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ARTICLE

MUC1 polymorphism confers increased risk for intestinal metaplasia in a Colombian population with chronic gastritis

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Gastric cancer (GC) stands as the second most common cause of cancer death for males worldwide, and intestinal metaplasia (IM) is a lesion that precedes GC development. In previous works it was shown that polymorphisms of MUC1 gene are associated with increased risk for GC and IM. The aim of the present study was to evaluate MUC1 gene polymorphism in patients with chronic gastritis from Colombia. A Portuguese population of patients with chronic gastritis was used for comparative purposes. A total of 67 Colombian cases and 52 Portuguese cases were analysed by restriction analysis and Southern blotting. MUC1 allele frequencies were significantly different between the two populations, with an overall prevalence of smaller alleles in Colombian samples. Colombian cases showed a lower prevalence of individuals homozygous for small MUC1 mucins in cases without IM (62.5%) when compared with cases with IM (86.0%). The same trend, although not statistically significant, is observed in the Portuguese population. In conclusion, our study shows that Colombian patients with chronic gastritis have a significantly higher prevalence of small MUC1 alleles than the Portuguese population. Our study also shows that small MUC1 genotypes are associated with increased risk for IM development in Colombian patients.
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Introduction

Gastric cancer stands as the second most common cause of cancer death for males worldwide.[1] The gastric carcinogenesis pathway is still poorly understood, but most studies indicate that intestinal metaplasia (IM) is a lesion of the gastric mucosa that precedes gastric carcinoma (GC) development.[2] Therefore, it is tempting to identify risk factors associated with IM as a way of progressing in the understanding of gastric carcinogenesis.

Mucins are heavily glycosylated proteins that constitute the major component of the mucous protective layer above mucosal surfaces.[3] MUC1 has been described as a membrane-associated molecule expressed on the apical membrane of various glandular epithelial cells and hematopoietic cells.[4,5] In 1997, our group has shown that individuals with MUC1 gene containing a small number of tandem repeats are at increased risk for GC development.[6] The rationale underlying that first study was that the genetic variation in size, common to most mucin genes,[7] might have an influence on the protective capabilities of

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the final mucin product. The MUC1 mucin was chosen as a target since it is expressed in gastric mucosa, and the gene polymorphism was demonstrated to have a direct effect on the size of the protein product.[8] We have then shown that, in the Portuguese population, the same MUC1 genetic polymorphism is associated with increased risk for development of IM, mostly incomplete IM subtype.[9]

In the present study we explore if the MUC1 susceptibility model for GC and IM is transposable to populations with distinct genetic background, in this case a series of individuals with chronic gastritis from a Colombian area with a high incidence of GC.

Materials and methods

Populations

We evaluated the MUC1 gene polymorphism in blood samples from two populations of patients with chronic gastritis. Colombian patients ($n=67$) were from Nariño, an Andean region of southern Colombia with an extremely high prevalence of GC. The Colombian patients population (age: 49.6 ± 8.8 years; male:female ratio 1.5:1) is mostly formed by 'Mestizos', a mixture of Amerindian and Spaniard ancestries. Portuguese patients ($n=52$) (age: 48.3 ± 8.1; male:female ratio 4.2:1) were recruited during a screening program for (pre)malignant lesions of the gastric mucosa in a northern region of Portugal with a high incidence of gastric cancer. Portuguese patients were all Caucasians.

MUC1 polymorphism analysis

DNA was isolated from blood samples (10 ml), using a salt–chloroform extraction method, as previously described by Mullenbach *et al*.[10] DNA samples were digested with *Alu*I (restriction sequence AG↓CT), which recognizes 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 were ranked and numbered from 1 to 20 according to their size and by comparison with a ladder of alleles previously characterized.[11] Allele 1 represents the largest allele and allele 20 represents the smallest allele. The sizes of the different MUC1 alleles were obtained by comparison with the mobility of the marker λ*Hind*III (Amersham).

Allele frequencies and Hardy–Weinberg equilibrium were assayed by GENEPOP software.[12] The high polymorphism of the MUC1 locus prompted us to group the alleles in large (L) and small (S) categories according to Carvalho *et al*.[6] Alleles 1–9 were grouped in the category of large alleles (L) and alleles 10–20 were grouped in the category of small (S) alleles. The partition of the alleles into large and small categories was performed using the median value of the distribution of the alleles in a control population of blood donors. Genotypes were thereafter recoded as LL (MUC1 large VNTR homozygotes), SS (MUC1 small VNTR homozygotes), and LS (heterozygotes for large and small alleles).

Endoscopy and histological characterization of the gastric lesions

From each patient biopsy specimens were taken from corpus ($n=1$) and antrum ($n=2$). Endoscopes were reprocessed after each procedure with adequate clearing and disinfection in a 2% glutaraldehyde solution according to the ESGE guidelines.[13]

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[14] into four distinct diagnostic classes: (a) chronic gastritis without atrophy, (b) chronic gastritis with atrophy, (c) chronic gastritis with atrophy and IM, and (d) chronic gastritis with atrophy, IM and dysplasia (Figure 1).

Statistical analysis

The frequencies of MUC1 alleles in the Colombian population were compared with the frequencies in the Portuguese population. Heterogeneity analysis between populations was based on allelic contingency tables using the STRUC program from the software package GENEPOP.

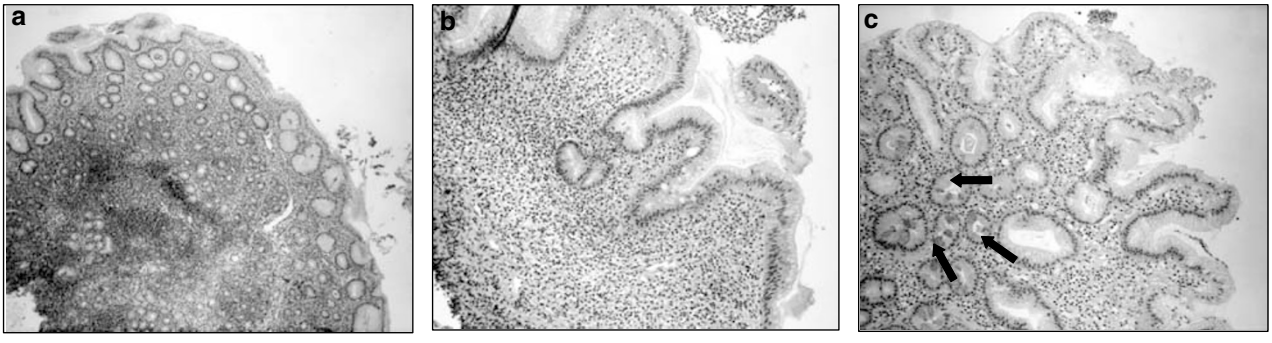
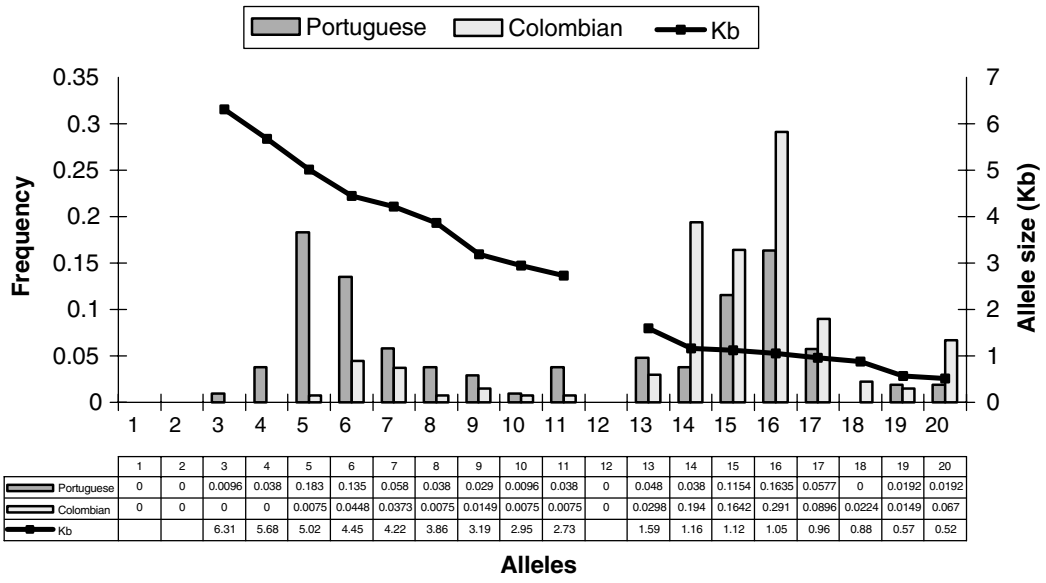


Figure 1 Histology of different diagnostic classes: (**a**) chronic gastritis without atrophy; (**b**) chronic gastritis with atrophy; (**c**) chronic gastritis with atrophy and IM (arrows).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Portuguese	0	0	0.0096	0.038	0.183	0.135	0.058	0.038	0.029	0.0096	0.038	0	0.048	0.038	0.1154	0.1635	0.0577	0	0.0192	0.0192
Colombian	0	0	0	0	0.0075	0.0448	0.0373	0.0075	0.0149	0.0075	0.0075	0	0.0298	0.194	0.1642	0.291	0.0896	0.0224	0.0149	0.067
Kb			6.31	5.68	5.02	4.45	4.22	3.86	3.19	2.95	2.73		1.59	1.16	1.12	1.05	0.96	0.88	0.57	0.52

Figure 2 Distribution of allele frequencies of MUC1 gene in Colombian ($n=67$) and Portuguese ($n=52$) patients with chronic gastritis. Alleles are numbered according to their size by decreasing order of molecular weight (allele 1 is the largest). Molecular weight of the different alleles is also plotted in the figure using the secondary *Y*-axis.

Associations between polymorphisms and the presence of atrophy and IM were evaluated using STATVIEW 4.02 software (SAS Institute, Cary, NC, USA). The Monte Carlo test was also applied (10 000 iterations) whenever expected values were less than 5 as described by Sham and Curtis[15] using the computer program CLUMP. Odds ratio and 95% confidence limits were determined using the BMDP 4F computer program (Statistical Package program BMDP; Los Angeles, CA, USA).

Results

The analysis of the MUC1 polymorphism in the 67 Colombian patients disclosed 15 alleles combined in 31 distinct genotypes with 85% of heterozygotes. All the alleles present in the Colombian patients had been previously described in other populations.[11] Data from Colombian patients were compared with those from a subsample of 52 Portuguese patients with chronic gastritis previously described.[9] The overall MUC1 allele frequencies distribution of the Colombian population is significantly different from the Portuguese population distribution ($P<0.001$) (Figure 2). The differences are mainly due to alleles 14 and 16 (small VNTRs), which are significantly over-represented in Colombian cases ($P<0.001$ and $P=0.022$, respectively), while alleles 5 and 6 (large VNTRs) are more prevalent in Portuguese cases ($P<0.001$ and $P=0.019$, respectively). The genotypic distributions are in Hardy–Weinberg equilibrium for both populations.

The gastric lesions observed in Colombian and Portuguese patients were classified in distinct categories (Table 1). The Colombian population showed atrophy in 56 cases (83.6%), and IM in 43 cases (64.2%), eight of them with dysplasia. The Portuguese population showed atrophy in 46 cases (88.5%), and intestinal IM in 35 cases (67.3%); no dysplastic lesions were observed in this population.

Table 1 Frequency of diagnostic categories in 67 patients from Colombia and 52 patients from Portugal with chronic gastritis

Diagnostic categories	*Colombia Number of patients (%)*	*Portugal Number of patients (%)*
Without atrophy	11 (16.4)	6 (11.5)
With atrophy	13 (19.4)	11 (21.2)
Atrophy with IM	35 (52.3)	35 (67.3)
Atrophy with IM and dysplasia	8 (11.9)	0 (0.0)

The frequency of *MUC1* recoded genotypes showed significant differences according to the presence or absence of IM in the Colombian population (Table 2). Colombian cases showed a lower prevalence of SS genotypes in cases without IM (62.5%) when compared with cases with IM (86.0%). The same trend, though not statistically significant as previously published for the whole population,[9] is observed in the Portuguese subsample (Table 2). No associations were found for classes with atrophy or dysplasia (data not shown).

The odds ratio for the development of IM was calculated according to the MUC1 genotypes – the cases homozygous

for large alleles (LL) were deleted from the analysis since only one case with an LL genotype was observed in the Colombian population. The presence of small VNTRs in homozygosity confers an increased risk for IM development (odds ratio 3.7) while the heterozygotes are 'protected' from the development of IM (odds ratio 0.2). The odds ratio for development of atrophy or dysplasia is not statistically significant (data not shown). A trend for an increased risk of developing IM in individuals with small VNTRs homozygosity was also observed in the Portuguese population (Table 3).

Discussion

In the present study we evaluated a cohort of Colombian patients with chronic gastritis from a region (Nariño) with a high incidence of gastric cancer.[16] We have shown that MUC1 allele frequencies of the Colombian patients are significantly different from the allele frequencies of a subsample of a previously described Portuguese population of patients with chronic gastritis.[9] Individual analysis of the alleles shows that Colombian patients have a significantly higher frequency of small VNTR alleles (alleles 14 and 16), while large VNTR alleles (alleles 5 and 6) have a significantly higher frequency in the Portuguese patients. The data show that Colombian and Portuguese patients' populations with chronic gastritis are genetically different for the MUC1 polymorphism, probably reflecting population ancestry and locus evolution.

Both the Colombian and the Portuguese patients with chronic gastritis have a high prevalence of IM (64.2 and 67.3%, respectively). The high frequency of IM in the two populations fits with the presence of a high incidence of GC in both settings.[17,18] The association between SS MUC1 genotypes and IM that we observed in the Colombian population (odds ratio 3.7) fits with previous observations in a Portuguese population.[9] Both observations suggest that in lesions of chronic gastritis, metaplastic transformation is associated with SS MUC1 genotypes. Similarly, a protective role for IM development is associated with MUC1 heterozygosity (LS) in the Colombian patients, as previously observed in the Portuguese population.[9]

The observations reported in the present study, together with previous publications,[6,9] reinforce that small MUC1 VNTRs homozygosity is associated with increased risk for the development of IM[9] and gastric carcinoma.[6] However, recent data from the literature show that allele size does not influence the levels of MUC1 expression in the gastric mucosa.[19] The putative biological significance of MUC1 VNTR variability remains elusive and should be addressed in future biochemical studies aiming to explore the effect of mucin size (VNTR alleles) in the adhesion of pathogens and mucin oligomerization.

In conclusion, our study shows that Colombian and Portuguese patients with chronic gastritis are genetically different for the MUC1 VNTR locus. Our study also shows that MUC1 small VNTRs genotypes confer increased susceptibility for IM development in the context of chronic gastritis in Colombian patients. The results support the involvement of MUC1 VNTR in the susceptibility for development of IM and GC in populations with different genetic background.

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Table 2 Frequency of chronic gastritis with and without IM according to MUC1 recoded genotypes in the Colombian and Portuguese populations

	Colombia		*Portugal*	
MUC1 recoded genotypes	*Without IM* N *(%)*	*With IM* N *(%)*	*Without IM* N *(%)*	*With IM* N *(%)*
LL	0 (0.0)	1 (2.3)	5 (29.4)	8 (22.9)
LS	9 (37.5)	5 (11.6)	8 (47.1)	17 (48.6)
SS	15 (62.5)	37 (86.0)	4 (23.5)	10 (28.6)
	P=0.037		*P*=0.856	

Table 3 Odds ratio (95% confidence limits) for the development of IM in patients with chronic gastritis from Colombia and Portugal, according to MUC1 polymorphism

MUC1 recoded genotypes with small VNTRs	*Colombia*		*Portugal*	
	Without IM (95% cl)	*With IM (95% cl)*	*Without IM (95% cl)*	*With IM (95% cl)*
Homozygous (SS)	0.2 (0.1–0.9)*	3.7 (1.1–12.2)*	0.7 (0.2–2.9)	2.2 (0.5–8.6)
Heterozygous (LS)	4.6 (1.3–15.9)*	0.2 (0.1–0.8)*	0.9 (0.3–3.0)	1.1 (0.3–3.4)

*Significant OR.

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