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Integrative Network-based Analysis of Colonic Detoxification Gene Expression in Ulcerative Colitis According to Smoking Status

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Abstract

Backgrounds and Aims: The effect of cigarette smoking [CS] is ambivalent since smoking improves ulcerative colitis [UC] while it worsens Crohn's disease [CD]. Although this clinical relationship between inflammatory bowel disease [IBD] and tobacco is well established, only a few experimental works have investigated the effect of smoking on the colonic barrier homeostasis focusing on xenobiotic detoxification genes.

Methods: A comprehensive and integrated comparative analysis of the global xenobiotic detoxification capacity of the normal colonic mucosa of healthy smokers [$n = 8$] and non-smokers [$n = 9$] versus the non-affected colonic mucosa of UC patients [$n = 19$] was performed by quantitative real-time polymerase chain reaction [qRT PCR]. The detoxification gene expression profile was analysed in CD patients [$n = 18$], in smoking UC patients [$n = 5$], and in biopsies from non-smoking UC patients cultured or not with cigarette smoke extract [$n = 8$].

Results: Of the 244 detoxification genes investigated, 65 were dysregulated in UC patients in comparison with healthy controls or CD patients. The expression of ≥ 45/65 genes was inversed by CS in biopsies of smoking UC patients in remission and in colonic explants of UC patients exposed to cigarette smoke extract. We devised a network-based data analysis approach for differentially assessing changes in genetic interactions, allowing identification of unexpected regulatory detoxification genes that may play a major role in the beneficial effect of smoking on UC.

Conclusions: Non-inflamed colonic mucosa in UC is characterised by a specifically altered detoxification gene network, which is partially restored by tobacco. These mucosal signatures

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could be useful for developing new therapeutic strategies and biomarkers of drug response in UC.

Key Words: Cigarette smoke; detoxification genes; inflammatory bowel diseases; network inference; ulcerative colitis

1. Introduction

The exact aetiology of ulcerative colitis [UC] remains poorly understood. More than 130 genetic risk loci involved in biological pathways including immune response, maintenance of intestinal barrier function, and endoplasmic reticulum stress, have been identified so far.[1,2] However, the concordance rate in monozygotic versus disygotic twins is 16% in control versus 4% in UC twins,[3,4] suggesting a role of environmental factors in the pathological process of UC. Western lifestyle seems strongly associated with the high incidence of UC [24.3/100 000 person-years in Europe and 6.3/100 000 person-years in Asia [5]] and the rising incidence of inflammatory bowel disease [IBD] in Western countries has generally pre-dated that in developing countries. The significant impact of industrialisation [exposure to pollutants, pesticides, etc.] on the occurrence of UC indicates that changes in the toxic environment are the major causes of this evolution and suggests that impaired ability to detoxify environmental toxicants could promote UC.

The most indisputable example of the influence of the environment on IBD is cigarette smoking [CS] which has a striking opposite effect on UC and Crohn's disease [CD].[6,7] Although cigarette use is an important risk factor for CD, UC patients are frequently non-smokers and smoking cessation increases the risk of developing UC. However, the protective molecular mechanisms of CS on UC remain obscure. Although some studies have demonstrated that nicotine has an anti-inflammatory function in UC,[8,9] the findings from clinical studies, systematic reviews, and meta-analyses do not support the efficacy of nicotine preparations on the induction of disease remission.[10]

Cigarette smoke contains numerous toxic compounds. The human xenobiotic-metabolising enzyme [XME] machinery is a major protective factor against environmental exposure[11] and can be induced by various exogenous and endogenous agents. Although the liver is the major organ for detoxification, colonic epithelial cells have a similar capacity to detoxify luminal and blood compounds.[12,13] The colon is exposed to various foreign chemicals [called xenobiotics], including drugs, food additives, pollutants, etc. Therefore, the proper functioning of the detoxification system is critical for protection of intestinal barrier. Xenobiotic metabolism and excretion are mediated by a large number of phase I, phase II, and phase III XMEs.[14] Phases I and II XMEs can synergistically transform lipophilic xenobiotics into hydrophilic metabolites to facilitate the excretion of toxicants. Phase I XMEs encompass a family of 50–100 enzymes [referred to as cytochrome P450, CYP] which mainly catalyse the oxidative metabolism of unwanted chemical compounds. Numerous CYP enzymes are expressed in the human normal colon and rectum.[12,15] Phase II reactions include the sulphation, glucuronidation, acetylation, methylation, and glutathione conjugation of xenobiotic derivatives which have undergone phase I XMEs. Phase III XMEs or transporters mainly consist in adenosine triphosphate [ATP]-binding cassette [ABC] transporters and a series of solute carriers [SLC] that facilitate the excretion of transformed xenobiotics. The gene expression of all these enzymes is regulated by transcriptional factors or nuclear receptors, including the aryl hydrocarbon receptor [AhR], pregnane X receptor [PXR], constitutive androstane receptor [CAR], liver X receptor [LXR], farnesoid X receptor [FXR], retinoid X receptor [RXR], and peroxisome proliferator activated receptor [PPAR].[14]

Evidence suggests that colonic epithelial cells become unable to detoxify toxic metabolites in cases of colitis.[16–18] The activation of the xenobiotic metabolic pathways improves dextran sodium sulphate [DSS]-induced colitis[19] and modulates the activity of immune and non-immune cells in the gut. [20] A few data from animal models[20,21] together with studies in IBD patients[13,18,22,23] suggest that detoxification enzyme depletion could be involved in UC initiation and/or progression. However, the information provided by these studies is limited and most often concerns a small number of XME genes. Furthermore, unlike the well-established relationship between IBD and CS, no experimental works have explored the effect of CS on the detoxification gene network in humans.

Using a combined approach integrating detoxification gene mRNA expression levels and network-based data analysis, our findings support an impaired detoxification capacity of the non-affected colonic mucosa of UC patients which was not found in healthy controls or patients with colonic CD. We developed a new method to infer a gene regulatory network from gene expression data, by integrating existing knowledge into the under-determined inverse network inference problem. Through this method, we identified new unexpected genes and gene interactions which could be responsible for the dysregulation of the detoxification system and the environmental susceptibility of UC.

2. Methods

2.1. Patients and biopsies

Human ascending colon biopsies were obtained from the IBD Gastroenterology Unit, Beaujon Hospital. The protocol was in agreement with the local ethics committee [CPP-Ile de France IV No. 2009/17] and written informed consent was obtained from all the patients before inclusion. The clinical characteristics of UC patients are shown in Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online. In all, 23 non-smoking and 5 smoking UC patients, 8 non-smoking and 9 smoking controls, and 18 CD patients [6 with Crohn's ileocolitis and 12 with Crohn's colitis] were selected and included in this study. Colonic CD patients were recruited to assess whether dysregulated detoxification gene expression profile was a general hallmark of IBD or a more specific feature of UC. Patients were consecutively recruited from January 2010 to October 2013. All patients were diagnosed based on classical clinical features as well as radiological, endoscopic, and histological findings. All biopsies were taken from the non-inflamed right colon to avoid variability in detoxification enzyme expression along the colon, and analysed by an expert gastrointestinal [GI] pathologist [DCH]. Unaffected areas were defined as mucosa regions without any macroscopic/endoscopic or histological signs of inflammation, as previously reported.[24] To preserve tissue transcriptional profiles, biopsy specimens were immediately kept at -80°C until RNA extraction.

2.2. Isolation of total mRNA and reverse transcription

Total mRNA was extracted from human ascending colon biopsies using RNAble® Kit [Eurobio Courtaboeuf, France]. RNA

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was quantified by a ND-1000 NanoDrop spectrophotometer [NanoDrop technologies Inc., France] and total mRNA integrity was verified with an Agilent 2100 Bionanalyser. Total mRNA [1 µg] was converted into cDNA using Moloney Murine Leukemia Virus Reverse Transcriptase [M-MLV RT] kit [Invitrogen, Carlsbad, CA, USA] according to the manufacturer protocol using Thermal Cyclers [Mastercycler®, Eppendorf, Germany].

2.3. Quantitative PCR

Quantitative real-time polymerase chain reaction [qRT-PCR] was performed with SYBR Green [Mastermix plus for SYBR® assay No ROX, Eurogentec, USA] using the Lightcycler 480 system [Roche, France]. Cycling conditions were as follows: 10 min at 95°C, followed by 50 cycles of 15 s at 95°C, 1 min at 65°C, followed by 5 s at 95°C and 1 min at 55°C. After the 50 cycles, a melting curve [10 min] at 40°C was run and then analysed with the Lightcycler® 480 gene scanning software. Cycle thresholds [Ct] obtained for target genes were normalised to those of the housekeeping gene [TATA box binding protein or TBP]. The 2-ΔΔCt method was used to calculate the fold change of target genes. The nucleotide sequences were obtained from the literature or designed using a primer design tool [NCBI Primer-Blast].

2.4. Preparation of cigarette smoke extract and treatment of biopsies

Water-soluble extracts of cigarette smoke were generated by bubbling smoke from research-grade standard filter cigarettes in serum-free culture medium using a vacuum pump smoking machine [Heinrich Borgwaldt, Hambourg, Germany] settled with standard puff parameters [35 ml volume, 2 s duration, 60 s frequency]. Under these conditions each cigarette is consumed after 10 puffs, approximately mimicking the human smoking pattern.[25] The aqueous smoke extract from one cigarette corresponded to 10 puffs [350 ml] bubbled in 5 ml culture medium. The final dilutions of cigarette smoke extract in culture media were expressed as puffs/ml. Non-affected colon biopsies obtained from non-smoking UC patients were cultured for 2 h in the presence or absence of cigarette smoke extract at a final concentration of 1.6% [0.36 puff/ml], rinsed twice with phosphate buffered saline [PBS], and then immediately kept at -80°C until RNA extraction. This concentration was chosen because viability studies demonstrated lack of non-specific toxicity as determined by lactate dehydrogenase [LDH] release [data not shown]. Though most of the effects presented here were obtained with Kentucky 2R4F cigarettes, similar effects were obtained using filter commercially available cigarettes.

2.5. Immunohistochemistry

Immunohistological methods were used on serial 4-µm paraffin-embedded sections from control and unaffected UC mucosa. All sections were deparaffinised in xylene, rehydrated incubated in 3% hydrogen peroxide, and heated for 10 min in 10 mM citrate buffer [pH 6.2]. Sections were incubated in a blocking buffer for 1 h at room temperature using the Vectastain universal elite ABC kit [Clinisciences, Nanterre, France], and then incubated with anti-ARNT, anti-ABCA1, anti-HIF3A, or anti-CYP1B1 antibodies overnight at 4°C [all primary antibodies were purchased from Abcam, UK]. Secondary biotinylated anti-mouse or anti-rabbit were detected using the Vectastain universal elite ABC kit.

2.6. Principal component analysis

Principal components [PC] were computed after a logarithmic transformation of the expression levels, using the MATLAB function princomp. The first two PCs, which accounted for 85% of the total variance, are shown in Supplementary Figure 1, available as Supplementary data at *ECCO-JCC* online. For each of the 39 patients, the projections of individual expression vectors on the first two PCs were computed.

2.7. Multiple correspondence analysis

Multiple correspondence analysis [MCA] was performed upon representation of the 65 dysregulated gene expression profiles as categorical variables based on their statistical *p*-values [see below]. The correspondence analysis [CA] was carried out by means of the MCA function within the R package 'FactoMineR'. The first two-correspondence dimensions were selected to best fit our model. These two dimensions captured 65% of the total pattern variations. This percentage is loosely comparable to that obtained in the principal component analysis, since the eigen values in an MCA can be much smaller than in a PCA.

2.8. Statistical analysis

Statistical analysis was performed using Prism Version 5.0 [GraphPad software, Inc., San Diego, CA]. The Mann-Whitney test was used to determine the intergroup statistical significance. Values were considered statistically significant when $p < 0.05$. Results are expressed as mean ± standard error of the mean [SEM]. Clustering was performed using dChip software.

2.9. Network-based analysis

2.9.1. Integrative network inference

Given a gene expression vector $\boldsymbol{x}$ whose components $\boldsymbol{x}_i$ represent the expression level of the gene i, we aimed at inferring a possible genetic interaction network consistent with the expression $\boldsymbol{x}$. This inverse problem has received a lot of interest in the past few years, and we refer to reference 8 for a recent report on this topic. Given the large number of unknown parameters [n^2 interaction pairs] and the low number of data points (n expression levels], our approach aimed at integrating existing knowledge on genetic interactions to add constraints [see Results] and reduce the under-determination of the problem. We direct the reader to Supplementary materials [available as Supplementary data at *ECCO-JCC* online] for a detailed full description of the algorithm.

2.9.2. Comparative analysis

A detailed description of the optimisation algorithm used to solve this constrained basis pursuit problem as well as metric and classification methods are presented in Supplementary materials.

3. Results

3.1. Detoxification gene expression profiles are impaired in the non-inflamed colonic mucosa of UC patients

The expression of 244 genes encoding for detoxification enzymes known to be expressed in the human colon[12] was quantified by qRT-PCR in non-inflamed colonic biopsies taken from the right colon of UC patients [$n = 19$] and healthy controls [$n = 8$] [Supplementary Tables 1 and 2, available as Supplementary data at *ECCO-JCC* online]. A total of 65 genes assigned to three different subgroups: XMEs, ABC or SLC transporters, and nuclear receptors were significantly dysregulated in UC patients compared with healthy subjects [fold change > |1.5|, *p*-value < 0.05]. Among these genes, ~ 70% [46/65] were down-regulated, and the expression of transcription factors and nuclear receptors was particular low [Fisher's exact test $p = 0.003$] [Table 1].

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Table 1. mRNA expression level of human phase I and II metabolising enzymes, transporters, and transcription factors in the ascending colon of ulcerative colitis [UC] and Crohn's disease [CD] patients.

Gene name	UC patients		CD patients	
	Fold change [mean±SEM]	*P*-value	Fold change [mean±SEM]	*P*-value
Phase I enzymes				
ADH4	**0.288 ± 0.052	0.009	1.78 ± 0.359	0.106
ADH6	*1.226 ± 0.077	0.035	0.994 ± 0.093	0.491
ADHFE1	*0.504 ± 0.069	0.002	0.842 ± 0.115	0.161
AKR1A1	*1.313 ± 0.091	0.033	*1.289 ± 0.105	0.037
AKR7A2	*1.565 ± 0.19	0.047	1.203 ± 0.125	0.232
ALDH1A3	*0.537 ± 0.073	0.019	1.212 ± 0.243	0.391
ALDH1L1	*2.297 ± 0.359	0.047	1.516 ± 0.217	0.097
ALDH7A1	*1.455 ± 0.149	0.030	1.057 ± 0.098	0.387
AOX1	*0.515 ± 0.156	0.023	0.757 ± 0.149	0.344
BCHE	*0.626 ± 0.115	0.038	**0.355 ± 0.073	0.0016
CBR3	*1.698 ± 0.22	0.026	1.25 ± 0.226	0.436
CES1	**2.695 ± 0.544	0.008	1.386 ± 0.218	0.173
CYP1B1	*0.301 ± 0.037	0.042	*1.988 ± 0.398	0.048
CYP2E1	*0.415 ± 0.106	0.044	1.22 ± 0.15	0.184
CYP2W1	*0.152 ± 0.088	0.032	*5.349 ± 1.652	0.036
CYP4F11	*0.588 ± 0.098	0.017	*2.346 ± 0.566	0.039
CYP51A1	*1.486 ± 0.117	0.016	**1.451 ± 0.086	0.007
ESD	*1.368 ± 0.115	0.032	0.983 ± 0.107	0.291
KCNAB2	*1.267 ± 0.0913	0.012	*0.493 ± 0.101	0.043
Phase II enzymes				
COMT	**1.506 ± 0.106	0.008	1.067 ± 0.085	0.291
GSTA4	*1.438 ± 0.178	0.047	0.931 ± 0.09	0.483
GSTP1	**1.673 ± 0.116	0.009	1.138 ± 0.111	0.113
INMT	**0.5359 ± 0.103	0.008	2.123 ± 0.612	0.241
MGST2	*1.356 ± 0.091	0.038	1.045 ± 0.084	0.454
SULT2A1	**0.06 ± 0.024	0.001	0.932 ± 0.0.335	0.100
TPMT	*0.64 ± 0.081	0.042	1.516 ± 0.217	0.097
UGT1A4	**0.147 ± 0.074	0.005	1.308 ± 0.228	0.211
UGT1A9	*0.633 ± 0.082	0.042	0.718 ± 0.122	0.076
UGT2B7	*0.46 ± 0.051	0.019	0.852 ± 0.109	0.310
Transporters				
ABCA1	**0.506 ± 0.061	0.001	0.601 ± 0.091	0.146
ABCA2	**0.471 ± 0.078	0.002	0.591 ± 0.048	0.075
ABCB1	*0.664 ± 0.082	0.026	**0.488 ± 0.102	0.009
ABCC1	*1.324 ± 0.108	0.042	0.921 ± 0.093	0.388
ABCC10	*0.757 ± 0.054	0.025	***0.508 ± 0.036	0.0004
ABCC5	**0.636 ± 0.08	0.009	0.916 ± 0.211	0.192
ABCC6	**0.63 ± 0.087	0.007	**0.511 ± 0.056	0.009
ABCG2	*0.68 ± 0.155	0.042	0.666 ± 0.134	0.065
ATP7A	**0.559 ± 0.067	0.008	0.989 ± 0.205	0.329
SLC1A3	*0.315 ± 0.069	0.022	1.107 ± 0.214	0.453
SLC7A5	**0.548 ± 0.104	0.004	1.047 ± 0.138	0.395
SLC10A2	**0.287 ± 0.085	0.008	0.931 ± 0.22	0.344
SLC15A1	*0.561 ± 0.136	0.041	*0.787 ± 0.297	0.010
SLC15A2	**0.264 ± 0.034	0.005	1.633 ± 0.480	0.221
SLC19A2	**0.576 ± 0.056	0.002	0.871 ± 0.217	0.065
SLC19A3	*0.707 ± 0.097	0.049	0.833 ± 0.118	0.167
SLC22A3	*0.643 ± 0.083	0.028	1.006 ± 0.143	0.380
SLC28A3	*2.234 ± 0.403	0.026	1.299 ± 0.280	0.068
SLC29A2	*1.566 ± 0.173	0.013	1.021 ± 0.054	0.491
SLC38A1	*2.925 ± 1.008	0.040	1.167 ± 0.10640	0.202
SLC38A5	**1.603 ± 0.153	0.009	1.373 ± 0.239	0.178
SLC47A1	**0.167 ± 0.071	0.005	7.482 ± 2.947	0.422
SLCO2B1	*0.66 ± 0.098	0.022	***0.441 ± 0.035	< 0.0001
SLCO4C1	*0.368 ± 0.082	0.012	0.630 ± 0.107	0.174

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Table 1. *Continued*

Gene name	UC patients		CD patients	
	Fold change [mean±SEM]	*P*-value	Fold change [mean±SEM]	*P*-value
Nuclear receptors and transcription factors				
ARNT	**0.775 ± 0.048	0.006	**0.747 ± 0.056	0.006
FOXO1	*0.594 ± 0.095	0.021	*0.786 ± 0.077	0.022
HIF3A	**0.089 ± 0.039	0.001	0.954 ± 0.284	0.418
NCOA2	*0.619 ± 0.039	0.014	1.035 ± 0.280	0.086
NCOR2	*0.462 ± 0.097	0.027	**0.557 ± 0.045	0.008
NR1H3	*0.55 ± 0.068	0.047	0.606 ± 0.065	0.086
NR3C1	*0.602 ± 0.068	0.017	*0.611 ± 0.048	0.024
PPARD	**0.443 ± 0.072	0.006	1.181 ± 0.330	0.413
PPARGC1A	*0.783 ± 0.075	0.025	0.954 ± 0.1614	0.262
RARB	**0.329 ± 0.067	0.002	1.017 ± 0.379	0.141
RXRB	*0.69 ± 0.091	0.042	**0.746 ± 0.042	0.004
THRB	**0.504 ± 0.063	0.002	**0.463 ± 0.082	0.002

Quantitative real-time polymerase chain reaction [RT-PCR] analysis of the mRNA expression level of phase I and II metabolising enzymes, transporters and transcription factors in the ascending colon of 19 UC patients and 20 CD patients compared with eight healthy controls. Values are expressed as mean±SEM [standard error of the mean].

*$P < 0.05$; **$p < 0.01$; ***$p < 0.001$.

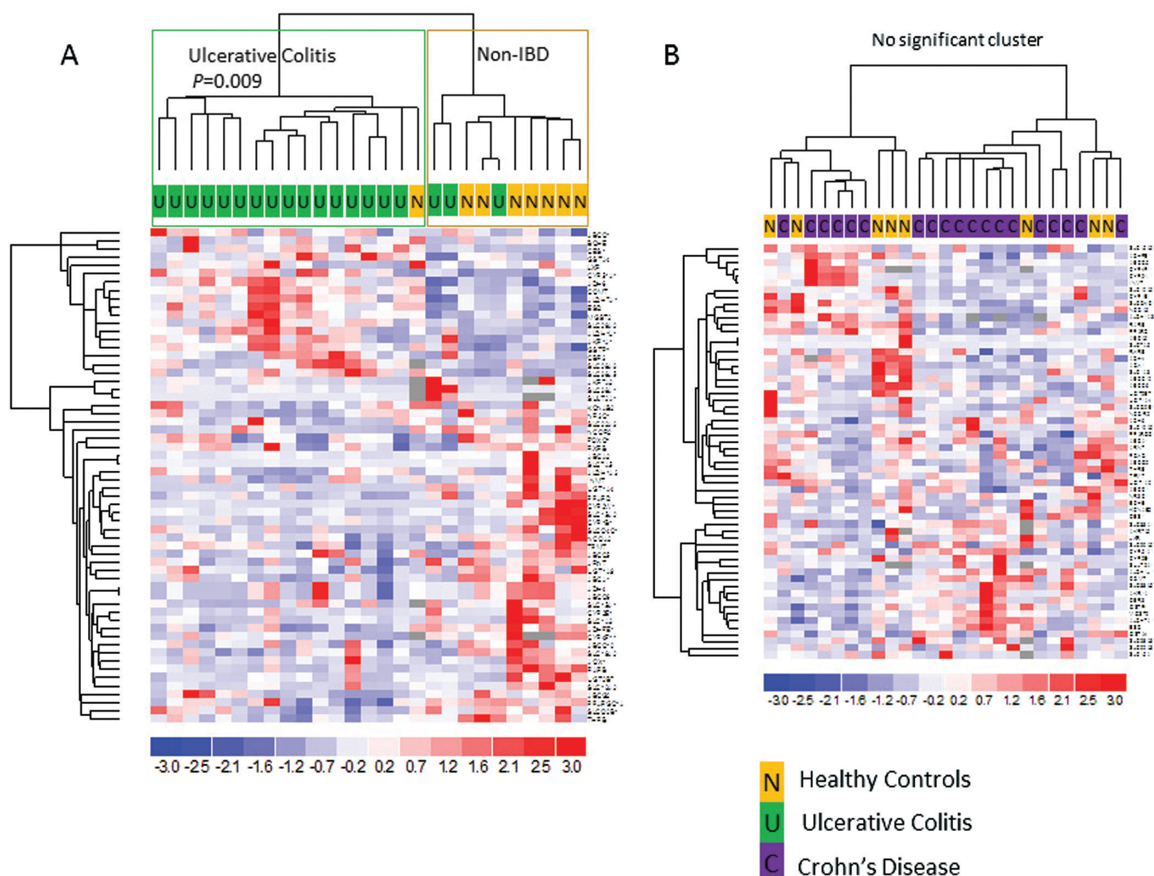

Figure 1. Hierarchical clustering of the 65 genes significantly dysregulated. A] In UC patients versus non-IBD healthy patients, and B] in CD patients versus non-IBD healthy patients [dChip software t-Test].; CD, Crohn's disease.

Using the gene function prediction tool Genemania [http://genemania.org/], we identified co-regulated genes belonging to the AhR [including cofactors *ARNT*, *NCOA2*, *NCOR2*, *NR3C1,* and a set of downstream target genes *ABCB1*, *ABCG2*, *ALDH1A3*, *ALDH7A1*, *AOX1*, *COMT*, *CYP1B1*, *CYP2E1*, *CYP2W1*, *INMT*, *UGT1A4*, *UGT1A9*, *SULT2A1*, *SLC7A5*], PXR/NR1I2 [*ABCB1/MDR1*, *ABCC1, SULT2A1*], and fatty acid metabolism [*PPARs*, *NR1H3/LXR*, *RXR]* clusters. Some of these genes have previously

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been reported to be dysregulated in both IBD patients and mouse colitis models.[20,23,26–28]

A hierarchical cluster analysis identified two distinct clusters based on the similarity of the 65 gene expression profiles, clearly distinguishing UC patients from healthy subjects [$P = 0.009$] [Figure 1A]. The detoxification gene expression profile was then analysed in the non-inflamed colonic mucosa of 18 CD patients. As shown in Figure 1B, the gene expression profiles in CD patients and healthy controls were quite similar, with only 15/65 similar gene expression profiles compared with UC patients [Table 1]. These data demonstrated a specific dysregulated detoxification gene expression in the non-inflamed colonic mucosa of UC patients.

To confirm these mRNA differences at the protein level, four down-regulated genes were analysed by immunohistochemistry on an independent set of biopsies. The tested genes included the aryl hydrocarbon receptor nuclear translocator [ARNT], the ATP-binding cassette transporter A1 [ABCA1], the cytochrome p450 1B1 [CYP1B1], and the hypoxia inducible factor 3A [HIF3A]. A significant decrease in their protein level was observed in the non-inflamed mucosa of UC patients compared with the normal mucosa of healthy subjects, confirming the mRNA results [Supplementary Figure 2, available as Supplementary data at *ECCO-JCC* online].

3.2. Effect of cigarette smoking

Regarding the protective effect of CS on UC, we investigated its impact on the expression of the 65 detoxification genes in the normal colonic mucosa of nine healthy smokers and eight healthy non-smokers. We found that 28 genes were differentially regulated in smokers: 15 XMEs [including *CYP1B1*, *CYP2W1*, *TPMT*, *SULT2A1*], seven transporters [including *ABCC1*, *SLC15A2*, *SLC47A1]* and six nuclear receptors and transcription factors [*HIF3A*, *NCOA2*, *PPARD, PPARGC1A*, *RARB*, *NR1H3*] [Supplementary Table 3, available as Supplementary data at *ECCO-JCC* online]. Interestingly, most of them [71%, 20/28] were inversely expressed in the colonic mucosa of UC patients [Supplementary Table 3]. It therefore seemed interesting to also assess the effect of CS on UC patients. However, it is usually a clinical challenge to obtain biopsies from UC patients in remission following smoking resumption because of colonoscopy repetition. We had the opportunity to study rare colonic biopsies from five UC patients in clinical, endoscopic, and histological remission following smoking resumption [Supplementary Table 3]. The expression of the 65 dysregulated genes was quantified in smoking patients with quiescent UC. Although the small number of patients analysed reduced the statistical power of our analysis, similarities and differences in gene expression levels between the different groups [UC and healthy non-smokers and smokers] were illustrated by principal component analyses [Figure 2A]. The subjects were easily classified according to their smoking habits, regardless of their disease status. This result suggests that CS strongly counter-regulates the altered detoxification gene expression in the colon of UC patients grouping together the smoking control and UC groups. Interestingly, the dysregulation of 43 out of the 65 genes observed in UC was inversed by CS, reaching the expression level observed in the control groups [Figure 2B and Supplementary Table 3].

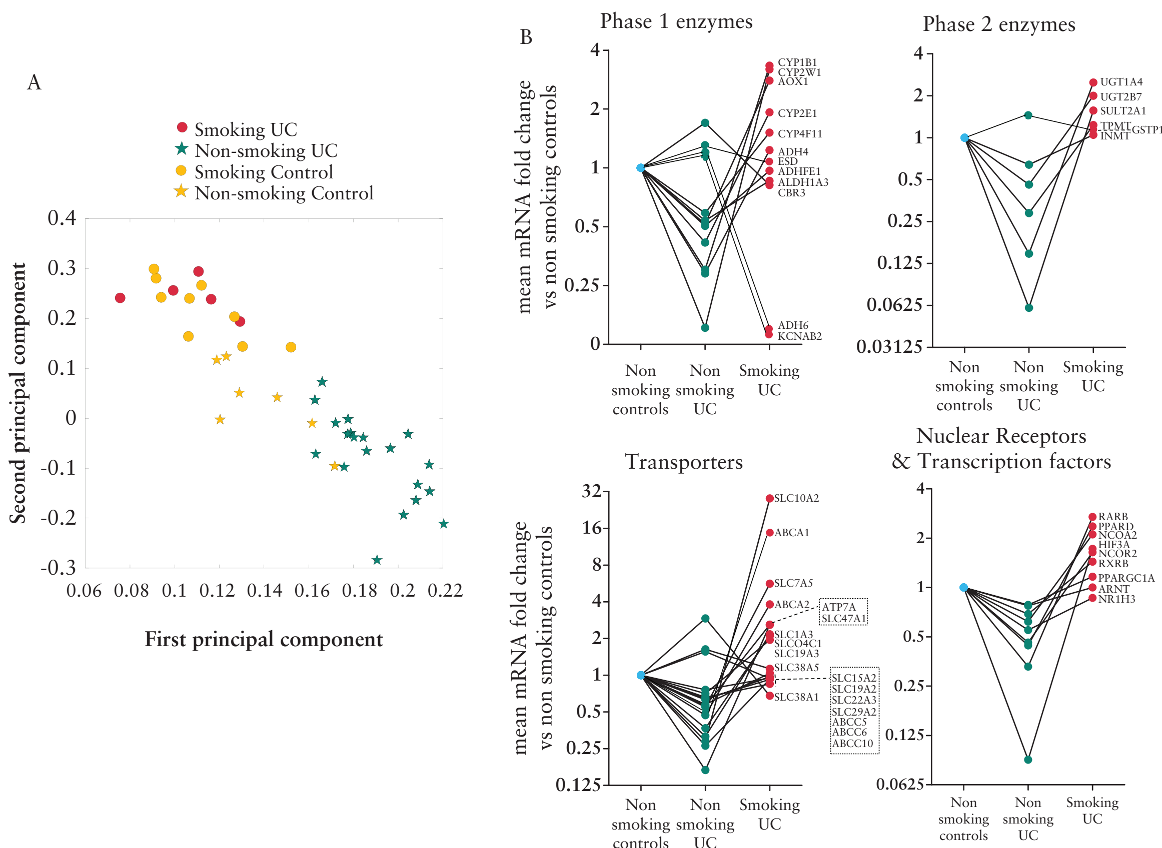

Figure 2. Effect of smoking on detoxification gene expression. A] Representation of the 41 patients through their projection onto the two first components of the principal component analysis applied to the logarithm of the gene expression data. The five smoking UC patients clearly belong to the upper-left group of non-IBD biopsies; B] 45 genes dysregulated in UC tend to be re-expressed at non-IBD levels in smoking quiescent UC patients. UC, ulcerative colitis; IBD, inflammatory bowel disease

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To overcome the limited amount of biopsies from smoking UC patients, non-affected biopsies of non-smoking UC patients were cultured in the presence or absence of cigarette smoke extract [CSE] to test the direct effects induced by CSE on the expression of the 65 dysregulated detoxification genes. CSE inversed the expression level of 47/65 dysregulated genes in non-smoking UC biopsies reaching the expression level observed in the control groups [Supplementary Table 3]. These data enhanced the very sharp concordance of cigarette smoke effects *in vivo* and *in vitro* on detoxification gene profiles. In order to visualise such an effect, we devised a way of looking at the corresponding pattern of variation between CSE-treated biopsies of non-smoking UC patients and biopsies of smoking UC patients. Since these two groups of data resulted from different normalised conditions [ie, *in vitro* and *in vivo*], we used a qualitative approach termed multiple correspondence analysis [MCA] to avoid any suspicion in considering the data as simply qualitative [Figure 3 and Supplementary Figure 3]. The MCA approach, visualised by means of a biplot with some overlying density curves, shows how highly concentrated zones based on the 65 dysregulated gene expression profiles (ie, no change [nc], and up- or down-regulated) systematically tend to have similar patterns of variations between CSE-treated biopsies of non-smoking UC patients and biopsies of smoking UC patients [Figure 3 and Supplementary Figure 3]. Upon interpreting these data as categorical, the similarity of the expression profiles labelled as up-regulated were found to be the closest between the two groups, followed immediately by those profiles labelled as down-regulated [Figure 3].

3.3. Network inference and comparative analysis

We next performed a differential network analysis to investigate the impact of the disease or smoking status on genetic interactions. To this end, our starting assumption was that the observed gene expression pattern was connected through a genetic regulation network [GRN].

Here, we introduced a novel method on the problem of GRN, based on a dynamic model of genetic interactions and aimed at integrating existing knowledge on genetic interactions by solving a constrained optimisation problem. Thus, we were able to infer a network compatible with the following assumptions and constraints:

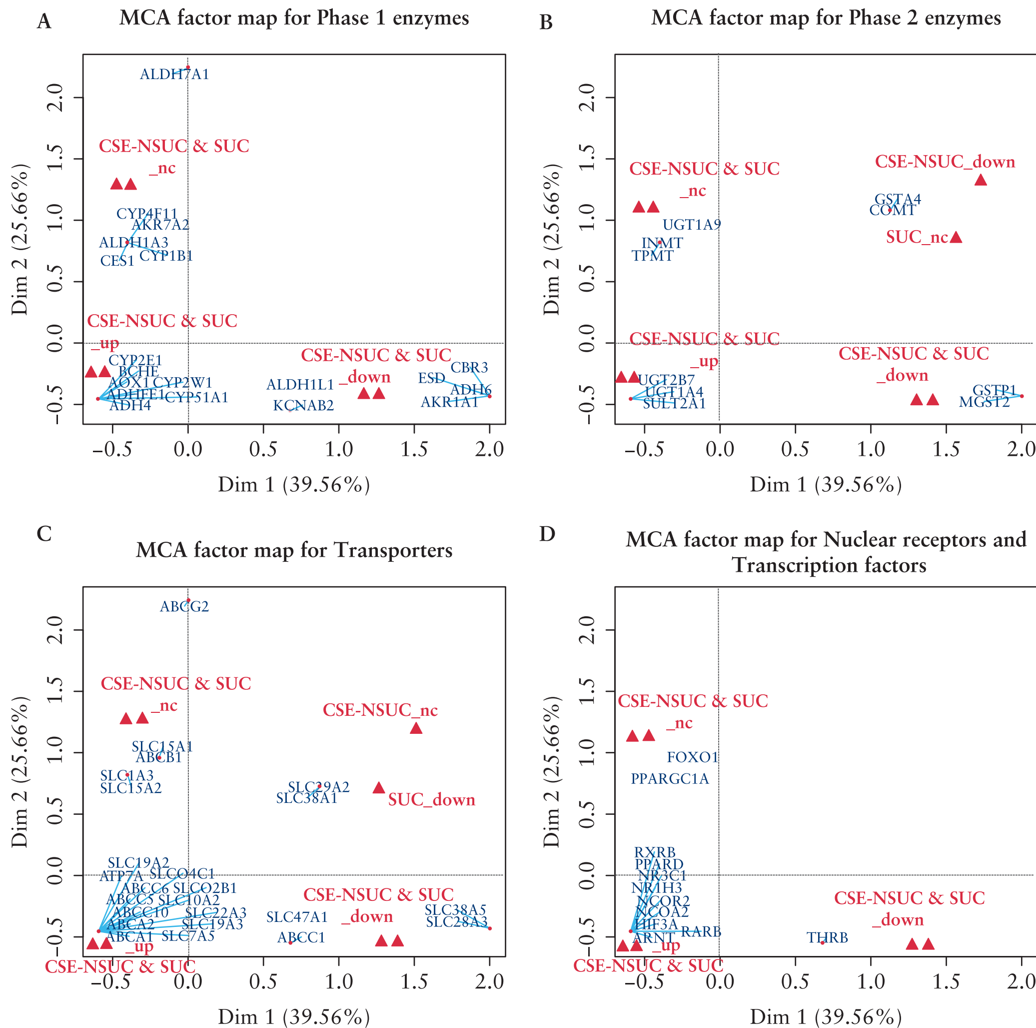

Figure 3. Representation of the 65 dysregulated gene expression categorical profiles as projected onto the first and second dimensions for the multiple component analysis [MCA]. The gene expression profiles are highlighted in blue [observations], whereas the categories are in red. A] MCA for enzymes of phase 1; B] MCA for enzymes of phase 2; C] MCA for transporters; D] MCA for nuclear receptors and transcription factors. CSE-NSUC, cigarette smoke extract-treated biopsies from non-smoking ulcerative colitis [UC] patients; SUC, biopsies from smoking UC patients; nc, no change.

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[i] the observed expression pattern is an equilibrium point for the dynamic model; [ii] some interactions are prohibited due to existing knowledge; and [iii] some interactions are forced to be activating and/or inhibiting. The obtained network, computed on the subset of regulatory genes and for the group of healthy non-smokers, is shown in Figure 4A. This representation highlights the potential role played by FOXO1 in the activation of NR1H3/LXR, and more importantly, the role played by NCOR2 [a repressive co-regulatory factor for multiple transcription factor pathways] in this subnetwork since it is the only gene able to repress other genes.

The resulting inference is a first step to a comparative approach between patients or group of patients, hence focusing on the relative meaning of these networks, as already done in the classical analysis which was more focused on fold changes than on absolute expression levels. To this end, we developed a technique whose aim was to find the minimal number of changes [in terms of sparsity] which should be applied to the control network to obtain a network compatible with the case gene expression profile. In Figure 4B and C, these changes in networks are shown for the comparative analysis between healthy non-smokers and smokers and between non-smoking controls and non-smoking UC patients. For instance, Figure 4B shows how to modify the network of Figure 4A with a minimal effort, to obtain a network compatible with the smoking control group expression patterns. It suggests in particular that the interactions with PPARGC1A are important, in terms of NCOA2 induction and NR1H3/LXR repression. Note that the PPARGC1A gene would not have been studied in a classical gene-based analysis since its fold change was close to 1, showing that the proposed method did not necessarily overlap the classical approach.

3.4. Classification and diagnosis

To validate our comparative analysis methodology, we tested its potential application to improve automated diagnosis based on gene expression data [Figure 5]. The diagnosis of UC is challenging and new biomarkers and diagnostic techniques are needed. To this end, we considered the classification problem where a binary variable [healthy subjects or UC patients] must be predicted using only the gene expression data of the 65 genes of interest. The classification performance was evaluated through a standard leave-one-out cross-validation whose score quantifies the proportion p of correct diagnosis. As a benchmark, we applied the Linear Support Vector Machine [L-SVM] classifier on the raw data, giving a classification score p[SVM] = 82%. To exploit our comparative network analysis, a similarity matrix taking the norm of the modification matrices dW between two patients is defined. Based on this similarity matrix, we first performed a multidimensional scaling, projecting data in two dimensions, and then applied the same L-SVM classifier [Figure 5]. The classification score was improved up to p[netSVM] = 89%. For the sake of comparison, if the same method was applied while the correlation of gene expression data is used as an inter-patient similarity measure, the score obtained was much lower (p[corrSVM] = 69%). This analysis showed that the comparative analysis based on the network inference we developed provides a sensitive, yet debatable, characterisation of the differences between two gene expression patterns.

4. Discussion

An impaired management of xenobiotics in the intestinal mucosa seems to be an important event in IBD initiation and progression.[13,17,18,22,29–33]

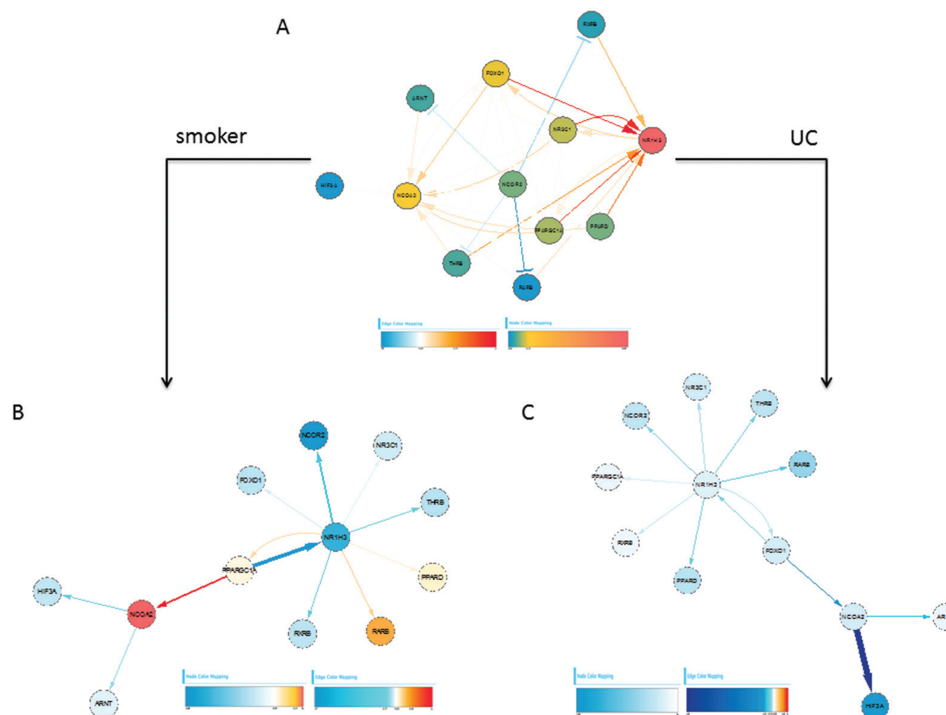

Figure 4. Detoxification gene network. A] Regulatory module network for control non-smoker [CNS] patients. Edge colour and width represent the weights *W*, arrow shapes indicate the type of interaction [activator/repressor], and node colours represent the level of gene expression. B] Minimal change in the regulatory module to switch from the control non-smoker network to the control smoker [CS] network. Edge colour and width represent the weight change d*W[CNS→CS]* [eg, the link from PPARGC1A to NCOA2 is increased]. Node colours represent the fold change [logarithm] between control smokers and control non-smokers [eg, NCOA2 is ove-expressed in the CS group compared with the CNS group]. C] Minimal change in the regulatory module to switch from the CNS network to the ulcerative colitis [UC] non-smoker [UNS] network. Edge colour and width represent the weight change d*W[CNS→UNS]*. Node colours represent the fold change [logarithm] between UNS and CNS patients.

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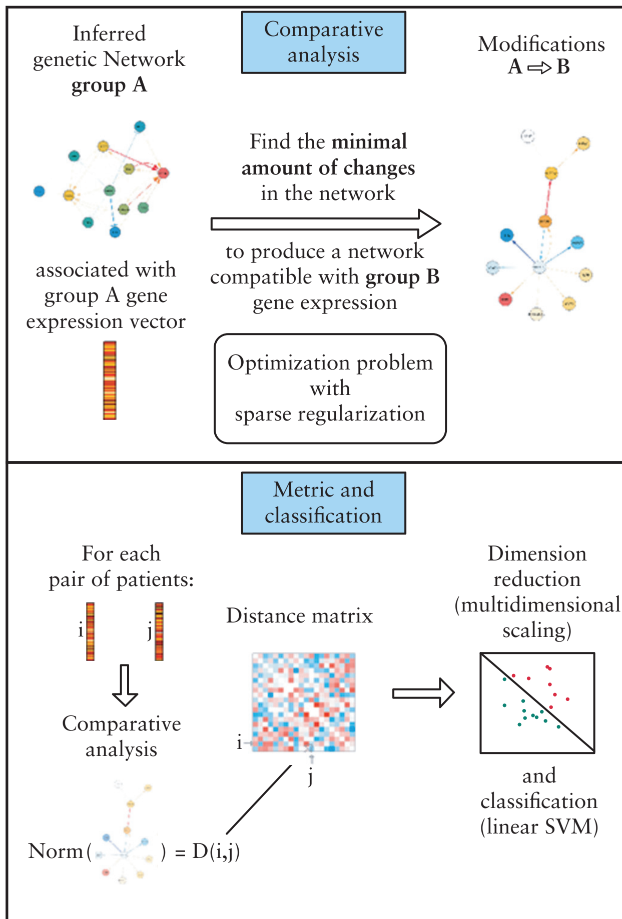

Figure 5. Schematic description of the comparative network analysis [upper panel] and metric-based classification approach [lower panel] described in the text.

However, the information provided by these studies remains very limited, sometimes conflicting,[34–36] and relates most often only to one or a small number of genes, without considering that the management of xenobiotics is usually due to the concerted action of many enzymes, transporters and/or factors, and nuclear cofactors in an integrated and interactive network. Moreover, to our knowledge the impact of cigarette smoke, which is known to protect against intestinal inflammation during UC, on the colonic detoxification capacity and coordinated expression of XMEs in humans has never been assessed.

In this study, we focused on the comprehensive expression of 244 XME genes in the non-inflamed mucosa of the ascending colon[12] of UC patients compared with healthy subjects or CD patients. We identified a specific dysregulation of the xenobiotic detoxification system in the non-inflamed colonic mucosa of UC patients, which is not found in healthy controls or patients with colonic CD, providing a clear-cut gene signature for UC. This dysregulation is likely to contribute to the pathophysiology of UC. Interestingly, the expression of several of these dysregulated genes was commonly modified by smoking in the colonic mucosa of healthy controls, supporting the idea that smoking could affect *per se* the colonic detoxification gene expression. Nuclear receptors and transcription factors which are overarching regulators of the xenobiotic response system, including detoxification enzymes and transporters, were strongly up-regulated in healthy smokers. Moreover, the expression of most genes modulated by tobacco in healthy controls was impaired in UC patients. One hypothesis could be that CS-induced colonic toxicity could up-regulate detoxification genes. This activation could reach a protective expression level threshold, allowing the colonic mucosa to better support and detoxify endogenous and/or environmental products in patients with susceptibility to develop UC. In this regard, we showed that the expression of the dysregulated detoxification gene set observed in UC patients was similarly reversed in both rare biopsies from UC patients in remission following smoking resumption and CSE-treated biopsies from non-smoking UC patients. Thus, CS could modulate the expression of XME genes in the colonic mucosa and help in normalising this dysregulation in UC, which is essential to the detoxification of xenobiotics.

One weakness of this study is the limited recruitment of patients with UC in remission after smoking resumption. However, the similar effect observed in CSE-treated biopsies of patients with UC helped offset this gap and validated the inducible effect of cigarette smoke on detoxification gene expression. We are also aware that CS could exert its protective effect on colonic mucosa in UC through other mechanisms. Smoking induces alterations to both the innate and the adaptive immune system and is associated with a distinct alteration in the intestinal microbiota both in patients with active CD and in healthy subjects.[37–39] However, in CSE-treated colonic explants of UC patients, detoxification gene network restoration was observed independently of any microbial/inflammation intervention.

In summary, our findings support that: i] the unaffected mucosa of UC patients exhibited an impaired epithelial detoxification capacity; ii] this signature was specific for UC and distinct from that observed in patients with CD and healthy controls, suggesting different reactivity of colonic mucosa to environmental threats; and iii] CS could selectively change the detoxification gene expression profile in the colonic mucosa of healthy subjects and overcome the impaired detoxification capacity in that of UC patients in connection with its protective effect. However, changes in the global detoxification gene expression profile in smoking patients with CD remains to be determined, to explain the opposite effect of tobacco in IBD.

In order to analyse these experimental data from a network point of view, we introduced a novel approach for the inference and the comparative analysis of genetic regulatory networks, integrating existing biological knowledge into a constrained optimisation problem. These methodological advances have enabled identification of not only new potentially interesting genes but also gene interactions which were not detectable when considering a classical single-gene differential expression analysis. This integrated analysis helped identify two unexpected genes which might play a central role in the overall dysregulation of detoxification genes in UC or in the beneficial effect of CS on UC, despite their limited fold change expression compared with healthy controls. The first gene identified was *PPARGC1A* [peroxisome proliferator-activated receptor-gamma coactivator 1-alpha], a transcriptional coactivator highly expressed in the intestinal epithelium, able to up-regulate the mitochondrial biogenesis, oxidative phosphorylation, and fatty acid β oxidation.[40] PPARGC1A interacts with several nuclear receptors, including PPARγ which plays key roles in the regulation of inflammation and immune response in UC.[41–44] Functional, biological, pharmacological, and chemical evidence has shown that PPARγ is an essential receptor mediating the common 5-aminosalicylic acid [5-ASA] anti-inflammatory activities in UC patients.[27,42] The second gene identified by the integrated analysis was *NR1H3/LXR* which is an important modulator of the inflammatory response in colonic epithelial cells. LXR activation reduces DSS-induced colitis severity[45] and LXR-deficient mice are more susceptible to colitis,[46] suggesting that LXR could reduce the inflammatory responses in IBD. Interestingly,

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LXR polymorphisms have been associated with an increased risk of IBD, especially among never smokers [4].

These results demonstrate that our integrated analysis allows identification of genes of interest which could be pathophysiologically relevant in UC due to their interactions within the network, and not only according to their change in fold expression.

In a context of increasingly available gene expression data, the approach introduced in this study opens new perspectives for the understanding of biological networks and their differential analysis, especially for complex diseases such as UC whose mechanisms are likely to occur at the network level. Our data indicate that smoking is an environmental factor modulating the detoxification capacity of the normal colonic mucosa and likely to counterbalance the impaired detoxification observed in the non-inflamed mucosa of UC patients.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

YPD planned and performed experiments, analysis, and interpretation of data; YL and AM performed colonic explant culture experiments; YB provided human materials and contributed to study design; DCH conducted histological assessments; GW conceived and designed the mathematical study, performed the analysis of data together with HZ and IM, and wrote the manuscript together with EOD, PS, FD; JPH participated in manuscript editing, XT collected clinical samples and clinical data, contributed to study design and data analysis, and participated in manuscript editing, EOD conceived and designed the study, analysed data, and wrote the manuscript.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

References

1. Graham DB, Xavier RJ. From genetics of inflammatory bowel disease towards mechanistic insights. *Trends Immunol* 2013;**34**:371–8.
2. Jostins L, Ripke S, Weersma RK, *et al*. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;**491**:119–24.
3. Halfvarson J, Jess T, Magnuson A, *et al*. Environmental factors in inflammatory bowel disease: a co-twin control study of a Swedish-Danish twin population. *Inflamm Bowel Dis* 2006;**12**:925–33.
4. Andersen V, Christensen J, Ernst A, *et al*. Polymorphisms in NF-kappaB, PXR, LXR, PPARgamma and risk of inflammatory bowel disease. *World J Gastroenterol* 2011;**17**:197–206.
5. Molodecky NA, Soon IS, Rabi DM, *et al*. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012;**142**:46–54 e42; quiz e30.
6. Mahid SS, Minor KS, Soto RE, Hornung CA, Galandiuk S. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clin Proc* 2006;**81**:1462–71.
7. Bergeron V, Grondin V, Rajca S, *et al*. Current smoking differentially affects blood mononuclear cells from patients with Crohn's disease and ulcerative colitis: relevance to its adverse role in the disease. *Inflamm Bowel Dis* 2012;**18**:1101–11.
8. Lakhan SE, Kirchgessner A. Anti-inflammatory effects of nicotine in obesity and ulcerative colitis. *J Transl Med* 2011;**9**:129.
9. Lunney PC, Leong RW. Review article: Ulcerative colitis, smoking and nicotine therapy. *Aliment Pharmacol Ther* 2012;**36**:997–1008.
10. Nikfar S, Ehteshami-Ashar S, Rahimi R, Abdollahi M. Systematic review and meta-analysis of the efficacy and tolerability of nicotine preparations in active ulcerative colitis. *Clin Ther* 2011;**32**:2304–15.
11. Nakata K, Tanaka Y, Nakano T, *et al*. Nuclear receptor-mediated transcriptional regulation in Phase I, II, and III xenobiotic metabolising systems. *Drug Metab Pharmacokinet* 2006;**21**:437–57.
12. Bourgine J, Billaut-Laden I, Happillon M, *et al*. Gene expression profiling of systems involved in the metabolism and the disposition of xenobiotics: comparison between human intestinal biopsy samples and colon cell lines. *Drug Metab Dispos* 2012;**40**:694–705.
13. Roediger WE, Babidge W. Human colonocyte detoxification. *Gut* 1997;**41**:731–4.
14. Wu H, Lu T, Xue H, Liang H. Sparse additive ordinary differential equations for dynamic gene regulatory network modeling. *J Am Stat Assoc* 2014;**109**:700–16.
15. Plewka D, Plewka A, Szczepanik T, *et al*. Expression of selected cytochrome P450 isoforms and of cooperating enzymes in colorectal tissues in selected pathological conditions. *Pathol Res Pract* 2014;**210**:242–9.
16. Chen G, Cairelli MJ, Kilicoglu H, Shin D, Rindflesch TC. Augmenting microarray data with literature-based knowledge to enhance gene regulatory network inference. *PLoS Comput Biol* 2014;**10**:e1003666.
17. Mencarelli A, Migliorati M, Barbanti M, *et al*. Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells. *Biochem Pharmacol* 2010;**80**:1700–7.
18. Langmann T, Moehle C, Mauerer R, *et al*. Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes. *Gastroenterology* 2004;**127**:26–40.
19. Takamura T, Harama D, Matsuoka S, *et al*. Activation of the aryl hydrocarbon receptor pathway may ameliorate dextran sodium sulfate-induced colitis in mice. *Immunol Cell Biol* 2010;**88**:685–9.
20. Monteleone I, Riszo A, Sarra M, *et al*. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. *Gastroenterology* 2012;**141**:237–48, 248 e1.
21. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, MDR1A, spontaneously develop colitis. *J Immunol* 1998;**161**:5733–44.
22. Deuring JJ, de Haar C, Koelewijn CL, Kuipers EJ, Peppelenbosch MP, van der Woude CJ. Absence of ABCG2-mediated mucosal detoxification in patients with active inflammatory bowel disease is due to impeded protein folding. *Biochem J* 2012;**441**:87–93.
23. Schwab M, Schaeffeler E, Marx C, *et al*. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* 2003;**124**:26–33.
24. Treton X, Pedruzzi E, Cazals-Hatem D, *et al*. Altered endoplasmic reticulum stress affects translation in inactive colon tissue from patients with ulcerative colitis. *Gastroenterology* 2011;**141**:1024–35.
25. Pryor WA. Biological effects of cigarette smoke, wood smoke, and the smoke from plastics: the use of electron spin resonance. *Free Radic Biol Med* 1992;**13**:659–76.
26. Furumatsu K, Nishiumi S, Kawano Y, *et al*. A role of the aryl hydrocarbon receptor in attenuation of colitis. *Dig Dis Sci* 2011;**56**:2532–44.
27. Bertin B, Dubuquoy L, Colombel JF, Desreumaux P. PPAR-gamma in ulcerative colitis: a novel target for intervention. *Curr Drug Targets* 2013;**14**:15017.
28. Heimerl S, Moehle C, Zahn A, *et al*. Alterations in intestinal fatty acid metabolism in inflammatory bowel disease. *Biochim Biophys Acta* 2006;**1762**:341–50.
29. Kaminsky LS, Zhang QY. The small intestine as a xenobiotic-metabolising organ. *Drug Metab Dispos* 2003;**31**:1520–5.

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30. Blokzijl H, Vander Borght S, Bok LI, *et al*. Decreased P-glycoprotein [P-gp/MDR1] expression in inflamed human intestinal epithelium is independent of PXR protein levels. *Inflamm Bowel Dis* 2007;**13**:710–20.
31. Brant SR, Panhuysen CI, Nicolae D, *et al*. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. *Am J Hum Genet* 2003;**73**:1282–92.
32. Martinez A, Marquez A, Mendoza J, *et al*. Role of the PXR gene locus in inflammatory bowel diseases. *Inflamm Bowel Dis* 2007;**13**:1484–7.
33. Dring MM, Goulding CA, Trimble VI, *et al*. The pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. *Gastroenterology* 2006;**130**:341–8; quiz 592.
34. Oostenbrug LE, Dijkstra G, Nolte IM, *et al*. Absence of association between the multidrug resistance [MDR1] gene and inflammatory bowel disease. *Scand J Gastroenterol* 2006;**41**:1174–82.
35. Wang J, Guo X, Yu S, *et al*. MDR1 C3435T polymorphism and inflammatory bowel disease risk: a meta-analysis. *Mol Biol Rep* 2014;**41**:2679–85.
36. Zintzaras E. Is there evidence to claim or deny association between variants of the multidrug resistance gene [MDR1 or ABCB1] and inflammatory bowel disease? *Inflamm Bowel Dis* 2011;**18**:562–72.
37. Allais L, Kerckhof FM, Verschuere S, *et al*. Chronic cigarette smoke exposure induces microbial and inflammatory shifts and mucin changes in the murine gut. *Environ Microbiol* 2016;**18**:1352–63.
38. Biedermann L, Zeitz J, Mwinyi J, *et al*. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. *PLoS One* 2013;**8**:e59260.
39. Montbarbon M, Pichavant M, Langlois A, *et al*. Colonic inflammation in mice is improved by cigarette smoke through iNKT cells recruitment. *PLoS One* 2013;**8**:e62208.
40. D'Errico I, Salvatore L, Murzilli S, *et al*. Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha [PGC1alpha] is a metabolic regulator of intestinal epithelial cell fate. *Proc Natl Acad Sci U S A* 2011;**108**:6603–8.
41. Dubuquoy L, Jansson EA, Deeb S, *et al*. Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. *Gastroenterology* 2003;**124**:1265–76.
42. Rousseaux C, El-Jamal N, Fumery M, *et al*. The 5-aminosalicylic acid antineoplastic effect in the intestine is mediated by PPARgamma. *Carcinogenesis* 2013;**34**:2580–6.
43. Bassaganya-Riera J, Reynolds K, Martino-Catt S, *et al*. Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastroenterology* 2004;**127**:777–91.
44. Su CG, Wen X, Bailey ST, *et al*. A novel therapy for colitis utilising PPAR-gamma ligands to inhibit the epithelial inflammatory response. *J Clin Invest* 1999;**104**:383–9.
45. Canavan M, McCarthy C, Larbi NB, *et al*. Activation of liver X receptor suppresses the production of the IL-12 family of cytokines by blocking nuclear translocation of NF-kappaBp50. *Innate Immun* 2013;**20**:675–87.
46. Jakobsson T, Vedin LL, Hassan T, *et al*. The oxysterol receptor LXRbeta protects against DSS- and TNBS-induced colitis in mice. *Mucosal Immunol* 2014;**7**:1416–28.

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