EXTENDED REPORT

Altered gut transcriptome in spondyloarthropathy

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Background: Intestinal inflammation is a common feature of spondyloarthropathy (SpA) and Crohn's disease. Inflammation is manifested clinically in Crohn's disease and subclinically in SpA. However, a fraction of patients with SpA develops overt Crohn's disease.
Aims: To investigate whether subclinical gut lesions in patients with SpA are associated with transcriptome changes comparable to those seen in Crohn's disease and to examine global gene expression in non-inflamed colon biopsy specimens and screen patients for differentially expressed genes.
Methods: Macroarray analysis was used as an initial genomewide screen for selecting a comprehensive set of genes relevant to Crohn's disease and SpA. This led to the identification of 2625 expressed sequence tags that are differentially expressed in the colon of patients with Crohn's disease or SpA. These clones, with appropriate controls (6779 in total), were used to construct a glass-based microarray, which was then used to analyse colon biopsy specimens from 15 patients with SpA, 11 patients with Crohn's disease and 10 controls.
Results: 95 genes were identified as differentially expressed in patients with SpA having a history of subclinical chronic gut inflammation and also in patients with Crohn's disease. Principal component analysis of this filtered set of genes successfully distinguished colon biopsy specimens from the three groups studied. Patients with SpA having subclinical chronic gut inflammation cluster together and are more related to those with Crohn's disease.
Conclusion: The transcriptome in the intestine of patients with SpA differs from that of controls. Moreover, these gene changes are comparable to those seen in patients with Crohn's disease, confirming initial clinical observations. On the basis of these findings, new (genetic) markers for detection of early Crohn's disease in patients with SpA can be considered.

The clinical association between spondyloarthropathy (SpA) and Crohn's disease is shown by the concurrence of similar arthropathy and intestinal inflammation in patients with either disease, indicating a shared aetiology and pathogenesis. Depending on the imaging technique used, up to one third of patients with Crohn's disease have peripheral or sacroiliac joint abnormalities similar to those seen in various subgroups with SpA.[1 2] In addition, 60% of patients with SpA who have no evidence of Crohn's disease exhibit endoscopic or histological signs of subclinical gut inflammation.[3] In general, two types of inflammation are observed: acute inflammation as seen in infectious colitis and chronic inflammation resembling that seen in Crohn's disease.[3] A striking parallel exists between the activity of inflammation at the joints and at the intestine. Moreover, long-term evolution to Crohn's disease was observed in 13% of patients with SpA with initial chronic gut inflammation, supporting the concept of preclinical Crohn's disease in those patients.[4] Since these clinical observations, several studies provided additional evidence for a joint–gut axis on the molecular and the genetic levels. The early immunological changes observed are up regulation of αEβ7 integrin on T cell lines from patients with SpA and an increase in lymphoid follicles and lamina propria mononuclear cells in intestinal biopsy specimens.[5–7] Increased expression of α*E*β7 and the E-cadherin–catenin complex was found in the gut mucosa from patients with Crohn's disease and in those with SpA.[5 8] A specific subset of CD163 macrophages is augmented in both groups of patients, supporting the hypothesis of a recirculation of similar clones in the intestinal mucosa and synovium.[9]

Both Crohn's disease and SpA are complex genetic traits, because many genes are probably associated in the pathogenesis, and environmental factors have a substantial influence on the outcome of the disease. Evidence exists for a common genetic risk factor in the development of subclinical intestinal inflammation in first-degree relatives of patients with ankylosing spondylitis, which is the prototype of SpA.[10] Furthermore, we found that *CARD15*, which was the first gene identified for susceptibility to Crohn's disease, is associated with chronic subclinical inflammation in patients with SpA.[11] In this regard, patients with SpA can serve as a unique model for detection of early genetic markers for Crohn's disease.

To determine whether the association between the two disorders occurs at the clinical and also at the transcriptome level, we compared global gene expression in non-inflamed colon biopsy specimens from patients with SpA and those with Crohn's disease. We propose that it is possible to identify a set of genes that distinguish patients with Crohn's disease and those with SpA having a history of chronic gut inflammation from patients with SpA without chronic gut inflammation and from controls.

PARTICIPANTS AND METHODS

Patients, tissue collection and histological classification

Colon biopsy specimens from patients with Crohn's disease and with SpA and from healthy controls were obtained during colonoscopy. All biopsy specimens were taken from

Abbreviations: ACOXI, Acyl-coenzyme A oxidase 1; EST, expressed sequence tag; IBD, inflammatory bowel disease; PCR, polymerase chain reaction; SpA, spondyloarthropathy

the non-inflamed sigmoid at 30 cm, immediately placed in RNA later (Ambion, Cambridgeshire, UK) and frozen at −80℃ until the sample was processed. Three specimens were obtained from each of 34 patients diagnosed with Crohn's disease according to clinical, endoscopic and histological criteria and from 20 patients diagnosed with SpA according to the criteria of the European Spondylarthropathy Study Group.[12] Sixteen patients without clinical manifestations of Crohn's disease or SpA, who were undergoing colonoscopy for colon cancer screening, were included as controls .

Histological classification of the ileum and colon in patients with SpA was carried out as in our previous studies.[3 4 13–15] We distinguished three classes: patients with normal histology, those with acute inflammatory lesions, and those with chronic inflammatory lesions.[16] In acute lesions, normal architecture was well preserved. Infiltration by neutrophils and eosinophils was seen, without a considerable increase in lymphocytes. Small superficial ulcers covered with fibrin and neutrophils overlying hyperplastic lymphoid follicles were occasionally observed. The lamina propria was oedematous and haemorrhagic, containing mainly polymorphonuclear cells. The pattern of inflammation was similar to that seen in acute self-limiting bacterial enterocolitis. The principal features of chronic lesions were crypt distortion, atrophy of the villous surface of the mucosa, villous blunting and fusion, increased mixed cellularity, and the presence of basal lymphoid aggregates in the lamina propria. Although several biopsy specimens were obtained from each patient, a diagnosis of chronic inflammation was made even if only one specimen showed chronic lesions, regardless of acute or active inflammation in the other specimens.

Patients with SpA who had chronic inflammation in the colon or ileum in previous examinations were termed patients with SpA with chronic gut inflammation.

RNA extraction

Total RNA was extracted from biopsy specimens using the Qiagen Rneasy Mini Kit (Westburg BV, Leusden, The Netherlands) with on-column DNAse treatment (Qiagen). Needle homogenisation was carried out. Quality and concentration of RNA were checked on the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

Macroarray hybridisation and analysis

Colony filters containing 74 828 expressed sequence tag (EST) clones (Human UniGene collection 2, RZPD, Germany) were used as initial screen. Radioactively labelled probes were produced by incorporation of $\alpha.^{33}$P-labelled deoxycytidine triphosphate during reverse transcription of 50 µg total RNA (MMLV, Promega, Leiden, The Netherlands), using oligodT as primer. ^{33}P-cDNA probes were purified on G-50 spin columns (Amersham Biosciences, Roosendaal, The Netherlands). Hybridisation was carried out at 10^6 cpm/ml at 65℃ for 20 h. Images were acquired after 6, 18 and 24 h of exposure, by using a Phosphorimager system (Amersham Biosciences). Spot definition and intensity measurement were carried out with Visualgrid (GPC Biotech AG, Munich, Germany). The raw expression data were processed with an in-house algorithm based on MS Access. Spot intensities were corrected for the local background, followed by a quality control of spots to exclude those influenced by intense signals of adjacent spots. The detection limit for expression values above background was calculated on the basis of the variation in the local background intensity. Constitutive genes (those that show the lowest coefficient of variation over all arrays) were used for normalisation. Subsequently, quantitative measures of each clone (gene) were calculated by $\log_2$ transformation of the ratio of the mean spot intensity in samples from patients with Crohn's disease or with SpA to the mean spot intensity in samples from controls.

Microarray hybridisation, scanning and analysis

Construction of a focus microarray chip, probe labelling, hybridisation, washing and scanning were carried out at the MicroArray Facility of the Flanders Interuniversity Institute for Biotechnology (MAF, Leuven, Belgium). Clones selected from the macroarray screen were amplified by polymerase chain reaction (PCR) from RZPD clones by using universal M13 primers. PCR fragments were purified on MultiScreen PCR plates (Millipore, Brussels, Belgium) and resuspended in 50% dimethyl sulfoxide (DMSO) at an average concentration of 100 ng/µl. The PCR products were arrayed in duplicate on Type VII silane-coated slides with a Molecular Dynamics Generation III printer (Amersham Biosciences, Buckinghamshire, UK). Total RNA (5 µg) was amplified using a modified protocol of in vitro transcription, as described previously.[17] In all, 5 µg of the amplified RNA was labelled with Cy3 or Cy5 as described at http:\\www.microarrays.be\service.htm. Arrays were scanned at 532 and 635 nm with a Generation III scanner (Amersham BioSciences). Images were analysed using ArrayVision (Imaging Research, Ontario, Canada). Each hybridisation was repeated in a dye swap experiment. Spot intensities were measured, corrected for local background, and those that exceeded the background by more than two standard deviation (SD) values were included. For each gene, ratios of red (Cy5) to green (Cy3) intensities (I) were calculated and normalised with a Lowess Fit of the $\log_2$ ratios ($\log_2$(ICy5/ICy3)) over the $\log_2$ total intensity ($\log_2$(ICy5×ICy3)).

For comparing the microarray datasets, a mixture of RNA from five patients with Crohn's disease, five patients with SpA and five controls was used as reference RNA. This reference sample provides a positive hybridisation signal at each probe element on the microarray, which is essential when calculating and comparing fluorescence ratios. The data were imported into GeneMaths XT (Applied Maths, St-Martens-Latem, Belgium). Weighted mean ratios and their corresponding errors (pixel SD) were calculated from the dye swap. Data were normalised over all arrays and missing values were imputed using the k-nearest-neighbour algorithm (20 neighbours). GeneMaths XT was used for all subsequent supervised and unsupervised analyses.

Statistics

All p values chosen for cut-off are subjective.

RESULTS

Design of the custom microarray

To provide a practical and cost-effective tool for conducting a large number of hybridisations, a self-designed focus microarray chip was constructed specifically for studying colonic gene expression in patients with SpA and with Crohn's disease. To accomplish this, a genomewide survey of gene expression in colon biopsy specimens from four patients with Crohn's disease, four patients with SpA and six controls was conducted using high-density nylon arrays containing 74 828 cDNA sequences (table 1, macroarrays).

Spots that showed aberrant morphology, encompassed variation in replicates or were impaired because of overshining (characteristic of radioactive signals) were filtered out and considered to be clones lost through experimental error. To select for clones that were differentially expressed in patients with Crohn's disease or with SpA, we arbitrarily selected for those that have a $\log_2$-transformed mean ratio of <-0.6 or $>+0.6$ (1.5-fold down regulated or up regulated). Genes that may be differentially expressed between groups (control *v* patients with Crohn's disease or control *v* those

Table 1 Study population

	Diagnosis	Sample	Sex	Age (years)	SpA	Gut histology	Clinical CD	CD location	Drug
Macroarrays	Control	1	F	59	No	Normal	No		–
		2	F	45	No	Normal	No		–
		3	F	55	No	Normal	No		–
		4	M	30	No	Normal	No		–
		5	F	58	No	Normal	No		–
		6	M	40	No	Normal	No		–
	CD	7	M	21	No	Chronic	Yes	IC	–
		8	F	48	No	Chronic	Yes	IC	5-ASA
		9	F	41	Yes	Chronic	Yes	C	–
		10	F	23	No	Chronic	Yes	C	AZA
	SpA	11	M	47	Yes	Normal	No		–
		12	F	85	Yes	Normal	No		–
		13	F	60	Yes	Normal	No		–
		14	F	43	Yes	Chronic	Yes		–
Microarrays	Control	1	M	54	No	Normal	No		–
		2	F	64	No	Normal	No		–
		3	F	72	No	Normal	No		–
		4	M	51	No	Normal	No		–
		5	F	21	No	Normal	No		–
		6	F	68	No	Normal	No		–
		7	F	32	No	Normal	No		–
		8	F	73	No	Normal	No		–
		9	F	66	No	Normal	No		–
		10	M	76	No	Normal	No		–
	CD	11	F	23	No	Chronic	Yes	IC	5-ASA
		12	F	39	Yes	Chronic	Yes	C	–
		13	M	51	No	Chronic	Yes	C	–
		14	M	43	No	Chronic	Yes	I	–
		15	M	36	No	Chronic	Yes	IC	–
		16	F	27	No	Chronic	Yes	IC	–
		17	F	46	Yes	Chronic	Yes	IC	AZA
		18	F	23	No	Chronic	Yes	IC	–
		19	M	19	No	Chronic	Yes	IC	–
		20	F	26	No	Chronic	Yes	IC	–
		21	F	36	No	Chronic	Yes	IC	–
	SpA	22	M	40	AS periph	Acute	No		–
		23	M	29	AS periph	Normal	No		NSAID
		24	M	42	AS periph	Acute	No		–
		25	M	31	AS periph	Acute	No		NSAID
		26	M	76	USpA	Normal	No		Steroids
		27	F	28	AS ax	Normal	No		NSAID
		28	M	58	AS periph	Normal	No		–
		29	M	38	AS ax	Normal	No		Sulfa
		30	F	49	AS periph	Chronic	No		Sulfa
		31	M	49	AS periph	Chronic	No		–
		32	M	45	AS periph	Chronic	No		Sulfa
		33	M	29	USpA	Acute	No		Sulfa+NSAID
		34	M	48	AS periph	Normal	No		NSAID
		35	F	44	AS periph	Chronic	Yes	IC	Sulfa+AZA
		36	M	36	AS ax	Normal	No		Sulfa+NSAID

5-ASA, 5-aminosalicylates; AS ax, ankylosing spondylitis with only axial involvement; AS periph, ankylosing spondylitis with peripheral involvement; AZA, azathioprine; C, colonic involvement only; CD, Crohn's disease; F, female; I, ileal involvement only; IC, ileocolonic; M, male; NSAID, non-steroidal anti-inflammatory drug; SpA, spondyloarthropathy; Sulfa, sulfasalazine; USpA, undifferentiated SpA.
Histology of patients with SpA is a historical classification.

with SpA) were identified using a simple algorithm based on the t test ($p<0.05$) and F values ($p<0.05$) as selection criteria, provided that at least three consistent intensity values were present in each group. F values were chosen for selection because we believed that differences in variances within groups might be important. A total of 2652 clones were identified as "potentially differentially expressed". These genes together with 4127 ESTs lost through experimental error—which may include, besides control ESTs, additional differentially expressed genes—were used to produce a glass-based microarray platform. This allowed us to screen more patients in a more accurate and sensitive manner.

Clustering of unfiltered data

We hybridised independent cohorts of patients: 15 with SpA, 11 with Crohn's disease and 10 controls to the focus microarray (table 1, microarrays). Unsupervised clustering (without prior knowledge of groups) using all genes showed no clustering with respect to disease or phenotype (eg, type of intestinal inflammation). The inability to find discriminatory genes by using unfiltered data is not surprising, as we are analysing the steady-state transcriptome in non-inflamed tissue samples of complex inflammatory diseases. Subtle differences that exist in only a few genes are lost in the vast number of random variations. The problem of detecting differentially expressed genes can be overcome by carrying out supervised clustering. To this end, we divided the patients into four main groups: Crohn's disease, SpA and chronic gut inflammation, SpA without chronic gut inflammation, and controls. Discriminant analysis can reduce n-dimensional data into a more visual two-dimensional or three-dimensional plot, with prior knowledge of groups (fig 1). With this approach, these groups became clearly separated, indicating that our full dataset contains genes that can differentiate between these disease states.

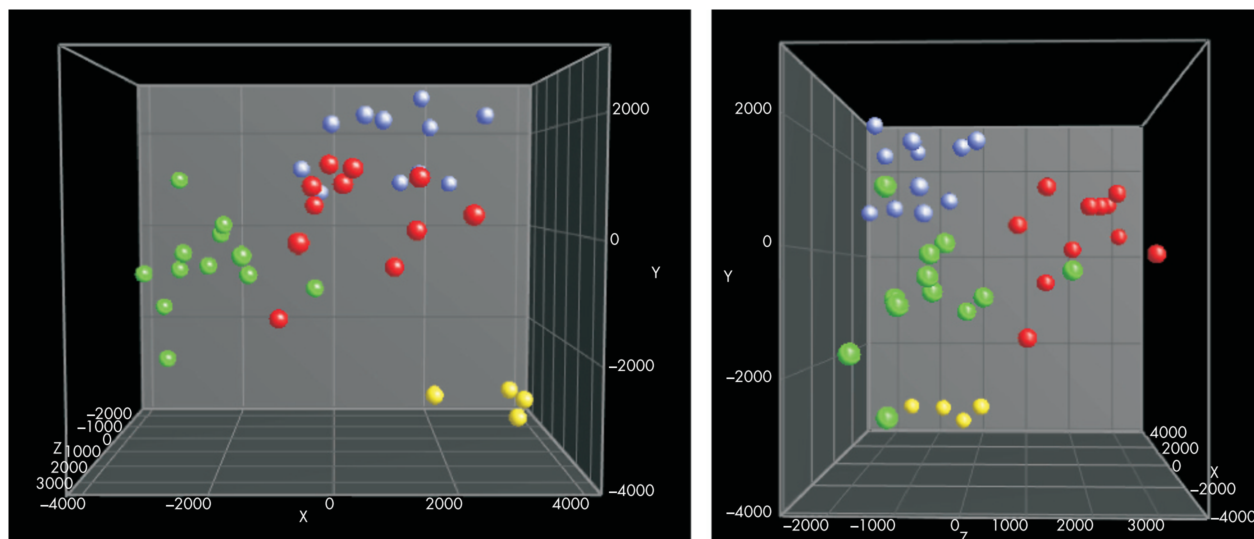

Figure 1 Discriminant analysis of all patients by using unfiltered data, shown in two directions. Four groups are clearly separated: Crohn's disease (green circles), SpA without chronic inflammation (blue circles), SpA with chronic inflammation (yellow circles) and controls (red circles).

Identification of genes whose differential regulation is common to both SpA with chronic gut inflammation and Crohn's disease

With an independent t test, we identified 123 genes that were expressed differentially between patients with Crohn's disease and controls ($p<0.01$). With this set, we were unable to distinguish patients with SpA from controls, although three of four of the patients with SpA with chronic gut inflammation clustered together, indicating the presence of changes similar to those observed in patients with Crohn's disease. Thus, it was logical to screen for genes modulated commonly in patients with Crohn's disease and controls on the one hand, and patients with SpA with chronic gut inflammation and controls on the other hand. To include a larger number of genes in this analysis, the level of significance was lowered from $p<0.01$ to $p<0.05$. This led to the identification of two sets of genes whose expression pattern differentiates patients with Crohn's disease from controls ($p<0.05$, $n = 630$) and those with SpA from controls ($p<0.05$, $n = 464$). The significance level for comparison of patients with SpA and controls was determined by analysis of variance, in which patients with SpA and chronic gut inflammation were defined as a distinct group. The set of 95 genes that were differentially expressed in patients with Crohn's disease and in those with SpA distinguished the three disease groups (fig 2A, table 2). In addition, patients with SpA and chronic gut inflammation clustered together and were more related to the cluster of patients with Crohn's disease than to the cluster of controls or patients with SpA, but remained a separate entity (fig 2A). Using this set of 95 genes, principal component analysis (another way of representing the data) clearly differentiates our patient groups (fig 2B). We attempted to identify the set of genes responsible for Crohn's disease that are also implicated in SpA in order to establish the genes that may render these people more susceptible to Crohn's disease.

Genes within the Crohn's disease–SpA chronic cluster

Table 2 shows the genes whose expression is aberrant in Crohn's disease and in SpA with chronic gut inflammation. Among them, two genes had already been described in the context of Crohn's disease. Acyl-coenzyme A oxidase 1 (ACOX1), which is the first enzyme of the fatty acid β-oxidation pathway, donates electrons directly to molecular oxygen, thereby producing hydrogen peroxide. The enzymatic activity of ACOX1 was diminished in both inflamed and non-inflamed areas in patients with Crohn's disease.[18] Our observation of down regulation of the ACOXI transcript corroborates this report and indicates a fault at the level of transcription or mRNA stability.

Glutathione peroxidase 2 (gastrointestinal glutathione peroxidase) is one of the four types of selenium-dependent glutathione peroxidases. Its exclusive expression in the gastrointestinal tract indicates that it functions as a barrier against the absorption of dietary hydroperoxides and protects against damage from endogenously formed hydroxyl peroxides. Its activity is increased in patients with ulcerative colitis in the active and in the remission stages.[19] Patients with Crohn's disease have increased plasma levels of gastrointestinal glutathione peroxidase.[20] We found that this gene is overexpressed in normal colon tissue in patients with Crohn's disease and in those with SpA and a history of chronic gut inflammation; thus, it can act as a marker expressed at non-pathological sites in the intestine of patients with Crohn's disease and of those in SpA susceptible to Crohn's disease.

DISCUSSION

Clinical study of intestinal abnormalities in patients with SpA has previously relied on cytokine profiles and immunological changes. In addition to analysing every protein, genomewide transcript profiles can be analysed by microarrays. Global gene expression analysis in non-inflamed colon tissue was used to find genes that are differentially expressed in patients with Crohn's disease and in those with SpA and a history of chronic gut inflammation. Previous studies on gene expression in patients with inflammatory bowel disease (IBD) have focused on biopsy specimens of actively inflamed tissues.[21–23] The use of samples from non-inflamed areas from patients with Crohn's disease offers the possibility of identifying early markers for Crohn's disease, which would permit prediction of the evolution to Crohn's disease in patients with SpA. Moreover, changes in the expression of genes that are regulated during inflammation would be more prominent than the subtle changes in non-inflammatory genes. It cannot be ruled out, however, that this procedure will also

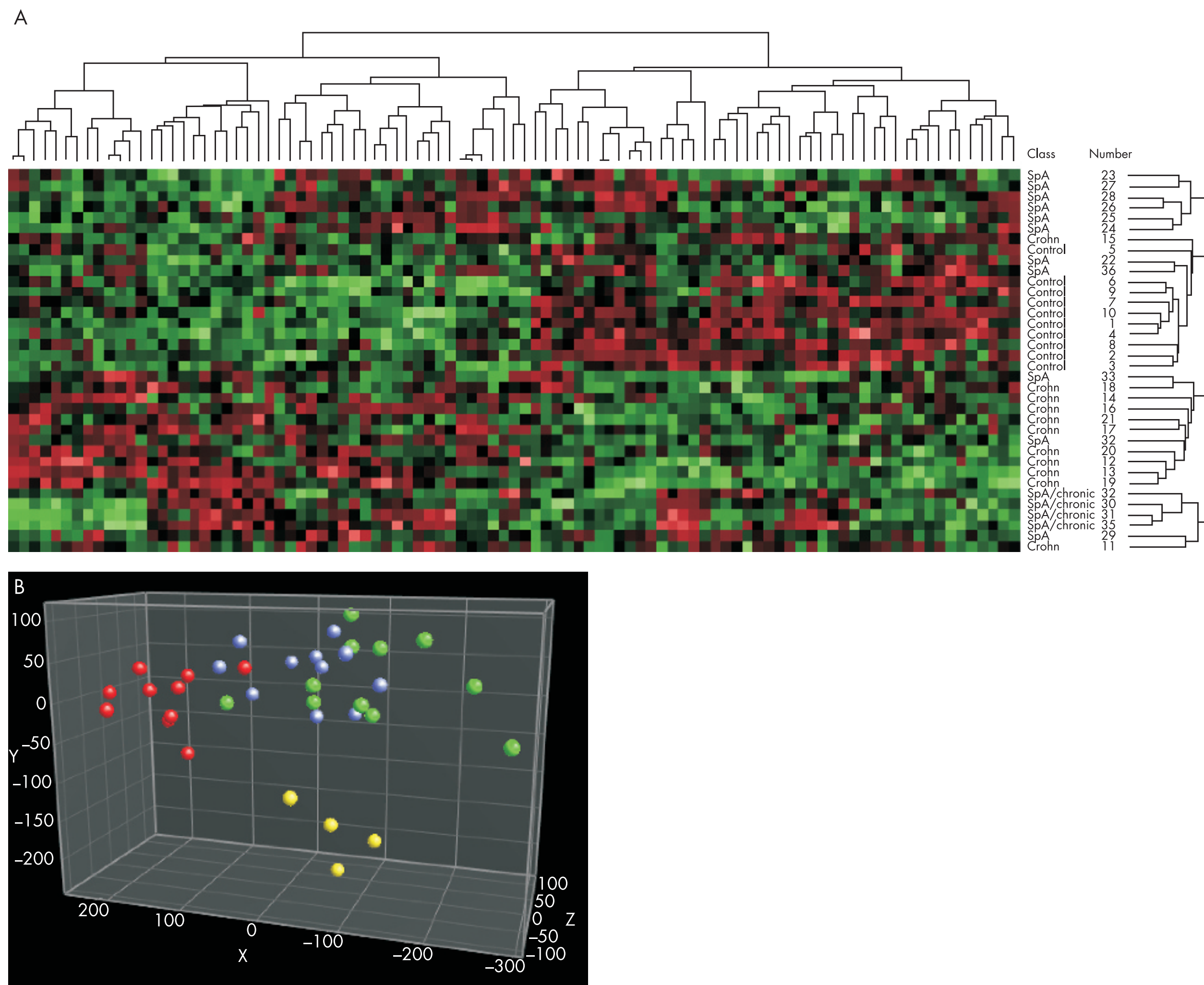

Figure 2 (A) Complete linkage clustering based on a set of 95 genes whose expression is deviant in patients with Crohn's disease (Crohn) and in those with spondyloarthropathy (SpA) and chronic gut inflammation as compared with healthy controls. Two main clusters mark an SpA–control cluster and a Crohn's disease–SpA with chronic inflammation cluster. (B) Principal component analysis view with a set of 95 genes whose expression is deviant in patients with Crohn's disease and in those with SpA and chronic gut inflammation. Crohn's disease (green circles), SpA without chronic inflammation (blue circles), SpA with chronic inflammation (yellow circles) and controls (red circles).

pick up genes whose differential expression is a consequence and not a cause of the disease. Additionally, looking at basal gene expression may enable us to consider genetic influences, as gene expression is highly heritable.[24] Therefore, future studies on markers for Crohn's disease should concentrate primarily on genes that are located near one of the known loci for Crohn's disease (table 2). Genes located within a region linked to Crohn's disease or IBD in general (if multipoint linkage was carried out), or within 5 cM of the markers that are linked to Crohn's disease or IBD (in case of two-point linkage), should be considered first. By using a model for early Crohn's disease when identifying Crohn's disease susceptibility genes, investigators can circumvent the heterogeneity of the disease, as only a small number of Crohn's disease genes will be implicated in patients with SpA.

Array analysis is a procedure for studying the expression of many genes in a limited number of samples. As the number of genes explored is usually large, this can lead to false-positive results. Nevertheless, array analysis enables us to explore gene expression with different computational tools. To confirm the importance of a set of genes associated with a phenotype, complementary techniques such as quantitative reverse transcriptase PCR are mandatory. Thus, arrays are not simply a way to find single differentially regulated genes; they can be used to compare global gene expression in distinct groups.

We show that patients with SpA have an aberrant gene expression profile when compared with healthy controls, indicating that changes in gene expression in the colon of patients with SpA is a biologically relevant concept. We identified a set of genes that are differentially expressed in both patients with Crohn's disease and those with SpA who are at higher risk of developing Crohn's disease. On the basis of the expression of these 95 genes, patients with SpA having subclinical chronic gut inflammation cluster with patients with Crohn's disease, confirming the clinical association between the two inflammatory disorders. We suggest a number of candidate genes for mutation screening. We are currently verifying a selection of genes by quantitative reverse transcriptase PCR and exploring the association of genes that are differentially expressed in patients with Crohn's disease and in those with SpA with a history of chronic gut inflammation, to find early (genetic) markers for Crohn's disease in patients with SpA.

Table 2 Ninety five ESTs that cluster patients with Crohn's disease and those with SpA and chronic gut inflammation

Accession	Unigene	Symbol	Gene description	Cytogenetic location	Expression in CD/SpA chronic	Marker	Score	Ref.
H81904	Hs.14779	ACAS2	Acetyl-coenzyme A synthetase 2 (ADP forming)	20q11.22	↓			
AI758270					↓			
R20596	Hs.101359	C6orf32	Chromosome 6 open reading frame 32	6p22.3–p21.32		D6S2439	LOD 2.6	Barmada *et al*[25]
AI349525	Hs.121692	FLJ34790	Hypothetical protein FLJ34790	17p13.1	↓			
AI445844	Hs.444422	PDK3	Pyruvate dehydrogenase kinase, 3	Xq22	↓	DXS1226-DXS1214	LOD 2.0	van Heel *et al*[26]
H56656	Hs.282958	FLJ13611		5q12.3	↓			
N22829	Hs.299055	GDI2	GDP dissociation inhibitor 2	10p15	↓			
AI274555	Hs.170568	TATDN1	TatD DNase domain containing	8q24.13		D8S198	NPL 2.47	Vermeire *et al*[27]
H61436	Hs.184668	SNX24	Sorting nexin 24	5q23.2				
H15751	Hs.30634	PARP16	Poly (ADP-ribose) polymerase family, member 16	15q22.31	↓			
H87107	Hs.216354	RNF5	Ring finger protein 5	6p21.3	↓	D6S1281-D6S1019	LOD 2.3	Rioux *et al*[28]
AI680066								
AI191504	Hs.510373	AK7	Adenylate kinase 7	14q32.2	↓			
R17390	Hs.80132	SNX15	Sorting nexin 15	11q12				
AA812701	Hs.343631	LOC340178	Hypothetical protein LOC340178	6q27				
AA825971	Hs.209561	KIAA1715		2q31				
AA291593	Hs.3402	SLC39A11	Solute carrier family 39 (metal ion transporter) member 11	17q24.3–q25.1				
	Hs.118836	MB	Myoglobin	22q13.1		D22S689	LOD 1.5	Barmada *et al*[25]
AA420968	Hs.98323	C6orf112	Chromosome 6 open reading frame 112	6q21				
R08643	Hs.350268	IRF2BP2	Interferon regulatory factor 2 binding protein 2	1q42.3				
T83584	Hs.115945	MANBA	Mannosidase, βA, lysosomal	4q22–q25		D4S406	NPL 2.17	Vermeire *et al*[27]
AA481482	Hs.67928	ELF3	E74-like factor 3 (ets domain transcription factor, epithelial specific)	1q32.2				
T78477	Hs.206770	ZNF297	Zinc finger protein 297	6p21.3		D6S121-D6S1019	LOD 2.3	Rioux *et al*[28]
H27674	Hs.238465	DAB2IP	DAB2-interacting protein	9q33.1–q33.3				
R13194	Hs.32935	BRF1	BRF1 homologue, subunit of RNA polymerase III transcription initiation factor IIIB	14q				
R98758	Hs.380474	FLJ32731	Hypothetical protein FLJ32731	8p11.1				
H96900	Hs.356467	MGC2747	MGC2747	19p13.11				
N62199	Hs.375193	KIF1B	Kinesin family member 1B	1p36.2	↓	D1S1597	LOD 3.01	Cho *et al*[29]
R51052	Hs.296169	SPHAR	S-phase response (cyclin-related)	1q42.11–q42.3	↓			
AI286348	Hs.443728	SH3RF2	SH3 domain containing ring finger 2	5q32				
R17478	Hs.497159	PIG13	Proliferation-inducing protein 13	1q25				
AI916359	Hs.205842	ATP2C2	Secretory pathway calcium ATPase 2	16q24.1				
H19034	Hs.5300	BLCAP	Bladder cancer-associated protein	20q11.2–q12				
N39050	Hs.93825	FLJ39784 fis	*Homo sapiens* cDNA FLJ39784 fis, clone SPLEN2002314					
R13544	Hs.7540	FBXL3A	F-box and leucine-rich repeat protein 3A	13q22	↓			
AI286163	Hs.122428				↓			
R07321	Hs.464137	ACOX1	Acyl-coenzyme A oxidase 1, palmitoyl	17q24–17q25	↓			
R12168	Hs.96870	STAU2	Staufen, RNA-binding protein, homologue 2 (*Drosophila*)	8q13–q21.1	↓			
H85472	Hs.75789	NDRG1	N-myc downstream-regulated gene 1	8q24.3	↓			
AA513663					↓			
R97820	Hs.325568	ZC3HAV1	Zinc finger CCCH type, antiviral 1	7q34	↓			
N62837	Hs.48647	ILT7	Leucocyte Ig-like receptor, subfamily A (without TM domain), 4	19q13.4	↓			
AA019615	Hs.145469	UCHL5	Ubiquitin carboxyl-terminal hydrolase L5	1q32	↓			
AI809092					↓			
AA758064	Hs.401400							
AI219353	Hs.89387	CASC2	Cancer susceptibility candidate 2	10q26.11	↓			
AI698801	Hs.202187							
R12632	Hs.109706	HN1	Haematological and neurological expressed 1	17q25.1				
AA468418								
	Hs.28777	HIST1H2AC	Histone 1, H2ac	6p21.3		D6S2439	LOD 2.6	Barmada *et al*[25]
H23734	Hs.522584	TMSB4X	Thymosin, β4, X linked	Xq21.3–q22				

Table 2 Continued

Accession	Unigene	Symbol	Gene description	Cytogenetic location	Expression in CD/SpA chronic	Marker	Score	Ref.
H75688	Hs.197015	SNX7	Sorting nexin 7	1p21.3				
N63669	Hs.81541	TRIO	Triple functional domain (PTPRF interacting)	5p15.1–p14				
AI819016					↑			
AI831827	Hs.211194	ARFGEF2	ADP-ribosylation factor guanine nucleotide exchange factor 2	20q13.13				
AI923299	Hs.213456	LOC158730	Hypothetical LOC158730	Xp21.1	↑			
AI286257	Hs.124553	ZNF263	Zinc finger protein 263	16p13.3				
AI863417	Hs.208726	RUNX1	Runt-related transcription factor 1 (acute myeloid leukaemia 1; aml1 oncogene)	21q22.3	↑			
AI921132								
AI274438					↑			
AI289775	Hs.387091		EST, weakly similar to 0412263A Ig G2 pFc′ PIG Gm		↑			
AI345173								
AI928384								
AA490519	Hs.16165		CDNA clone IMAGE:5286005					
AI696951	Hs.355460		CDNA: FLJ21763 fis					
AI339536	Hs.348436		*Homo sapiens*, clone IMAGE:5286812, mRNA					
AI245190								
AI261428	Hs.145523							
AI809310	Hs.210385	HERC1	Hect domain and RCC1 (CHC1)-like domain 1	15q22				
AI831536	Hs.413608	IL12RB2	Interleukin 12 receptor, β2	1p31.3–p31.2				
T79944					↑			
AA480677	Hs.161954		*Homo sapiens*, clone IMAGE:4865533		↑			
AA009461	Hs.172210	MUF1	MUF1 protein	1p34.1	↑	D1S197	NPL 2.07	Vermeire *et al*[27]
R61783	Hs.5097	SYNGR2	Synaptogyrin 2	17q25.3	↑			
AI282992	Hs.2704	GPX2	Glutathione peroxidase 2 (gastrointestinal)	14q24.1	↑			
W65310								
AA588676	Hs.162575	PDLIM3	PDZ and LIM domain 3	10q22.3–q23.2				
AI811147					↑			
AI357718								
N58523	Hs.137274							
W74496					↑			
AI352320					↑			
AI280708	Hs.7155	LOC129607	Thymidylate kinase family LPS-inducible member	2p25.2				
AI815599	Hs.90744	PSMD11	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	17q11.2				
AI718161								
N98921	Hs.85844	TPM3	Tropomyosin 3	1q21.2		D1S305	NPL 2.97	Vermeire *et al*[27]
AI367201	Hs.127496	HSPC135	HSPC135 protein	3q13.2				
AA425545	Hs.104613	RP42	RP42 homologue	3q26.3				
H44213	Hs.374986		Hypothetical gene supported by BC031266					
N58936	Hs.339283	NCOA7	Nuclear receptor coactivator 7	6q22.32				
N90208	Hs.344478	FLJ32440	Hypothetical protein FLJ32440	8q24.13		D8S198	NPL 2.47	Vermeire *et al*[27]
AA427403	Hs.366	IFITM1	Interferon-induced transmembrane protein 1 (9-27)	11p15.5				
R25648	Hs.23920	PI4K2B	Phosphatidylinositol 4-kinase type IIβ	4p15.2				
N93265	Hs.75428	SOD1	Superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	21q22.11				
H99865	Hs.91954	NR2F2	Nuclear receptor subfamily 2, group F, member 2	15q26				

BRF1, butyrate response factor 1; CD, Crohn's disease; Zo1, zonula occludens-1; DAB2, disabled homolog 2; Dlg, discs-large protein; EST, expressed sequence tag; Ig, immunoglobulin; LIM, Lin-11, Is1-1, Mec-3; LOD, logarithm of the ratio of the odds that two loci are linked; LPS, lipopolysaccharide; NPL, non-parametric LOD score; PSD, postsynaptic density protein; SpA, spondyloarthritis.
Genes with aberrant expression in patients with Crohn's disease and in those with SpA and chronic gut inflammation (↓ down regulated and ↑ up regulated as compared with the controls and those with SpA; $p<0.05$).
Genetic markers are cited for genes that are located within or near (5 cM) one of the Crohn's disease locus, together with the score supporting linkage of the loci.

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