Genetic polymorphisms of MMP1, MMP3 and MMP7 gene promoter and risk of colorectal adenoma.
Astrid Lièvre, Jacqueline Milet, Jérôme Carayol, Delphine Le Corre, Chantal Milan, Alexandre Pariente, Bernard Nalet, Jacques Lafon, Jean Faivre, Claire Bonithon-Kopp, et al.

To cite this version:
Astrid Lièvre, Jacqueline Milet, Jérôme Carayol, Delphine Le Corre, Chantal Milan, et al.. Genetic polymorphisms of MMP1, MMP3 and MMP7 gene promoter and risk of colorectal adenoma.. BMC Cancer, BioMed Central, 2006, 6, pp.270. 10.1186/1471-2407-6-270 . inserm-00122090

HAL Id: inserm-00122090
https://www.hal.inserm.fr/inserm-00122090
Submitted on 26 Dec 2006

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Genetic polymorphisms of MMP1, MMP3 and MMP7 gene promoter and risk of colorectal adenoma

Astrid Lièvre1, Jacqueline Milet2,3, Jérôme Carayol2, Delphine Le Corre1, Chantal Milan4, Alexandre Pariente5, Bernard Nalet6, Jacques Lafon7, Jean Faivre4, Claire Bonithon-Kopp4, Sylviane Olschwang8, Catherine Bonaiti-Pellié2,3, Pierre Laurent-Puig*1 and members of the ANGH group

Address: 1INSERM U775 et Université René Descartes, Paris, France, 2INSERM, U535, Villejuif, France, 3Université Paris-Sud, IFR69, Villejuif, France, 4INSERM E106, Faculté de Médecine, Dijon, France, 5Centre Hospitalier, Pau, France, 6Centre Hospitalier, Montélimar, France, 7Centre Hospitalier, Aix-en-Provence, France and 8INSERM U599, Institut Paoli Calmettes, Marseille, France

Email: Astrid Lièvre - astrid-lievre@orange.fr; Jacqueline Milet - milet@vjf.inserm.fr; Jérôme Carayol - carayol@vjf.inserm.fr; Delphine Le Corre - delphine.lecorre@univ-paris5.fr; Chantal Milan - chantal.milan@u-bourgogne.fr; Alexandre Pariente - alex.pariente@wanadoo.fr; Bernard Nalet - bernard.nalet@ch-montelimar.fr; Jacques Lafon - jacques.lafon@ch-aix.fr; Jean Faivre - jean.faivre@chu-dijon.fr; Claire Bonithon-Kopp - bonithon@u-bourgogne.fr; Sylviane Olschwang - olschwangs@marseille.fnclcc.fr; Catherine Bonaiti-Pellié - bonaiti@vjf.inserm.fr; Pierre Laurent-Puig* - pierre.laurent-puig@univ-paris5.fr; members of the ANGH group - Pierre.laurent-puig@univ-paris5.fr

* Corresponding author

Abstract

Background: Matrix metalloproteinases (MMP) have been shown to play a role in colorectal cancer (CRC). More recently, MMP1, MMP3 and MMP7 functional gene promoter polymorphisms have been found to be associated with CRC occurrence and prognosis. To document the role of MMP polymorphisms in the early step of colorectal carcinogenesis, we investigated their association with colorectal adenomas in a case-control study comprising 295 patients with large adenomas (LA), 302 patients with small adenomas (SA) and 568 polyp-free (PF) controls.

Methods: Patients were genotyped using automated fragment analysis for MMP1 -1607 ins/del G and MMP3 -1612 ins/delA (MMP3.1) polymorphisms and allelic discrimination assay for MMP3 -709 A/G (MMP3.2) and MMP7 -181 A/G polymorphisms. Association between MMP genotypes and colorectal adenomas was first tested for each polymorphism separately and then for combined genotypes using the combination test. Adjustment on relevant variables and estimation of odds ratios were performed using unconditional logistic regression.

Results: No association was observed between the polymorphisms and LA when compared to PF or SA. When comparing SA to PF controls, analysis revealed a significant association between MMP3 -1612 ins/delA polymorphism and SA with an increased risk associated with the 6A/6A genotype (OR = 1.67, 95%CI: 1.20–2.34). Using the combination test, the best association was found for MMP3.1-MMP1 (p = 0.001) with an OR of 1.88 (95%CI: 1.08–3.28) for the combined genotype 2G/2G-6A/6A estimated by logistic regression.

Conclusion: These data show a relation between MMP1 -1607 ins/del G and MMP3 -1612 ins/delA combined polymorphisms and risk of SA, suggesting their potential role in the early steps of colorectal carcinogenesis.
Matrix metalloproteinases (MMPs) are an important family of metal-dependant enzymes that are responsible for the degradation of extracellular matrix components. MMPs are involved in various physiologic processes, such as embryogenesis and tissue remodeling [4,5]. They also play a key role in invasion and metastasis of tumor cells which require proteolysis of basal membranes and extracellular matrix [6]. For a long time, MMPs were considered to be important almost exclusively in these two steps of carcinogenesis. However, recent studies suggested that MMPs are involved in several other processes associated with cancer development. Indeed, they regulate tumor growth and apoptosis, they promote angiogenesis, loss of cell adhesion, invasion and metastasis. Finally, some of them are also required in immune responses to cancer [7].

The role of MMPs in CRC has been described [8,9]. Numerous, including MMP1, MMP3 and MMP7 are overexpressed in colorectal tumors [10,11]. The expression of MMP7 was shown to correlate with Dukes' stage and increased metastatic potential [12,13] while MMP-1 expression was shown to be related to invasion, metastasis and prognosis [14-16]. Moran et al. demonstrated that MMP3 expression was significantly lower in CRC with high microsatellite instability which are known to have a better clinical outcome than CRC without microsatellite instability [17]. This observation suggests that MMP3 could be implicated in tumor invasion, lymph node involvement and metastatic spread in CRC. MMPs are overexpressed in a variety of premalignant tumor tissues, including colorectal adenoma [18-20] and MMP7 has been shown to be important in the growth of early colonic adenomas and their transformation into invasive cancer [21].

A functional single nucleotide polymorphism (SNP) has been reported in the MMP1 gene promoter that consists in a guanosine (G) insertion at position -1607. This SNP generates a new 5'-GGA-3' core recognition sequence for members of the Ets family of transcription factors [22]. In vitro, the homozygous 2G/2G genotype results in an increased transcription activity compared to the 1G/1G genotype. In vivo, an association was found between the 2G allele and MMP1 overexpression in ovarian cancer tissue [23]. The MMP1 2G/2G genotype was reported to be linked to an increased risk of CRC [24,25]. Indeed, the 2G allele was shown to favor invasion, metastasis and prognosis [25,26]. A SNP corresponding to an insertion/deletion of an adenosine (A) at position -1612 of the MMP3 gene promoter was also described and shown to interfere with transcriptional activity [10]. In a case-control study, Hinoda et al. found that the frequency of the 6A allele was significantly higher in CRC patients than in controls, as the frequency of the 6A/6A genotype compared to the 5A/6A + 5A/5A genotypes [24]. In this study, MMP1 and MMP3 were in significant linkage disequilibrium and the most frequent haplotype 2G-6A was significantly increased in CRC patients compared to controls. Concerning the other MMP3 polymorphisms, four are in substantial linkage disequilibrium with the -1612 ins/del A (-1986 T/C, -1346 A/C, -376 G/C, and +802 A/G), except one (-709 A/G) which is not of proven functional importance [27]. In the MMP7 gene promoter region, two SNPs (-181 A/G and -135 C/T) have been shown to modify the gene transcription activity [28]. Recently, both SNPs have been associated with CRC susceptibility and the -181 A/G genotype was linked to distant metastasis [29]. These findings are in favor of the role of MMP1, MMP3 and MMP7 functional gene polymorphisms in colorectal carcinogenesis. They are localized in a MMP cluster of 400 kb at 11q21–23 that counts 9 MMPs (MMP-1, 3, 7, 8, 10, 12, 13, 20, 27).

To explore whether MMP1, MMP3 and MMP7 gene promoter polymorphisms are involved in the early step of colorectal carcinogenesis, we investigated the relation between these polymorphisms and the risk of colorectal adenoma in a case-control study.

**Methods**

**Selection of cases and controls**

The GEADE study is a case-control and family study of patients with high-risk adenomas (larger than 9 mm). The data were obtained from 18 participating gastroenterology units of general hospitals in France (table 3). From September 1995 to March 2000, 306 consecutive patients with newly diagnosed colorectal large adenoma (LA) were enrolled in the study. Subjects with history of cancer, familial adenomatous polyposis, established hereditary non-polyposis colorectal cancer and inflammatory bowel disease had been excluded. In order to be able to distinguish genetic factors involved in the occurrence of adenomas or in their growth, 307 cases with small adenomas (SA) (smaller than 0.5 cm) and 572 polyp-free (PF) controls (with normal colonoscopy) were enrolled in the same units. All patients and controls were Caucasian.
Information on indication of colonoscopy, family history of CRC, and completeness of colonoscopy was also collected for all patients and controls. Controls (2 per case) were matched to LA cases by age, gender, and geographic area (five regions: Paris area, North-East, Centre, South-West and South-East, France) (table 3). Patients with SA were relatively rare and could not be matched with LA cases. Blood samples were obtained at the time of the colonoscopy and were kept only if adenoma was diagnosed after histological examination (for LA and SA cases). Twenty individuals could not be genotyped because biological material was not sufficient: 11 cases with LA, 5 cases with SA and 4 PF controls. The final sample was thus: 295 cases with LA, 302 cases with SA and 568 PF controls. A complete description of the patients sample is given in Cottet et al. [30]. All patients and controls gave their informed consent and the study was approved by an ethics committee for biomedical research (Le Kremlin-Bicêtre) and by the National Committee CNIL (Commission nationale de l’informatique et des libertés).

**MMP1, MMP3 and MMP7 genotyping**

The MMP1 -1607 ins/del G, MMP3 -1612 ins/del A, MMP3 -709 A/G and MMP7 -181 A/G polymorphisms were named respectively MMP1, MMP3.1, MMP3.2 and MMP7 polymorphisms. MMP1 and MMP3.1 have been studied by genotyping lymphocyte DNA by multiplex PCR-based approach using the following primers: 5’ [6FAM]-GCCCCTTTGAACTCACAATGTTATG-3’ and 5’-ACTTCCCTCCCCATGATGATCC-3’ for MMP1, 5’-GTCTTTC-3’ and 5’-[FAM]-GTCATATCTGCGCAAAGAGAGAGA-3’ and 5’-[FAM]-CGGCACCTGGCTAAAGAC-3’ for MMP7 polymorphism as previously described [31]. After amplification and dilution, fragments were separated on an ABI Prism 3700 DNA Genetic Analyzer (Applied Biosystems).

The MMP3.2 and MMP7 polymorphisms were studied by real-time PCR allelic discrimination assay on ABI 7900 HT Sequence Detection System (Applied Biosystems) as previously described [31]. For the MMP7 DNA amplification was performed with forward 5’-AGTCAATTTATGCAGCAAGA-3’ and reverse 5’-GTGTTATTTTCTCAT-TAACCTAAGGAGA-3’ primers and allelic discrimination was performed by the use of specific probes for each allele respectively labelled with fluorescence reporter dyes VIC and FAM: 5’-CAATGTATTGTCTTTC-3’ and 5’-CAATGTATTTCGTCTTTC-3’.

For the MMP3.2 polymorphism, a mix containing specific primers and fluorescent probes designed by Applied Biosystems (Taqman SNP Genotyping Assay, ID C_3047714_10) was used. Some QC blinded samples were distributed throughout the runs and genotyped for concordance of results and control samples without DNA samples were systematically included in each MMP genotyping plate.

**Statistical analysis**

Hardy-Weinberg proportions were tested for each polymorphism. Linkage disequilibrium (LD) between pairwise loci was estimated using the measure D’ [32].

Association was first tested for each polymorphism separately. For each polymorphism, genotype-specific odds ratio (OR) and 95% confidence intervals (CI) were computed using unconditional logistic regression adjusted on matching factors and Wald test was used to assess the global effect of this SNP. The homozygous genotype for the more frequent allele among controls was set as the reference class. Homogeneity of allele frequencies within regions was previously tested. Tests of homogeneity and unconditional logistic regression were done using STATA.

The association was further examined using the combination test, a method developed by Jannot et al. [33] that allows for the analysis of all possible combinations of 1 to n tightly linked SNPs in order to test their association with the disease. For each SNP combination, the method computes a statistic test contrasting the genotypic (or haplotypic) distribution between cases and controls. Because all these tests are correlated (many of them are nested in each other), a permutation procedure is implemented which displays a significance level adequately adjusted for multiple testing. In a second step, among the significantly associated combinations, the most parsimonious one can be identified by comparing nested combinations using chi2 tests.

With the 4 SNPs of the present study, a total of 15 SNP combination were tested (4 single SNP tests, 6 two-SNP tests, 4 three-SNP tests and 1 four-SNP test).

| Table 1: Characteristics of the study population |
|-----------------------------------------------|
| Large adenomas | Small adenomas | Polyp free controls |
| N | 295 | 302 | 568 |
| Age, mean (SD) | 62.3 (13.1) | 60.6 (12.1) | 60.7 (12.4) |
| Sex ratio (male/female) | 1.68 | 1.24 | 1.52 |

SD: standard deviation
First, we used the FAMHAP12 software to apply this method by performing haplotypic tests [34]. Then, the COMBINTEST (Jannot, personal communication) was used to perform genotypic tests.

As the combination test does not yet allow an adjustment on confounders, when association was found significant, the combination was further tested using unconditional logistic regression and the risks associated with the different genotypes were estimated.

**Results**

Table 1 shows some characteristics of the cases and controls. LA cases were slightly older than SA and PF controls, but there was no age difference between LA and SA patients, despite the absence of matching. The sex ratio (male/female) was similar in LA and PF because of matching, and slightly higher in LA than in SA patients.

The distribution of genotypes for the 4 polymorphisms was consistent with Hardy-Weinberg equilibrium (p = 0.65 for MMP1, p = 0.13 for MMP3.1, p = 0.36 for MMP3.2 and p = 0.10 for MMP7). As shown in table 2, three of the four polymorphisms (MMP1, MMP3.1 and MMP3.2) were in LD but MMP7 was only in slight LD with MMP3.2 polymorphism (D' = 0.18) and was not found in LD with the other ones (D'< 0.05).

Some differences in allele frequencies were found between centres inside the regions defined for matching (the homogeneity of allele frequencies was rejected for 5 tests among the 20 ones performed). Although such differences might be due to chance, there might exist some kind of stratification within the 5 regions initially defined, as found for other polymorphisms [35]. Therefore, we adjusted analyses on smaller groups of centres to protect against spurious association due to possible remaining population admixture. Centres with too few participants were pooled with the geographically closest ones, which led to eleven categories (table 3).

Results of analyses by single polymorphisms for LA vs PF, LA vs SA and SA vs PF are presented in table 4. No association was observed between the polymorphisms and LA when compared to PF or SA. Conversely, a significant effect of MMP3.1 polymorphism was found when comparing SA to PF controls (p = 0.008, Wald test). The data suggest an opposite effect of 6A allele depending on whether one or two copies were present. Compared with the 5A/5A genotype, the 6A/6A variant genotype appeared associated with an increased risk of SA whereas 5A/6A was inversely associated but the ORs were not significantly different from 1.0. When we considered a recessive model, the genotype 6A/6A was this time clearly associated with a higher risk of SA compared with the genotypes 5A/5A and 5A/6A pooled together (OR = 1.67, 95%CI: 1.20–2.34).

The haplotypic analysis with the combination test did not reveal any effect of the 4 polymorphisms on LA. When comparing SA vs PF, the test was globally significant after correction for multiple testing (p = 0.012). The combination of polymorphisms found associated with SA with the lowest p-value was MMP1-MMP3.1 (p = 0.001, uncorrected). This was the best combination as no other combinations of 1 or 2 SNP was significantly associated with SA (in particular, when MMP3.1 was considered alone, the p-value was 0.059). The genotypic analysis by the combination test provided quite similar results with a p-value of 0.03 after correction for multiple testing. The lowest p-value was found for the same combination MMP1-MMP3.1 (p = 0.003, uncorrected). The only difference was that MMP3.1 SNP alone was significantly associated with SA (p = 0.011). As in the haplotypic analysis, the comparison between statistics of MMP1-MMP3.1 and MMP3.1 combinations showed that the combination MMP3.1-MMP1 was the most significantly associated one.

An association between combined MMP1-MMP3.1 genotypes and SA was confirmed by logistic regression adjusted on centre (p = 0.002) (table 5). The same opposite effect of 5A and 6A alleles shown in the SNP by SNP analyses was found for MMP3.1 but only for the carriers of genotype 2G/2G at the MMP1 locus.

**Table 2: Linkage disequilibrium (D') between pairwise polymorphisms**

| Polymorphisms D' (p-value) | MMP3.1 | MMP7 | MMP3.2 |
|---------------------------|--------|------|--------|
| MMP1                      | 0.429 (<0.001) | 0.016 (0.686) | 0.761 (<0.001) |
| MMP3.1                    | 0.040 (0.231) | 0.946 (<0.001) | 0.185 (0.005) |
| MMP7                      |        |      |        |
The distribution of genotypes for the four polymorphisms was consistent with Hardy-Weinberg equilibrium. MMP1, MMP3.1 and MMP3.2 polymorphisms were found in LD, as previously in several studies, the MMP1 2G and MMP3.1 6A alleles as well as the MMP1 1G and MMP3.1 5A alleles were in LD with a D’ coefficient between 0.50 and 0.92 according to the ethnic origin of the patients [24,26,31]. Concerning the MMP3.2 -709 A/G polymorphism, few data are available but one study reported a substantial LD between five MMP3 promoter polymorphisms, except for this one, which had a lower frequency of the minor allele (0.2 vs > 0.4 for the minor allele of the other polymorphisms) [27]. The MMP7 polymorphism was not found in LD with the others (D’ < 0.05), which is consistent with the literature [31].

In this case-control study, the MMP3.1 6A/6A genotype was significantly associated with an increased risk of SA when compared to PF controls (OR = 1.67, 95%CI: 1.20–2.34, under a recessive model) while no association was observed between the MMP polymorphisms analyzed and LA when compared to PF or SA. Moreover, the combination test revealed a stronger effect of the MMP1-MMP3.1 2G/2G-6A/6A combined genotype. These results suggest that MMP1 polymorphism, which was not found to influence adenoma risk when taken alone, may have a role by interacting with the effect of MMP3.1. This finding underlines the importance of using the combination test for demonstrating combined effects of polymorphisms with little or no individual effect. Indeed, this test has been shown to be particularly powerful to detect the effect of polymorphisms when several polymorphisms interact with low marginal effect of each SNP and when one of the SNPs masks the expression of the other ones [33].

Our data suggest that the MMP3.1 polymorphism, and especially the MMP1-MMP3.1 2G/2G-6A/6A combined genotype might play a role at the earliest step of colorectal carcinogenesis by promoting the development of adenomas from normal colon epithelial cells. However, the following steps of colorectal carcinogenesis, in particular adenoma growth, could require the contribution of other factors. These factors could mask the effect of MMP1-MMP3.1 combined genotype and explain the absence of association when LA patients are compared to PF controls. However, these preliminary results must be taken with caution and have to be confirmed.

The MMP3.1 6A/6A genotype has already linked to the development of CRC. Indeed, its frequency was significantly higher in CRC patients than in controls when compared to the 5A/5A + 5A/6A genotypes in a case-control study [24]. These results are inconsistent with the hypothesis that the increased transcriptional level of MMP3 increases CRC susceptibility as the 6A/6A genotype is associated with the lowest transcriptional level [36]. One

Table 3: Distribution of cases and controls in the different centres and groups of centres

| Region         | Centre         | Number of individuals by centre | Number of individuals by group of centres |
|----------------|----------------|---------------------------------|------------------------------------------|
|                |                | All cases | Controls | All cases | Controls |
| Paris area     | Le Chesnay    | 10        | 15       | 55        | 47       |
|                | Meaux          | 45        | 32       | 55        | 47       |
|                | Orleans        | 16        | 7        | 12        | 9        |
|                | Le Mans        | 49        | 49       | 65        | 56       |
|                | Dreux          | 12        | 39       | 12        | 39       |
| North-East     | Evreux         | 25        | 16       | 58        | 49       |
|                | Beauvais       | 8         | 19       | 51        | 43       |
|                | Soissons       | 1         | 1        | 1         | 1        |
|                | Compiègne     | 24        | 16       | 58        | 49       |
|                | Metz           | 45        | 49       | 45        | 49       |
| Centre         | Vichy          | 38        | 2        | 37        | 4       |
|                | Bourg en Bresse| 76        | 51       | 114       | 53       |
|                | Beaune         | 37        | 37       | 37        | 37       |
| South-West     | Tarbes         | 31        | 29       | 31        | 29       |
|                | Pau            | 67        | 54       | 67        | 54       |
| South-East     | Toulon         | 10        | 10       | 10        | 10       |
|                | Aix en Provence| 58        | 61       | 68        | 71       |
|                | Montélimar     | 45        | 36       | 45        | 36       |

The distribution of genotypes for the four polymorphisms was consistent with Hardy-Weinberg equilibrium. MMP1, MMP3.1 and MMP3.2 polymorphisms were found in LD, as previously in several studies, the MMP1 2G and MMP3.1 6A alleles as well as the MMP1 1G and MMP3.1 5A alleles were in LD with a D’ coefficient between 0.50 and 0.92 according to the ethnic origin of the patients [24,26,31]. Concerning the MMP3.2 -709 A/G polymorphism, few data are available but one study reported a substantial LD between five MMP3 promoter polymorphisms, except for this one, which had a lower frequency of the minor allele (0.2 vs > 0.4 for the minor allele of the other polymorphisms) [27]. The MMP7 polymorphism was not found in LD with the others (D’ < 0.05), which is consistent with the literature [31].

In this case-control study, the MMP3.1 6A/6A genotype was significantly associated with an increased risk of SA when compared to PF controls (OR = 1.67, 95%CI: 1.20–2.34, under a recessive model) while no association was observed between the MMP polymorphisms analyzed and LA when compared to PF or SA. Moreover, the combination test revealed a stronger effect of the MMP1-MMP3.1 2G/2G-6A/6A combined genotype. These results suggest that MMP1 polymorphism, which was not found to influence adenoma risk when taken alone, may have a role by interacting with the effect of MMP3.1. This finding underlines the importance of using the combination test for demonstrating combined effects of polymorphisms with little or no individual effect. Indeed, this test has been shown to be particularly powerful to detect the effect of polymorphisms when several polymorphisms interact with low marginal effect of each SNP and when one of the SNPs masks the expression of the other ones [33].

Our data suggest that the MMP3.1 polymorphism, and especially the MMP1-MMP3.1 2G/2G-6A/6A combined genotype might play a role at the earliest step of colorectal carcinogenesis by promoting the development of adenomas from normal colon epithelial cells. However, the following steps of colorectal carcinogenesis, in particular adenoma growth, could require the contribution of other factors. These factors could mask the effect of MMP1-MMP3.1 combined genotype and explain the absence of association when LA patients are compared to PF controls. However, these preliminary results must be taken with caution and have to be confirmed.

The MMP3.1 6A/6A genotype has already linked to the development of CRC. Indeed, its frequency was significantly higher in CRC patients than in controls when compared to the 5A/5A + 5A/6A genotypes in a case-control study [24]. These results are inconsistent with the hypothesis that the increased transcriptional level of MMP3 increases CRC susceptibility as the 6A/6A genotype is associated with the lowest transcriptional level [36]. One
explanation may be that MMP3 is indirectly involved in the development of CRC [24]. The presence of an increased risk of adenomatous polyps of the colon in coronary atherosclerosis patients [37,38] suggested that the association of the MMP3 6A/6A genotype and CRC is due to its link with an increased atherosclerosis risk [39-41]. These speculations should obviously be taken with caution and the mechanisms underlying the potential involvement of MMP3.1 polymorphism in colorectal carcinogenesis remain to be clearly determined.

Different studies showed that some MMP, including MMP2, MMP7 and MMP9 are expressed in colorectal adenomas that are well established premalignant lesions of colorectal cancers [18-20,42], implying their role other than extracellular matrix destruction and metastasis in cancer development and progression. The expression of E1AF, an Ets family transcriptional factor that plays a role in the progression of CRC, has been shown to be associated with the expression of MMP1 and MMP7 in CRC tissues [43,44]. Moreover, E1AF has been reported to be a potent activator of cyclooxygenase-2 (COX-2) transcription [45] and it is established that COX-2 plays an important role in the early stages of colorectal carcinogenesis [46]. Finally, absence of MMP7 may result in a significant reduction in mean tumour number and average tumour diameter in Min (multiple intestinal neoplasia) mice deficient in this MMP [21]. These findings suggest that some MMP might contribute to early tumour development, especially in tumours of gastrointestinal tract. Although several studies analyzed the expression of different MMP in colorectal adenomas, none had investigated the role of functional MMP polymorphisms in the development of these premalignant lesions despite evidence of their implication in colorectal carcinogenesis.

In the present study, the MMP3.1 6A/6A genotype was significantly associated with an increased risk of SA. Moreover, the analysis of the effects of combined MMP genotypes by the means of the combination test allowed to find an indirect role of MMP1 polymorphism in this early step of colorectal carcinogenesis since a potentialisation of the effect of MMP3.1 polymorphism by this polymorphism has been shown. Such an effect would not have been identified if a separated analysis of each MMP genotype had been performed, underlining the relevance of the combination test.

**Conclusion**

These data show a relation between MMP1 -1607 ins/delG and MMP3 -1612 ins/delA combined polymorphisms and risk of colorectal SA, suggesting their potential role in the early steps of colorectal carcinogenesis.

**Abbreviations**

CRC; colorectal cancer

LA; large adenoma

MMP; matrix metalloproteinases

**Table 4: Association between genotypes of MMP and colorectal adenomas**

|                     | Large adenoma patients (LA) | Polyp free controls (PF) | Small adenoma patients (SA) | LA vs PF | LA vs SA | SA vs PF |
|---------------------|----------------------------|--------------------------|----------------------------|----------|---------|---------|
|                     | OR† (95%CI) | P** | OR† (95%CI) | P** | OR† (95%CI) | P** |
| MMP1 1G/1G         | 81 | 154 | 81 | 1 | 1 | 1 |
| 1G/2G              | 139 | 285 | 139 | 0.94 (0.66–1.33) | 1.04 (0.69–1.56) | 0.96 (0.67–1.37) |
| 2G/2G              | 72 | 122 | 79 | 1.18 (0.78–1.78) | 0.491 | 0.90 (0.56–1.43) | 0.792 | 1.22 (0.81–1.84) | 0.431 |
| MMP3.1 5A/5A       | 70 | 130 | 68 | 1 | 1 | 1 |
| 5A/6A              | 139 | 291 | 132 | 0.87 (0.60–1.25) | 1.10 (0.72–1.69) | 0.85 (0.58–1.24) |
| 6A/6A              | 73 | 126 | 93 | 1.11 (0.73–1.69) | 0.391 | 0.78 (0.48–1.25) | 0.250 | 1.50 (0.99–2.28) | 0.008 |
| MMP3.2 AA          | 182 | 358 | 194 | 1 | 1 | 1 |
| AG                 | 105 | 187 | 92 | 1.10 (0.81–1.50) | 1.20 (0.83–1.73) | 0.87 (0.64–1.20) |
| GG                 | 7 | 19 | 16 | 0.72 (0.29–1.78) | 0.615 | 0.49 (0.19–1.27) | 0.175 | 1.34 (0.65–2.75) | 0.464 |
| MMP7 AA            | 92 | 187 | 99 | 1 | 1 | 1 |
| AG                 | 138 | 259 | 136 | 1.08 (0.77–1.50) | 1.06 (0.72–1.56) | 0.98 (0.70–1.38) |
| GG                 | 64 | 119 | 67 | 1.17 (0.78–1.77) | 0.742 | 1.08 (0.67–1.72) | 0.944 | 1.14 (0.76–1.71) | 0.746 |

* OR adjusted on age, sex, and group of centres
** P: p-value for the Wald test assessing the global effect of SNP, uncorrected for multiple testing
Table 5: Combined effect of MMP1 and MMP3.1 loci in small adenomas

| Genotypes  | MMP free (n) | Small adenoma (n) | OR* (95% CI) |
|------------|--------------|-------------------|--------------|
| MMP1       | MMP3.1       |                   |              |
| 1G-1G      | 5A-5A        | 76                | 47           | 1            |
| 1G-1G      | 5A-6A        | 58                | 26           | 0.77 (0.41–1.43) |
| 1G-1G      | 6A-6A        | 19                | 5            | 0.37 (0.12–1.12) |
| 1G-2G      | 5A-5A        | 46                | 18           | 0.68 (0.34–1.38) |
| 1G-2G      | 5A-6A        | 174               | 88           | 0.83 (0.52–1.33) |
| 1G-2G      | 6A-6A        | 56                | 33           | 1.13 (0.62–2.04) |
| 2G-2G      | 5A-5A        | 8                 | 3            | 0.63 (0.15–2.62) |
| 2G-2G      | 6A-6A        | 59                | 18           | 0.45 (0.23–0.89) |
| 2G-2G      | 6A-6A        | 51                | 55           | 1.88 (1.08–3.28) |

* OR adjusted on age, sex and group of centres

PF; polyp free

SA; small adenoma

SNP; single nucleotide polymorphism

Competing interests

The author(s) declare that they have no competing interests.

Authors’ contributions

AL and DLC carried out the genotyping under the supervision of PLP. AP, BN, JL and the ANGH Group participated to the inclusion of patients in the study and performed colonoscopies with polypectomy. SO carried out the DNA extraction. JM, JC and CBP performed the statistical analysis. CBP, CM, JF, CBK, SO, AP and JL participated in the design of the study and/or the management of the data. AL, CBP, JM and PLP drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank all the following members of the ANGH Group for their participation in the GEADE study: TP. Berthelémy (Pau), P. Cassan (Vichy), M. Blanchard (Châteauroux), J. Andrieu (Le Chesnay), JL. Staub (Niort), D Labarrière (Orléans), D Legoux (Orléans), J. Naudy (Toulouse), D. Goldfain (Dreux), C. Bories (Beauvais), J. Andreu (Le Chesnay), J. L. Staub (Niort).

The study was supported by grants from the “Fondation de France”, the French National Society of Gastroenterology, the “Association pour la Recherche sur le Cancer”, the Regional Council of Burgundy and the French Ministry of Health (PHRC). The U775 unit is supported by the Ligue Nationale contre le Cancer.

References

1. Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 1990, 61:759-767.
2. Gilbertsen VA: Proctosigmoidoscopy and polypectomy in reducing the incidence of rectal cancer. Cancer 1974, 34:suppl.936-9.
3. Winawer SJ, Zauber AG, Ho MN, O’Brien MJ, Gottlieb LS, Sternberg SS, Waje JD, Schapiro M, Bond JH, Panish JF, et al.: Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. N Engl J Med 1993, 329:1977-1981.
4. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM: Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000, 18:1135-1149.
5. Visse R, Nagase H: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003, 92:827-839.
6. Forget MA, Desrosiers RR, Beliveau R: Physiological roles of matrix metalloproteinases: implications for tumor growth and metastasis. Can J Physiol Pharmacol 1999, 77:465-480.
7. Egeblad M, Warb Z: New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002, 2:161-174.
8. Leeman MF, Curran S, Murray GI: New insights into the roles of matrix metalloproteinases in colorectal cancer development and progression. J Pathol 2003, 201:528-534.
9. Zuckcr S, Vacirca J: Role of matrix metalloproteinases (MMPs) in colorectal cancer. Cancer Metastasis Rev 2004, 23:101-117.
10. Ye S: Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. Matrix Biol 2000, 19:623-629.
11. Hewit RE, Leach IH, Powe DG, Clark IM, Cawston TE, Turner DR: Distribution of collagenase and tissue inhibitor of metalloproteinases (TIMP) in colorectal tumours. Int J Cancer 1991, 49:666-672.
12. Morii M, Barnard GF, Minnori K, Ueo H, Akiyoshi T, Sugimachi K: Overexpression of matrix metalloproteinase-7 mRNA in human colon carcinomas. Cancer 1995, 75:1516-1519.
13. Yamamoto H, Ishi F, Hinoda Y, Imai K: Suppression of matrixins inhibits colon cancer cell invasion in vitro. Int J Cancer 1995, 61:182-222.
14. Shiozawa J, Ito M, Nakayama T, Nakashima M, Kohno S, Sekine I: Expression of matrix metalloproteinase-1 in human colorectal carcinoma. Mod Pathol 2000, 13:925-933.
15. Murray GI, Duncan ME, O’Neil P, Melvin WT, Fothergill JE: Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. Nat Med 1996, 2:461-462.
16. Baker EA, Bergin FG, Leaper DJ: Matrix metalloproteinases, their tissue inhibitors and colorectal cancer staging. Br J Surg 2000, 87:1215-1221.
17. Moran A, Iniesta P, de Juan C, Gonzalez-Quevedo R, Sanchez-Perroteau A, Diaz-Rubio E, Ramon y Cajal S, Torres A, Balibrea JL, Benito Nagashima Y, Miyazaki K, Koshikawa N, Mitsuhashi M, Shimada H: Expression of matrixins mRNA in colorectal adenomas and its induction by truncated fibronectin. Biochem Biophys Res Commun 1999, 264:365-366.
Matrilysin gene expression in sporadic and familial colorectal adenomas. Mol Carcinog 1997, 19:225-229.
21. Watanabe CL, Heppner KJ, Labosky PA, Hogan BL, Matrissian LM: Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci U S A 1997, 94:1402-1407.
22. Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, Brinkhoffer CE: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 1995, 55:3321-3325.
23. Kanamori Y, Matsushima M, Minaguchi T, Sagae S, Kudo R, Takakuna N, Nakamura Y: Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Res 1999, 59:4225-4227.
24. Hinoda Y, Okayama N, Talano N, Fujimura K, Suehiro Y, Hamanaka R, Terakawa N, Nakamura Y: The 5A/6A polymorphism of matrix metalloproteinase (MMP)-1 and MMP-3 genes with colorectal cancer. Int J Cancer 2002, 102:526-529.
25. Ghiardi G, Biondi ML, Mangoni J, Leviti S, De Monti M, Guagnellini E, Berga A, Blons H, Laurent-Puig P: Prognostic significance of MMP-1 and MMP-3 functional promoter polymorphisms in colorectal cancer. Clin Cancer Res 2005, 11:594-599.
26. Beyzade S, Zhang S, Wong YK, Day IN, Eriksson P, Ye S: Influence of matrix metalloproteinase-1 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. J Am Coll Cardiol 2003, 41:2130-2137.
27. Jormsjo S, Whatling C, Walter DH, Zeiher AM, Hamsten A, Eriksson J: Association of Ets-related transcriptional factor EFA1 expression with tumour progression and overexpression of MMP-1 and matrilysin in human colorectal cancer. J Pathol 2003, 200:568-576.
28. Nosho K, Yoshida M, Yamamoto H, Taniguchi H, Adachi Y, Mikami M, Hinoda Y, Imai K: Association of Ets-related transcriptional factor EFA1 expression with tumour progression and overexpression of MMP-1 and matrilysin in human colorectal cancer. J Pathol 2003, 200:568-576.
29. Horiuschi S, Yamamoto H, Min Y, Adachi Y, Itoh F, Imai K: Association of Ets-related transcriptional factor EIAF expression with tumour progression and overexpression of MMP-1 and matrilysin in human colorectal cancer. J Pathol 2003, 200:568-576.
30. Jormsjo S, Whatling C, Walter DH, Zeiher AM, Hamsten A, Eriksson J: Allele-specific regulation of matrix metalloproteinase-7 promoter activity is associated with coronary artery luminal dimensions among hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2001, 21:1834-1839.
31. Ghiardi G, Biondi ML, Erario M, Guagnellini E, Sorca R: Colorectal carcinoma susceptibility and metastases are associated with matrix metalloproteinase-7 promoter polymorphisms. Clin Cancer Res 2003, 9:4940-4942.
32. Costet V, Pariente A, Nalet B, Lafon J, Milan C, Olschewang S, Paire J, Bonatti-Pellie C, Bonhout-Kopp C: Low compliance with colonscopic screening in first-degree relatives of patients with large adenomas. Aliment Pharmacol Ther 2006, 24:101-109.
33. Alonsos H, Gad S, Zini-zinohoue F, Maniere I, Beauregard J, Trepoquet D, Brusn D, Beaune P, Laccourreye O, Laurent-Puig P: Matrix metalloproteinase 3 polymorphism: a predictive factor of response to neoadjuvant chemotherapy in head and neck squamous cell carcinoma. Clin Cancer Res 2004, 10:2594-2599.
34. Janssens CM, Van den Berghe H, Delespesse G, De Vos G: The Interaction of Selection and Linkage. I. General considerations: heterotic models. Genetics 1964, 49:49-67.
35. Jannot AS, Essiou X, Reese MG, Clerget-Darpoux F: Improved use of SNP information to detect the role of genes. Genet Epidemiol 2003, 25:158-167.
36. Becker T, Knapp M: A powerful strategy to account for multiple testing in the context of haplotype analysis. Am J Hum Genet 2004, 75:561-570.
37. Lonjou C, Clayton J, Cambon-Thomsen A, Raffoux C: HLA-A, B, C, DQ haplotype frequencies in France—implications for recruitment of potential bone marrow donors. Transplantation 1995, 60:375-383.
38. Ye S, Eriksson P, Hamsten A, Kurbeken M, Humphries SE, Henney AM: Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem 1996, 271:13055-13060.
39. Correa P, Strong JP, Johnson WD, Pizzolato P, Haenszel W: Atherosclerosis and polyps of the colon. Quantification of precursors of coronary heart disease and colon cancer. J Chronic Dis 1982, 35:313-320.
40. Stemmermann GN, Heilbrun LK, Nomura A, Yano K, Hayashi T: Adenomatous polyps and atherosclerosis: an autopsy study of Japanese men in Hawaii. Int J Cancer 1986, 38:789-794.
41. Ye S, Watts GF, Mandalia S, Humphries SE, Henney AM: Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. Br Heart J 1995, 73:209-212.
42. Humphries SE, Luong LA, Talmud PJ, Frick MH, Kesaniemi YA, Pasternack A, Taskinen MR, Syvanne M: The 54A/6A polymorphism in the promoter of the stromelysin-1 (MMP-3) gene predicts progression of angiographically determined coronary artery disease in men in the LOCAT gemfibrozil study. Lpid Coro- nary Angiography Trial. Atherosclerosis 1998, 139:49-56.
43. Gnasso A, Motti C, Iarcce Carallo L, Liberatoscioli L, Bernardini S, Massoud R, Mattioli PL, Federici G, Cortese C: Genetic variation in human stromelysin gene promoter and common carotid geometry in healthy male subjects. Arterioscler Thromb Vasc Biol 2000, 20:1600-1605.
44. Newell KJ, Wityt JP, Rodgers WH, Mattrissian LM: Expression and localization of matrix-degrading metalloproteinases during colorectal tumorigenesis. Mol Carcinog 1994, 10:199-206.
45. Horiuschi S, Yamamoto H, Min Y, Adachi Y, Itoh F, Imai K: Association of Ets-related transcriptional factor EIAF expression with tumour progression and overexpression of MMP-1 and matrilysin in human colorectal cancer. J Pathol 2003, 200:568-576.
46. Williams CS, Mann M, DuBois RN: The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 1999, 18:7908-7916.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/6/270/pre-pub

Publish with BioMed Central and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."
Sir Paul Nurse, Cancer Research UK

Your research papers will be:
- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp