, Filipe MI, Pinho A: Variants of intestinal metaplasia in the evolution of chronic atrophic gastritis and gastric ulcer. A follow up study. *Gut* 1990; **31**: 1097–1104.
19 Filipe MI: Natural history of precursor lesions to gastric carcinoma: growth factors and oncogenes in the metaplasia-dysplasia-carcinoma sequence. *Eur J Cancer Prev* 1994; **3**: 19–23.