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ORIGINAL ARTICLE

Genetic Variation and Gene Expression Levels of Tight Junction Genes Indicates Relationships Between *PTEN* as well as *MAGI1* and Microscopic Colitis

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Abstract

Background and Aim Microscopic colitis (MC) has been associated with increased paracellular permeability. Therefore, we aimed to investigate potential associations between MC and several genes encoding tight junction (TJ) proteins reported to interact with each other.

Methods The association between MC and single nucleotide polymorphisms (SNP; $n = 63$) within TJ genes (*F11R, MAGI1, MAGI2, MAGI3, PARD3, PTEN,* and *TJP1*) were investigated in a case–control study ($n_{\text{MC patients}} = 104$ and $n_{\text{controls}} = 423$). The genes that exhibited an association with MC were further investigated for gene expression related to genotype, MC phenotype, and gender using colonic biopsies from MC patients ($n = 25$) and controls ($n = 58$).

Results Based on the number of investigated genes and after correction for multiple testing, an association was detected between a SNP marker in *PTEN* (rs1234224) and both MC overall (OR = 1.70, 95% CI 1.23–2.34, $p = 0.001$) and collagenous colitis (CC; OR = 1.79, 95% CI 1.22–2.62, $p = 0.003$). Further, SNP markers in *MAGI1* (rs17417230) and *F11R* (rs790055) were associated with MC overall (OR = 1.58, 95% CI 1.14–2.19, $p = 0.006$) and with CC (OR = 2.58, 95% CI 1.27–5.25, $p = 0.007$), respectively. However, none of the associated SNPs contributed markedly to the expression of the respective genes. Nonetheless, decreased *MAGI1* ($p = 3.47 \times 10^{-4}$) and *PTEN* ($p = 0.004$) expression was associated with lymphocytic colitis (LC) and CC, respectively, compared to controls.

Conclusions Decreased expression of *PTEN* and *MAGI1* in the colonic mucosa might contribute to the pathogenesis of MC and its sub-phenotypes. Furthermore, our study indicates that genetic variants of TJ components are predisposing factors in the etiology of MC. Finally, *F11R, MAGI1,* and *PTEN* are new candidate genes that exhibit an association with MC.

Keywords Microscopic colitis · Genetic predisposition · Genotype · Gene expression · Single nucleotide polymorphism · Tight junctions

Introduction

Microscopic colitis (MC), including lymphocytic colitis (LC) and collagenous colitis (CC), is a chronic inflammation of the colon, that is characterized by chronic bloodless and watery diarrhea. The mucosal appearance at endoscopy is normal, or near normal, but has typical histopathological abnormalities [1–4]. CC is characterized by a thickened sub-epithelial collagen layer and an increased number of lymphocytes, whereas LC is associated with an increased number of intraepithelial lymphocytes [3–7]. The etiologies of LC and CC are multifactorial and largely unknown, but factors including female gender, increasing age, smoking habits, autoimmune disorders, genetic susceptibility, and environmental factors are implicated [2, 8–10].

Sven Almer and Jan Söderman have shared senior authorship.

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Further, MC is associated with increased intestinal permeability as measured using ^{51}CrEDTA and horseradish peroxidase [11] or by performing impedance spectroscopy [12]. Paracellular permeability across the paracellular space is primarily regulated by the tight junction (TJ) structures [13]. In addition, decreased expression of TJ proteins, such as claudin-4 and occludin, has been observed in CC [14].

Genetic variation of the TJ-related gene *MAGI2* is associated with Crohn's disease (CD) [15], ulcerative colitis (UC) [15, 16], and celiac disease [16], and the TJ-related gene *PARD3* with celiac disease [16]. In a previous study on IBD [17], a network consisting of seven TJ-related genes [see Fig. 1 in 17] were investigated since we noted that proteins encoded by *MAGI2* and *PARD3* interacted with proteins encoded by *F11R* (encoding JAM-A), *MAGI1*, *MAGI3*, *PTEN*, and *TJP1* according to the STRING search tool [18], thus indicating that these proteins jointly contribute to a shared function. Here, we extend these observations to encompass MC. Additionally, we investigated the corresponding gene expressions in relation to genotype, MC phenotype, and gender.

Methods

Study Subjects

Swedish patients with an established diagnosis of MC ($n_{total} = 104$, median age 65 years; 39 LC patients, 22 women and 65 CC patients, 51 women) and controls ($n = 423$, median age 59 years, 223 women) from an anonymized cohort consisting of randomly selected individuals living in the same geographic region were included in a case–control study. Controls suffering from gastrointestinal symptoms were not included in the investigation.

A second MC cohort (Table 1), from which both RNA from intestinal biopsies and DNA samples were available, was used to further explore significant findings from the case–control study.

Colonic biopsies were obtained from 25 MC patients (Table 1) of which 19 (76%) underwent primary investigation for diarrhea; the other six already had an established diagnosis of MC. The patients under primary investigation came to colonoscopy because of ongoing non-bloody diarrhea, while patients with an established diagnosis had colonoscopy either as follow-up after medical treatment or due to an increase in bowel symptoms. There were 14 LC patients, 10 CC patients, and one female patient with an undefined sub-phenotype of MC. For the newly diagnosed cases, study biopsies were sampled at the same occasion as the diagnostic biopsies. In addition, colonic biopsies were obtained from 58 controls (Table 1), who underwent endoscopy in the diagnostic workup of suspected gastrointestinal disorders, mainly suspicion of other inflammatory bowel diseases or cancer. The biopsy specimens were primarily obtained from the transverse colon. When samples from this segment were not available, other colonic segments were used, with a preference for specimens obtained from the ascending colon. One biopsy per individual was selected and categorized as LC, CC, or as a non-inflamed control based on histopathological assessment. The phenotype was based on routine histopathological assessment where the established diagnostic criteria according to Langner et al. [3] were applied. The key histological features are thickened sub-epithelial collagen band in CC (> 10 μm) and an increased number of intraepithelial lymphocytes in LC (> 20 per 100 epithelial cells). All control biopsies were devoid of inflammatory cellular infiltrate and collagen deposition.

Table 1 Summary of the study participants and biopsy locations in the second Swedish MC cohort that was used to analyze the significant genetic associations found in the first cohort

Disease and subgroups[a]	Number of individuals/biopsies
MC (n = 25)[a,b]	
Ascending colon	2
Transverse colon	19
Left-sided colon	4
CC (n = 10)	
Ascending colon	1
Transverse colon	6
Left-sided colon	3
LC (n = 14)	
Ascending colon	1
Transverse colon	12
Left-sided colon	1
Non-MC controls (n = 58)[c]	
Ascending colon	5
Transverse colon	36
Left-sided colon	17

[a]Includes 10 CC patients (median 63, range 47–85, years of age; 10 women), 14 LC patients (median 62, range 49–88, years of age; 10 women), and one female patient with an undefined sub-phenotype (44.6 years of age)

[b]One of the CC patients also had biopsy verified celiac disease

[c]Two of the non-MC controls ($n = 58$; median age 51 years, range 18–84) had biopsy verified celiac disease

Single Nucleotide Polymorphism Selection for Genetic Association Studies

TJ-related genes (*F11R, MAGI1, MAGI2, MAGI3, PARD3, PTEN,* and *TJP1*) encoding products interacting with each

other [18–20] were investigated. All SNP markers are given in Supplementary Table 1.

SNP markers (minor allele frequency ≥ 10%, pairwise $r^2 \geq 0.8$) of *F11R*, *MAGI1*, *MAGI3*, and *PTEN* were selected using SNPbrowser Software version 4.0 (Applied Biosystems, Foster City, CA, USA), as previously described [17].

Genotyping and Gene Expression Analysis

The DNA isolation and the allelic discrimination (Supplementary Table 1) was performed as previously described [17].

Genes exhibiting an association with MC overall, CC, or LC were further investigated by gene expression analysis in relation to genotype, MC phenotype, and gender. The biopsies were submerged in RNA*later* (Qiagen, Düsseldorf, Germany) and stored at either + 4 °C overnight and afterward at − 20 °C, or at room temperature for 1 h and then at − 80 °C until extraction. The different storage temperatures did not affect the expression of genes, neither in biopsies from the MC patients nor the controls (data not shown). RNA purification was performed as previously described [17].

Gene expression of *F11R* (Hs00170991_m1), *MAGI1* Hs00191026_m1, and *PTEN* Hs02621230_s1) was analyzed using TaqMan Gene Expression Assay (Applied Biosystems), TaqMan Universal Mastermix (Applied Biosystems), and a 7500 Fast Real-Time PCR system (Applied Biosystems). Each individual reaction contained 10 ng cDNA in a total reaction volume of 20 µL. Threshold cycle (C_T) values were established (ExpressionSuite Software version 1.0.3; Applied Biosystems) and normalized to the average of selected reference genes, as previously described [17].

Statistical Analysis

Allelic odds ratios (OR) and p values, based on the Chi-squared (χ^2) test, were calculated using the JMP Genomics 6.0 software (JMP Genomics 6.0; SAS Institute Inc., Cary, NC, USA). Deviation from Hardy–Weinberg equilibrium was tested separately in controls and MC patients using the exact test implemented in Haploview version 4.2 (https://www.broad.mit.edu/haploview). For the analysis of genetic associations, a Bonferroni adjusted p value < 0.007 (based on the number of genes analyzed; $n = 7$) was considered significant.

The expressions of genes exhibiting significant associations to MC, CC, or LC were further analyzed. Group differences in gene expression were investigated using Kruskal–Wallis analysis of variance (ANOVA) with Mann–Whitney U post hoc test using Statistica 12.7 (StatSoft Inc., Tulsa, OK, USA). Gene expressions in relation to genotype were investigated using Spearman's rank correlation test, whereas gene expression in relation to MC phenotype and gender were analyzed using logistic regression (Statistica 12.7; StatSoft Inc.). Significant findings were further explored by multiple logistic regression (Statistica 12.7; StatSoft Inc.), where adjustment for possible confounders (gender and age) was performed one at a time. Correlations between gene expressions were determined using Spearman's rank correlation (Statistica 12.7; StatSoft Inc.). Scatter plots were created for the relationship between gene expression and phenotype using Statistica 13.1 (StatSoft Inc.). All statistical analyses with respect to gene expression were performed using ΔC_T values, and a Bonferroni adjusted p value < 0.017 (based on the number of genes analyzed; $n = 3$) was considered significant.

Ethical Considerations

The study was conducted under approval by the ethics committees of Linköping University (Dnr M35-07, Dnr 2011/201-31) and Karolinska Institutet (Dnr 2007/791-31/3).

Results

Genetic Associations

Seven out of the 63 selected SNP markers were excluded due to failed genotyping or the absence of Hardy–Weinberg equilibrium (Supplementary Table 1). The strongest association in the case–control study was observed between a *PTEN* SNP marker (rs1234224; susceptibility allele G) and both MC overall (OR = 1.70, 95% CI 1.23–2.34, $p = 0.001$) and CC (OR = 1.79, 95% CI 1.22–2.62, $p = 0.003$) (Supplementary Table 2). Additionally, a *MAGI1* SNP marker (rs17417230; susceptibility allele C) was significantly associated with MC overall (OR = 1.58, 95% CI 1.14–2.19, $p = 0.006$), whereas a *F11R* marker (rs790055; susceptibility allele G) was significantly associated with CC (OR = 2.58, 95% CI 1.27–5.25, $p = 0.007$).

Gene Expression

No significant differences in the expression of *F11R* or *MAGI1* were observed between biopsies from the ascending colon, transverse colon, or left-sided colon among controls. *PTEN* exhibited a slightly increased expression (Kruskal–Wallis ANOVA; $p = 0.015$) in the proximal colon (ascending colon and transverse colon) compared to

the left-sided colon (Mann–Whitney U test; $p = 0.006$). Since no or only a slight difference in gene expression (1.1 fold on a linear scale) was observed, all biopsies were analyzed together, regardless sampling location.

Gene Expression in Relation to Genotype

No significant correlations were observed between the susceptibility allele of the identified SNP markers and the expression of the corresponding genes (*F11R, MAGI1,* and *PTEN*; Table 2). However, a trend ($p = 0.103$) toward reduced *PTEN* expression in relation to the G/G susceptibility genotype was observed among CC patients (Fig. 1).

Gene Expression in Relation to Phenotype

Significant associations were observed between decreased *MAGI1* expression and MC overall ($p = 2.58 \times 10^{-5}$) as well as in CC ($p = 0.002$) and LC ($p = 3.47 \times 10^{-4}$) compared to controls (Table 3, Fig. 2). Furthermore, significant associations were observed between decreased *PTEN* expression and both MC overall ($p = 0.002$) and CC ($p = 0.004$) compared to controls and with a similar trend for LC ($p = 0.018$). Significant findings, in relation to MC, were confirmed using multiple logistic regression and adjustment, one at a time, for possible confounders (gender and age) (data not shown).

The expression of *MAGI1* and *PTEN* were positively correlated among the MC patients ($p = 4.10 \times 10^{-4}$, $r_s = 0.65$), but not among the controls ($p = 0.358$, $r_s = 0.12$).

Gene Expression in Relation to Gender

No significant associations were observed between gene expression (*F11R, MAGI1,* and *PTEN*) and gender among MC patients or controls (Table 4, Fig. 2).

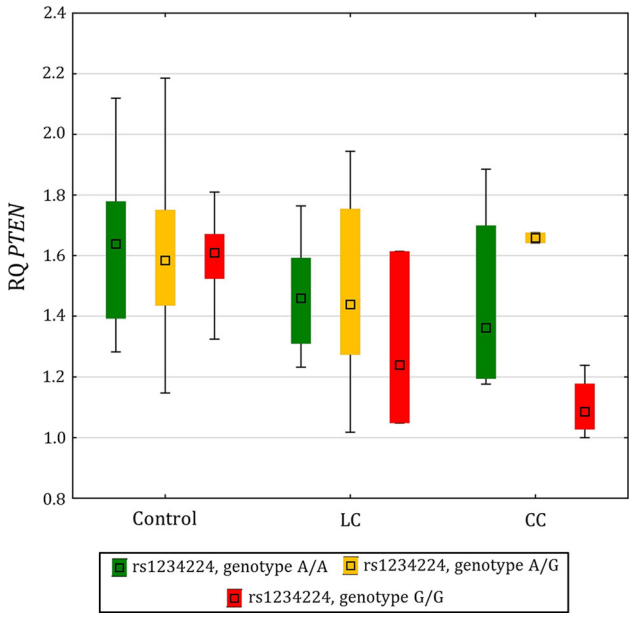

Fig. 1 Box pot of *PTEN* expression in the colonic mucosa from non-MC controls, LC patients, and CC patients stratified by the rs1234224 genotype

Discussion

Microscopic colitis constitutes multifactorial conditions with mainly watery diarrhea, chronic inflammation of the colon, and an enhanced intestinal permeability [11, 12]. The etiology is largely unknown, but a genetic predisposition might exist [10]. Because of the accompanying increased paracellular permeability, we aimed to investigate the genetic associations between MC and genes that encode tight junction proteins.

The strongest associations were observed between a SNP marker in *PTEN* (rs1234224) and both MC and CC, and a decreased expression of *PTEN* was apparent in both MC and CC, compared to controls. Recently, we described significantly lower level of *PTEN* expression in biopsies from inflamed IBD mucosa, compared to non-inflamed

Table 2 For genes with a significant genetic association with investigated phenotypes, gene expression (ΔC_T values) was analyzed in relation to genotype in colonic biopsies from the different subgroups

Gene	SNP marker	Non-MC controls (number of controls)	p value	MC patients (number of patients)	p value	CC patients (number of patients)	p value	LC patients (number of patients)	p value
F11R	rs790055	AA (2), AG (16), GG (39)	0.676	AA (3), AG (8), GG (14)	0.482	AA (2), AG (3), GG (5)	0.428	AA (0), AG (5), GG (9)	0.851
MAGI1	rs17417230	AA (6), AC (33), CC (19)	0.480	AA (6), AC (13), CC (6)	0.621	AA (2), AC (4), CC (4)	0.590	AA (4), AC (9), CC (1)	0.825
PTEN	rs1234224	AA (21), AG (31), GG (6)	0.978	AA (10), AG (8), GG (7)	0.081	AA (4), AG (2), GG (4)	0.103	AA (5), AG (6), GG (3)	0.535

Table 3 Gene expression (ΔC_T values) was analyzed in relation to phenotype using logistic regression

	Single logistic regression			
	p value	Estimate	Estimate (95% CI)	Nagelkerke R^2
MC versus non-MC controls				
F11R[a]	0.042	2.14	0.08 to 4.21	0.08
MAGI1[b]	**2.58×10^{-5}**	6.54	3.49 to 9.59	0.64
PTEN[c]	**0.002**	3.61	1.37 to 5.86	0.19
CC versus non-MC controls				
F11R[d]	0.084	2.34	− 0.32 to 4.99	0.08
MAGI1[e]	**0.002**	9.02	3.33 to 14.72	0.74
PTEN[f]	**0.004**	4.99	1.63 to 8.35	0.25
LC versus non-MC controls				
F11R[g]	0.181	1.66	− 0.77 to 4.08	0.04
MAGI1[h]	**3.47×10^{-4}**	5.62	2.54 to 8.70	0.53
PTEN[i]	0.018	3.44	0.59 to 6.30	0.13
LC versus CC				
F11R	0.422	− 1.90	− 6.54 to 2.74	0.04
MAGI1	0.323	− 1.00	− 2.98 to 0.98	0.06
PTEN	0.419	− 1.21	− 4.14 to 1.72	0.04

ΔC_T values are inversely related to gene expression values. The estimates represent the natural logarithm of the odds ratio with a negative value corresponding to increased odds, while a positive value corresponds to decreased odds. Significant values ($p < 0.017$) are marked in bold

[a] $n_{MC} = 25$; $n_{non\text{-}MC} = 57$

[b,c] $n_{MC} = 25$; $n_{non\text{-}MC} = 58$

[d] $n_{CC} = 10$; $n_{non\text{-}MC} = 57$

[e,f] $n_{CC} = 10$; $n_{non\text{-}MC} = 58$

[g,h,i] $n_{CC} = 14$; $n_{non\text{-}MC} = 58$

IBD mucosa [17]. PTEN is involved in the regulation of fibroblast viability [21], and an overexpression of *PTEN* inhibits lipopolysaccharide-induced fibroblast proliferation, differentiation, and collagen secretion in mice [22]. Decreased PTEN expression is associated with pulmonary fibrosis that is characterized by collagen secretion and the activation and proliferation of fibroblasts [23]. In parallel, CC is associated with a distinctive, thickened sub-epithelial collagen layer [5], which might be a consequence of the decreased colonic *PTEN* expression observed in our study.

A SNP marker in *MAGI1* (rs17417230) was associated with MC. Compared to controls, decreased *MAGI1* expression was associated with MC overall, CC, and LC. The expression of *MAGI1* and *PTEN* was positively correlated to each other, which is in accord with observations of the recruitment of PTEN by MAGI-1b at adherens junctions [24]. Similar to MC, inflamed IBD mucosa expressed lower levels of *MAGI1* and *PTEN* compared to both non-inflamed IBD as well as controls. Compared to the MC mucosa, the expression levels of *MAGI1* and *PTEN* were, however, further reduced in inflamed IBD mucosa (data not shown). Based on these expression levels, there are both similarities and differences between MC and IBD.

A SNP marker in *F11R* was significantly associated with CC, whereas a marginally significant association was observed between decreased *F11R* expression and MC overall. Aside from the thickened sub-epithelial collagen layer, CC is also associated with increased mononuclear inflammation in the lamina propria [3]. Further, reduced JAM-A (encoded by *F11R*) expression is associated with increased infiltration of polymorphonuclear leukocytes (PMN) to the colonic mucosa in patients with colitis [25]. An increased PMN infiltration and lymphoid aggregates are observed in the colonic mucosa from JAM-A$^{-/-}$ mice [26]. Finally, reduced JAM-A expression in the colonic mucosa from patients with inflammatory bowel disease has also been observed [27]. It therefore seems plausible that the regulation of *F11R* is relevant for the etiology of CC.

None of the associated SNP markers contributed significantly to the expression of their respective genes in MC patients or in controls, i.e, the study was unable to demonstrate an allelic effect on gene expression using the current sample size and/or analyzing whole intestinal mucosa biopsies (representing a heterogeneous collection of cell types). Nevertheless, a nonsignificant decrease in *PTEN* expression among MC and CC patients was

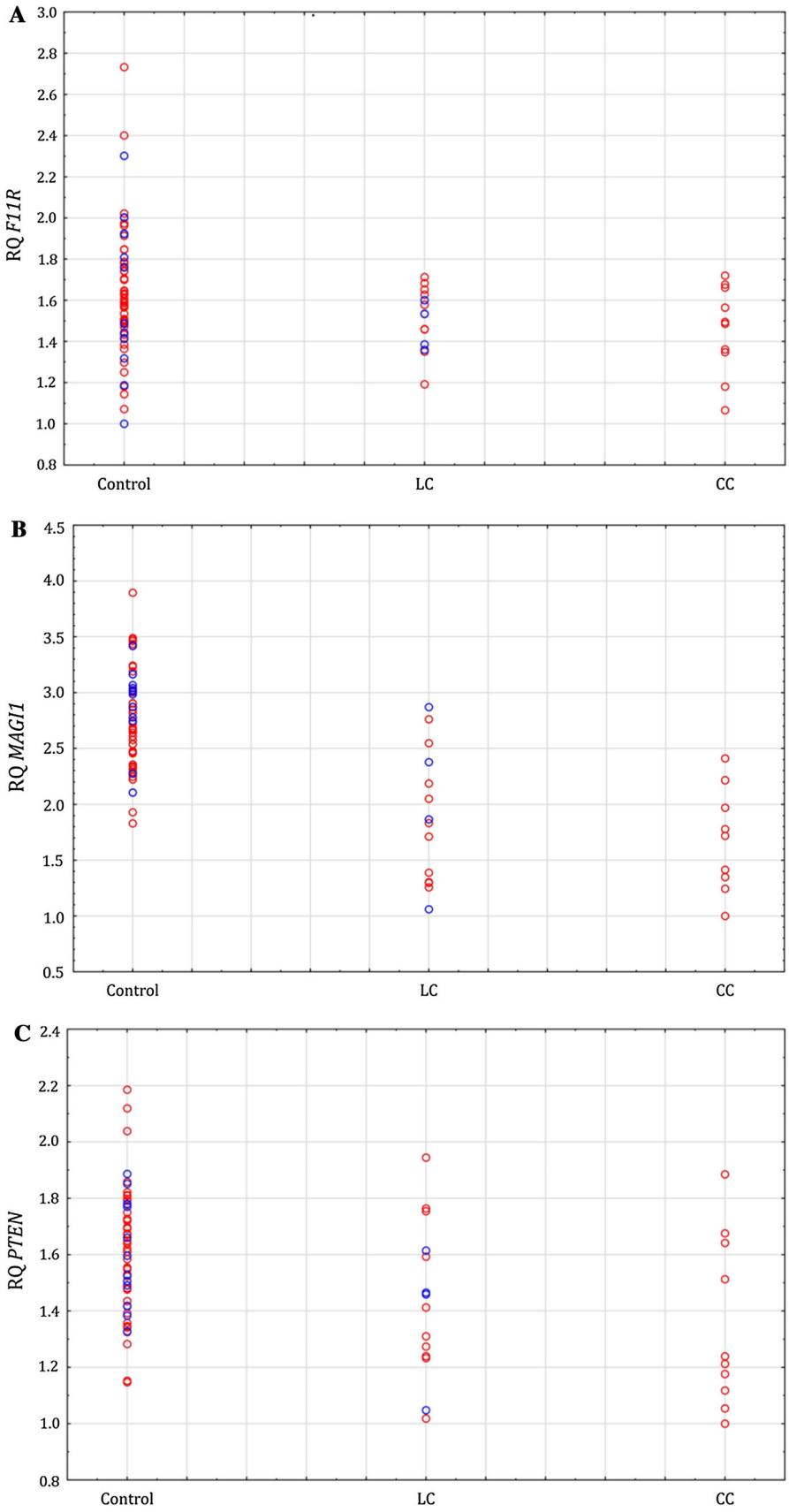

Fig. 2 Colonic mucosal expression of *F11R* (**a**), *MAGI1* (**b**), and *PTEN* (**c**) in relation to phenotype and further stratified by gender (women are illustrated with open red circles and men with open blue circles)

Table 4 Gene expression (ΔC_T values) was analyzed in relation to gender using single logistic regression

	p value	Estimates	Estimate (95% CI)	Nagelkerke R^2
Non-MC controls				
F11R[a]	0.751	− 0.38	− 2.74 to 1.98	< 0.01
MAGI1[b]	0.218	1.82	− 1.08 to 4.72	0.04
PTEN[c]	0.711	0.60	− 2.58 to 3.78	< 0.01
MC patients				
F11R[d]	0.898	− 0.39	− 6.38 to 5.60	< 0.01
MAGI1[e]	0.453	1.02	− 1.64 to 3.69	0.04
PTEN[f]	0.979	− 0.05	− 3.84 to 3.74	< 0.01

ΔC_T values are inversely related to gene expression values. The estimates represent the natural logarithm of the odds ratio with a negative value corresponding to increased odds, while a positive value corresponds to decreased odds

[a] $n_{female} = 45$; $n_{male} = 12$

[b,c] $n_{female} = 45$; $n_{male} = 13$

[d,e,f] $n_{female} = 21$; $n_{male} = 4$

observed in relation to homozygosity for the *PTEN* SNP marker susceptibility allele (rs1234224, susceptibility allele G; Table 2 and Fig. 1). The majority of hitherto identified risk alleles is noncoding and likely exerts their effects via regulation of gene expression, possibly in a tissue-/cell-type-dependent manner [28, 29]. We have previously established risk allele–gene expression relationships using a disease-relevant sample type (i.e, intestinal mucosa biopsies) and a similar sample size [30]. However, ultimately, success of identifying a risk allele–gene expression relationship using a heterogeneous biological sample will depend on factors such as effect size, disease status, medication, and cell type.

In conclusion, decreased *PTEN* and *MAGI1* expression in the colonic mucosa might contribute to the pathogenesis of MC and its sub-phenotypes. Furthermore, our study indicates that genetic variants of a number of TJ components might be predisposing factors for MC. Similar to the so far identified risk alleles for CD and UC [29, 31–34], we observed small to modest effect sizes of the investigated SNP markers in relation to MC, however, with relatively wide confidence intervals. The identified SNP markers require further investigation in a larger, independent cohort, to both validate and more precisely determine their influence in relation to MC. Nonetheless, *F11R, MAGI1*, and *PTEN* are new candidate genes associated with MC.

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Author's contribution EN, SA, and JS conceived and designed the study; EN contributed to all laboratory work, performed the statistical analysis, and contributed to data analysis and drafted the manuscript; MRM and SA provided blood samples and patient data; all authors contributed to the context of the manuscript and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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Affiliations

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