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MAGI2 genetic variation and inflammatory bowel disease (IBD)

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Abstract

Despite recent advances the majority of IBD susceptibility ‘genes’ remain undiscovered. Recent data suggest that autoimmune conditions may ‘share’ susceptibility loci. Epidemiological evidence indicate an association between celiac disease and IBD and both conditions demonstrate increased gut permeability. *MAGI2*, recently implicated in UC and celiac disease, encodes a scaffolding protein involved in epithelial integrity. Our aim was to test *MAGI2* variants for association with IBD and also their role in determining intermediate hereditary phenotypes defined by antibody production to microbial antigens. We genotyped 113 *MAGI2* SNPs in 681 cases of Crohn’s disease (CD), 259 ulcerative colitis (UC) cases and 195 controls. The most significant IBD association was in intron 6 (rs2160322, $p=0.009$) and both UC ($p=0.006$) and CD ($p=0.03$) contributed to this association. The most significant CD association was with an intron 2 haplotype (rs7785088/rs323149/rs13246026, $p=0.002$). We observed highly significant associations with UC in intron 6 (rs7803276/rs7803705, $p=0.002$) and also significant associations in introns 2, 6 and 20. Significant associations were seen with: IgG ASCA positive CD in intron 3 ($p=0.003$), intron 6 ($p=0.003$), and intron 20 ($p=0.001$); anti-CBir1 positive CD in intron 3 ($p=0.0001$) and intron 6 ($p=0.008$); and anti-OMPc positive CD in intron 3 ($p=0.0009$), and intron 9 ($p=0.007$). Quantitative antibody levels were also associated with variants in intron 4 (anti-IgA ASCA, $p=0.0003$ and anti-IgG ASCA, $p=0.0002$). These findings support the significance of the epithelial barrier in IBD pathogenesis.

Keywords

Inflammatory bowel disease; *MAGI2*; genetic susceptibility; serology

INTRODUCTION

It is clear from both genetic and clinical perspectives that the inflammatory bowel diseases (IBD) in general and Crohn’s disease (CD) in particular are heterogenous conditions. Early successes in the field of IBD genetics implicated genetic variants that alter innate immunity responses in IBD pathogenesis (1,2). More recently, through utilizing the technological advances of genome-wide association scans (GWAS), novel ‘pathways’ have been implicated in disease pathogenesis including autophagy(3–5) and the IL23/Th17(6) (7) pathway. Intriguingly recently published data have suggested that a number of autoimmune conditions may ‘share’ genetic susceptibility loci such as the gene encoding the interleukin-23 receptor (*L23R*), which has not only been implicated in IBD pathogenesis (6)

but also in ankylosing spondylitis(8) and psoriasis(9). These findings support earlier genetic data suggesting that there is likely to be a 'shared' genetic predisposition to some autoimmune conditions(10,11) with the exact phenotype being modulated through some other mechanism such as phenotype determining genes and/or environmental factors.

In searching for disease genes one can investigate the relationship with clinical disease and with intermediate phenotypes. Serological immunophenotypes defined by antibody production to microbial antigens (e.g. antibodies to oligomannan, anti-Saccharomyces Cerevisiae (ASCA),(both IgG and IgA), the Pseudomonas fluorescens-related protein (anti-I2), Escherichia Coli outer membrane porin C (anti-OMPC) and CBir1 flagellin (anti-CBir1)) are heritable quantitative traits in IBD(11–14). Serotypes are associated with distinct phenotypes and with a more severe course of CD(15,16) and it is also recognized that more severe phenotypes of genetically complex diseases may contain individuals with a greater genetic contribution. The data that demonstrate that individuals who have a greater reactivity to microbial antigens and have a more severe course of disease carry more *NOD2* CD associated genetic variants supports this hypothesis(17). These data also provide support for the association between a defect in innate immunity/bacterial handling and reactivity to microbial antigens.

Despite the powerful nature of the GWASs, it is clear that these studies have not yet identified even the majority of susceptibility alleles for CD, and other genetic approaches are still warranted. Alternative approaches include; the investigation of susceptibility alleles identified from related conditions or diseases; the study of more homogenous IBD groups defined by demographic or clinical phenotypes (e.g. early age of disease onset, more severe disease etc) or immunophenotypes (as discussed above); the study of candidate genes under peaks of linkage; the investigation of human genes implicated from animal models of mucosal inflammation; and the investigation of implicated pathways that contribute to the pathophysiology of IBD. Previously published data from both 'functional' (18) and genetic studies(19,20) have implicated epithelial barrier disruption as a risk factor for developing IBD, and epidemiological evidence suggests an association between IBD and celiac disease(21). In both disorders there is evidence of enhanced intestinal permeability(20,22,23) and in CD increased intestinal permeability is apparent in unaffected relatives of people with CD suggesting a primary genetic etiology to this permeability(24,25). Epithelial barrier integrity is a primary mode of innate defense against commensal bacteria.

Recently an association between a variant in the *MYO9B* gene that encodes an epithelial scaffolding protein, and the risk of both celiac disease and ulcerative colitis has gone some way to explain this epidemiological association between gluten sensitivity and IBD(26,27). A further study by Wapenaar et al described an association between variants within the gene encoding membrane associated guanylate kinase, WW and PDZ containing protein 2 (*MAGI2*) and celiac disease(28). The study also describes a borderline association between a single *MAGI2* SNP and UC, but no association was seen between *MAGI2* and Crohn's disease (CD) (28).

MAGI2 is a large gene of approximately 1.4 megabases consisting of 21 exons that encode a protein of 2410 amino acids. *MAGI2* is located on chromosome 7 in a genetic region implicated by linkage analyses as harboring susceptibility gene(s) for IBD(29). The aim of our study was to further investigate the role that genetic variants in the tight junction pathway gene *MAGI2* may have in the development of CD and UC and also to responses to CD related microbial antigens.

MATERIALS AND METHODS

Subjects

Six hundred and eighty-one CD cases, 259 UC cases and 195 control subjects were included in the study. IBD phenotype was assigned using a combination of standard endoscopic, histological, and radiographic features (15). Controls were included in the study that had no personal or family history of IBD. All study subjects were Caucasian.

Selection of SNPs

The single nucleotide polymorphisms (SNPs) for genotyping were selected using data from the Caucasian data of the International 'HapMap' (30–32) and through utilizing the "Tagger" software program (33). SNPs that were shown to tag the major Caucasian haplotypes and that were also compatible with Illumina technology were genotyped. Our aim was to identify SNPs in linkage disequilibrium with all SNPs in the HapMap data with a minor allele frequency $\geq 5\%$. One hundred and thirteen SNPs were included in the study and HAPLOVIEW predicted that these SNPs formed 19 haplotypes.

Genotyping

DNA was extracted from Epstein Barr virus transformed lymphoblastoid cell lines using a standard technique of proteinase K digestion, organic extraction, and ethanol precipitation (34). The SNPs were genotyped using the validated oligonucleotide ligation assay, Illumina Golden Gate technology (35) (Illumina, San Diego, CA). The full list of 113 SNPs genotyped in this study is listed in supplementary Table 1.

IBD Serological status

Sera were analyzed at Cedars-Sinai Medical Center for expression of antibodies to oligomannan (anti-Saccharomyces Cerevisiae (ASCA), both IgG and IgA), the Pseudomonas fluorescens-related protein (anti-I2), Escherichia Coli outer membrane porin C (anti-OMPC), and CBir1 flagellin (anti-CBir1) in a blinded fashion by enzyme-linked immunosorbent assay (ELISA), as previously described (15,16). Any antibody level determined as equal to or more than 2 standard deviations above the population mean was designated as positive. In addition, since seroactivity to microbial antigens is a quantitative trait, the antibody level was assessed 'across' each genotype (e.g. homozygote for common allele versus heterozygote versus homozygote for rare allele) using linear regression (see statistical methods).

Statistical Analyses

Case control analyses with the Chi squared test (Haploview v4 (31)) were used to test for association with any given phenotype. The p values for association with any phenotype have been represented graphically by calculating the Logarithm of the inverted P value ($1/P$). All p values reported are two-tailed p values and are not corrected for multiple testing. However, we have calculated the number of expected results to have occurred by chance and those actually observed positive associations for a cut-off p value of ≤ 0.01 (see results).

We used Haploview v4 (31) to determine haplotype blocks using the Gabriel et al confidence interval method (36). Association with IBD phenotypes and immunophenotypes with all haplotypes was determined within Haploview using the Chi squared test. Association with quantitative values of the IBD serologies was calculated in PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>) (37) using linear regression. SNPs were excluded from the analysis if they failed to meet Hardy-Weinberg equilibrium ($p \leq 0.01$). Since MAGI2 is a large gene, with many SNPs and haplotypes, we have conferred each SNP

or haplotype a 'locus', so that relationships regarding disease and intermediate phenotypes can be compared (see results section and tables).

ETHICAL CONSIDERATIONS

Subjects and controls were recruited at Cedars-Sinai Medical Center following approval of the Cedars-Sinai Medical Center Institutional Review Board. Blood samples for serological and genetic analyses were drawn following informed consent.

Results

We genotyped 113 SNPs across the *MAGI2* gene in 681 CD cases, 259 UC cases and 195 control subjects. One *MAGI2* SNP was excluded from the analyses as it failed to meet our criteria for Hardy-Weinberg equilibrium. These SNPs generate 19 haplotype blocks. Overall we observed 34 positive associations with disease and/or with intermediate phenotype at a *p* value of 0.01 or less, by chance we should have observed 15 associations with this statistical cut-off. Thus 19 of the associations were likely to be true positives.

IBD, UC and CD and *MAGI2*—The association between the *MAGI2* SNPs and haplotypes with IBD, CD and UC are demonstrated graphically in figure 1a and listed in full in table 1. A three marker intron 2 *MAGI2* haplotype (*rs7785088*, *rs323149* and *rs13246026*, *MAGI2* locus 1) was protective against IBD susceptibility (IBD haplotype frequency 8.2% vs. control haplotype frequency 12.9%, OR 0.61 (95% CI 0.44–0.86, *p* = 0.006). CD was the main contributor to this association (OR 0.57, CI 0.40 – 0.81, *p* = 0.002) with a trend towards association in UC with this haplotype (*p* = 0.11). CD was also associated with alleles in introns 2, 5, 6 and 20 (table 1). An intron 6 SNP (*rs2160322*, 'locus' 4) was significantly associated with UC, with the common C allele being the risk allele (UC 72.2% vs control allele frequency 63.6% (OR 1.49, 1.12 – 1.97, *p* = 0.006). Two further SNPs (*rs7788384*, 'locus' 5, and *rs2110871*, 'locus' 9) and a 2 marker haplotype (*rs7803276/rs7803705*, 'locus' 8) from intron 6 were associated with UC susceptibility. Intron 20 (*p* = 0.03, 'locus' 10) and intron 2 (*p* = 0.02, 'locus' 7) SNPs were also associated with UC (table 1 and figure 1A).

IBD serotype and *MAGI2*—Since serotypes have been shown to define particular IBD phenotypes, are heritable traits, and have been associated with genetic variation in innate immunity(17) we hypothesized that CD specific microbial antibody responses could be related to epithelial barrier defects. A number of *MAGI2* alleles were associated with CD immunophenotypes; these are listed in table 2 and demonstrated in Figure 1.b and 1.c. Anti-CBir1 positive CD was most highly associated with an intron 3 SNP (*T* allele of *rs10239917*, *P* = 0.0002, locus 12) and a 2 marker haplotype constructed from this SNP (*rs10239917*) and *rs11773635* produces both risk a haplotype (allele *TA*, *P* = 0.0002) and a protective haplotype (allele *CA*, *P* = 0.0001) for anti-CBir1 positive CD. IgG ASCA positive CD is associated with 2 separate haplotype blocks within intron 6. The *GC rs7803705/rs7803276* haplotype (locus 8) is protective for IgG ASCA positive CD (*P* = 0.003), and the *rs6951193/rs759332* haplotype (locus 15) demonstrates both risk alleles (*AA*, *P* = 0.01) and protective alleles (*GA*, *P* = 0.009) for IgG ASCA positive CD. Anti-OMPC positive CD is associated with the *rs11773635* SNP in intron 3 (locus 13, OR 1.56, CI 1.20 – 2.04, *P* = 0.0009) with no fewer than 4 separate SNPs in intron 9 (loci 23, 25, 26, 27)(*rs798285*, *P* = 0.007; *rs798287*, *P* = 0.008; *rs798292*, *P* = 0.01 and *rs798279*, *P* = 0.02), as well as an intronic 4 SNP (locus 24, *rs725555*, *P* = 0.001).

A number of studies have demonstrated that increasing numbers of seropositivity for antibodies to microbial antigens are associated with a more severe course of disease in CD (reviewed in(17)). In addition, combinations of seropositivity may create yet more

genetically homogenous groups for testing association. The *T* allele of *rs10239917* (intron 3, locus 12) is associated with both anti-CBir1 and IgG ASCA positive CD ($P = 0.000038$) when compared to those not positive for both serotypes. Similarly the intron 3 two marker haplotype (*rs10239917/rs11773635*, locus 12) shows both a risk (CC, $P = 0.0055$) and protective haplotype (CA, $P = 0.0008$) with anti-CBir1 and anti-OMPc positive CD.

Quantitative data—Antibody levels were analyzed as a quantitative trait using linear regression and the positive associations are shown in table 3 and in figure 1.d.. Interestingly, the *T* allele of *rs10239917* (locus 12, see above) in intron 3 is also associated with IgG ASCA level.

Discussion

Using a haplotype tagging approach, an efficient method of investigating such a large gene, we have identified associations between variants within *MAGI2* and IBD, UC and CD, as well as with immunophenotypes defined by seroreactivity to microbial antigens. The findings presented here support, by extension, previous work that identified a weak association with *MAGI2* and UC(28) but also identify novel associations with CD. In the previous study a ‘targeted’ approach to the *MAGI2* gene was adopted given its size. The investigators tagged SNPs located in the exons and exon-intron boundaries with a mean allele frequency greater than 20%. The single *MAGI2* marker (*rs6962966*, intron 14) that demonstrated association with CD in the paper by Wapenaar et al was not included in our list of tagging SNPs as we had devised this list prior to the publication of the Wapenaar et al paper. Not one of the SNPs that we have demonstrated to be associated with IBD were tested in the Dutch IBD collection and none of the SNPs we tested showed strong LD with *rs6962966* ($D' < 0.50$).

Given the approach we have taken to this large gene it is unlikely that we have identified actual ‘causative’ polymorphisms and are more likely to have identified the SNPs that are in linkage disequilibrium (LD) with the disease and antibody phenotype related polymorphisms. This may be particularly true as, with one exception (*rs3735442*, synonymous exon 6 SNP, locus 20), the SNPs associated with disease or phenotype are all located within introns. However it is increasingly recognized that many disease associated SNPs may be intronic or even occur at regulatory sites that are thought to be in ‘gene deserts’(38). One IBD GWAS utilized a genome-wide study of global gene expression (39) to demonstrate that a SNP in a gene desert on chromosome 5p that was associated with CD susceptibility was also associated with quantitative expression of the prostaglandin receptor EP4, PTGER4(38). Utilizing the same expression database(39), we have found that two of the disease associated *MAGI2* SNPs are in LD with SNPs associated with changes in *MAGI2* gene expression (the intron 3 SNP *rs17437602*, locus 14) is in LD with *rs10485909* ($D' = 0.58$) which is associated with *MAGI2* quantitative gene expression levels ($P = 0.0009$); the intron 20 SNP *rs4727608* (locus 16) is in LD with *rs2471597* ($D' = 0.60$) which is associated with *MAGI2* quantitative gene expression levels ($P = 0.001$). Furthermore it can be seen that the associations with both CD and UC as well as with disease associated immunophenotypes demonstrated in this study cluster in regions such as intron 3, intron 6 and intron 20. The stronger association with haplotypes than individual SNPs, as seen with locus 1 and CD, locus 8 and UC, and locus 15 and CBir1 positive CD, is further evidence that we have ‘tagged’ risk or protective haplotypes rather than ‘causative’ SNPs suggesting that further fine-mapping and or sequencing targeted at individuals carrying the disease associated haplotypes is warranted.

It is recognized that there is impaired gut barrier function in IBD(20,22,25) and increased epithelial permeability may result in a break down in the first line of defense against

commensal bacteria by allowing increased translocation of bacteria from the gut lumen. This coupled with other 'permissive' genetic variants could lead to an increase in innate and adaptive immune responses to disease related microbial antigens. *MAGI2* encodes a scaffolding protein that is involved in epithelial tight junction assembly(40) and hence a defect in *MAGI2* expression or function may explain the association between *MAGI2* SNPs and antibodies to microbial antigens. The associations with immunophenotypic sub-groups are particularly strong, a finding that reinforces the approach of using homogenous groups based on physiological intermediate phenotypes and not just clinical and demographic parameters as a powerful method for detecting disease associations as has recently been demonstrated in identifying a novel asthma susceptibility locus(41).

The previous association between CD and genetic variation within *DLG5*(19) had already implicated genetic variants within scaffold proteins involved in epithelial integrity as important molecules in IBD pathogenesis. The scaffolding proteins enable proteins to 'come together' thereby allowing them to interact. *MAGI2* is known to contain nine potential protein-protein interaction domains including a Guk (guanylate kinase-like) domain, two WW domains (domains that mediate protein-protein interaction through recognizing proline-rich peptide motifs and phosphorylated serine/threonine-proline sites(42)) and six PDZ domains (modular protein interaction domains that bind in a sequence-specific manner to short C-terminal peptides). In addition to its effect on epithelial integrity, *MAGI2* has been demonstrated to inhibit cell migration and proliferation via PTEN(phosphatase and tensin homolog)(43). PTEN is a key regulator of cell growth and apoptosis and PTEN protein stability is increased following binding to *MAGI2*(44). Defects in apoptosis have been implicated in IBD pathogenesis(45).

While the demonstrated *MAGI2* associations presented in this paper have not been adjusted by a conservative Bonferroni correction for multiple testing given the large number of SNPs needed to 'tag' this gene, we would propose that the excess of significant associations (i.e. more than double than would be expected), the previous finding of association with UC and celiac disease, and the function of the *MAGI2* protein as an epithelial scaffolding molecule, as well as the genetic location of the gene, make a convincing argument for the role of *MAGI2* variants in both UC and CD pathogenesis. These findings also support the emerging hypothesis of the pleiotropic effects of some genes in predisposing to a number of autoimmune conditions. There is an increased risk of celiac disease in people with type 1 diabetes(46), and interestingly a recent study has suggested that not only is there increased intestinal permeability in type 1 diabetes but this abnormality precedes the development of disease onset(47). These data suggest that *MAGI2*'s role in IBD pathogenesis and other autoimmune conditions should be further investigated and future studies should concentrate on fine-mapping the gene, although this will be a considerable body of work given that *MAGI2* is approximately 1.4MB in length. Investigation on the expression and function of *MAGI2* in IBD should also be considered, the results of which would give us further insight into the underlying processes that lead to chronic gastrointestinal inflammation. Furthermore therapeutic strategies that can reverse increased intestinal permeability may restrict antigen presentation to a genetically 'primed' immune system thereby 'preventing' the development of conditions such as IBD and celiac disease.

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Abbreviations

CD	Crohn's disease
GWAS	genome wide association study
IBD	inflammatory bowel disease
MAGI2	membrane associated guany late kinase, WW and PDZ domain containing 2 (dbGENE id 9863)
OR	odds ratio
CI	confidence intervals
SNP	single nucleotide polymorphism
ASCA	Anti-Saccharomyces cerevisiae antibodies
IgG	immunoglobulin G
IgA	immunoglobulin A
anti-I2	antibody to Pseudomonas fluorescens-related protein
anti-OmpC	antibody to Escherichia Coli outer membrane porin C
anti-CBir1	antibody to CBir1 flagellin
ELISA	enzyme-linked immunosorbent assay

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Figure 1. Graphical representation of the association between MAGI2 and IBD and IBD phenotypes

1a. Association between MAGI2 SNPs and haplotypes and IBD, UC and CD. 1b. Association between MAGI2 SNPs and haplotypes and IBD immunophenotypes defined by anti-microbial serology. 1c. Association between MAGI2 SNPs and haplotypes and IBD immunophenotypes defined by anti-microbial serology from intron 3 to intron 6. 1d. Association between MAGI2 SNPs and quantitative values of anti-microbial serologies. The $\times$ axis represents a 'true' scale of genetic distance with the start and finish of the gene marked by blue diamonds. The gene is represented in a graphic form at the top of the individual diagrams (not to scale). The y axis demonstrates the degree of statistical association in a logarithmic scale through calculating the $\text{LOG}_{10} 1/p$ value where the p value has been calculated from the Chi squared test used to test association with *MAGI2* alleles. The red dotted lines represent degrees of statistical significance: a – $p = 0.05$; b – $p = 0.005$; c – $p = 0.0005$; and d – $p = 0.00005$

Table 1
Association between *MAGI2* SNPs and haplotypes and IBD, UC and CD

The *MAGI2* locus column identifies loci across the gene to enable the reader an easier method of cross-referencing across the tables.

<i>MAGI2</i> SNP or Haplotype	<i>MAGI2</i> locus	'Risk' allele	Gene location (intron etc)	Phenotype	'Disease' allele Hz	Control allele Hz	OR	95% CI	P value
<i>rs7785088/rs323149/rs13246026</i>	1	GTC	Intron 2	IBD	8.2%	12.9%	0.61	0.44 – 0.86	0.006
<i>rs10242163/rs10808167/rs1207881/rs10264296</i>	2	GGGC	Intron 2	IBD	3.5%	5.9%	0.59	0.36 – 0.96	0.04
<i>rs10260232</i>	3	T	Intron 5	IBD	55.9%	50.3%	1.25	1.01 – 1.56	0.05
<i>rs2160322</i>	4	C	Intron 6	IBD	70.3%	63.6%	1.35	1.08 – 1.70	0.009
<i>rs7788384</i>	5	G	Intron 6	IBD	61.7%	56.2%	1.26	1.01 – 1.57	0.05
<i>rs7785088/rs323149/rs13246026</i>	1	GTC	Intron 2	CD	7.7%	12.9%	0.57	0.40 – 0.81	0.002
<i>rs10242163/rs10808167/rs1207881/rs10264296</i>	2	GGGC	Intron 2	CD	3.1%	5.9%	0.51	0.30 – 0.85	0.01
<i>rs1026023</i>	3	T	Intron 5	CD	56.1%	50.3%	1.27	1.01 – 1.58	0.04
<i>rs2160322</i>	4	C	Intron 6	CD	69.6%	63.6%	1.31	1.03 – 1.66	0.03
<i>rs3807736</i>	6	T	Intron 20	CD	56.3%	50.3%	1.28	1.02 – 1.60	0.04
<i>rs7799287</i>	7	C	Intron 2	UC	39.8%	32.1%	1.40	1.06 – 1.84	0.02
<i>rs17151725/rs7799287</i>	7	GC	Intron 2	UC	39.8%	32.1%	1.40	1.06 – 1.84	0.02
<i>rs7803276/rs7803705</i>	8	GC	Intron 6	UC	58.0%	50.2%	1.37	1.05 – 1.78	0.02
<i>rs7803276/rs7803705</i>	8	AG	Intron 6	UC	21.5%	30.4%	0.62	0.46 – 0.84	0.002
<i>rs2160322</i>	4	C	Intron 6	UC	72.2%	63.6%	1.49	1.12 – 1.97	0.006
<i>rs7788384</i>	5	G	Intron 6	UC	64.3%	56.2%	1.40	1.07 – 1.84	0.01
<i>rs2110871</i>	9	C	Intron 6	UC	73.7%	66.7%	1.40	1.05 – 1.88	0.02
<i>rs7791394</i>	10	G	Intron 20	UC	57.5%	50.0%	1.35	1.04 – 1.76	0.03

IBD – inflammatory bowel disease, CD – Crohn's disease, UC – ulcerative colitis, Hz – frequency, OR – odds ratio, CI – confidence intervals.

Table 2
Association between MAGI2 SNPs and haplotypes and CD associated immunophenotype

The MAGI2 locus column identifies loci across the gene for ease of cross-referencing.

MAGI2 SNP or Haplotype	MAGI2 locus	'Risk' allele	Gene location (intron etc)	Phenotype	Case allele Hz	Control allele Hz	OR	95% CI	P value
rs7791394	10	C	Intron 20	anti-ASCA+ve CD ¹	57.6%	50.6%	1.32	1.07 – 1.64	0.01
rs38121	11	C	Intron 2	anti-IgG ASCA +ve CD ²	72.2%	66.2%	1.35	1.07 – 1.70	0.02
rs10239917	12	T	Intron 3	anti-IgG ASCA +ve CD ²	44.2%	36.3%	1.39	1.12 – 1.73	0.003
rs319870	13	A	Intron 3	anti-IgG ASCA +ve CD ²	80.1%	74.7%	1.36	1.05 – 1.76	0.02
rs17437602	14	T	Intron 3	anti-IgG ASCA +ve CD ²	70.1%	64.4%	1.30	1.03 – 1.63	0.03
rs10239917/rs1773635	13	TA	Intron 3	anti-IgG ASCA +ve CD ²	44.2%	36.3%	1.39	1.12 – 1.73	0.003
rs7803705	8	A	Intron 6	anti-IgG ASCA +ve CD ²	74.2%	66.7%	1.43	1.13 – 1.82	0.003
rs6951193	15	A	Intron 6	anti-IgG ASCA +ve CD ²	72.2%	65.8%	1.35	1.07 – 1.71	0.01
rs7803276	8	A	Intron 6	anti-IgG ASCA +ve CD ²	50.7%	44.2%	1.30	1.05 – 1.61	0.02
rs7803705/rs7803276	8	GC	Intron 6	anti-IgG ASCA +ve CD ²	25.7%	33.3%	0.69	0.55 – 0.88	0.003
rs6951193/rs759332	15	AA	Intron 6	anti-IgG ASCA +ve CD ²	35.5%	29.0%	1.35	1.07 – 1.70	0.010
rs6951193/rs759332	15	GA	Intron 6	anti-IgG ASCA +ve CD ²	27.4%	34.0%	0.74	0.58 – 0.93	0.009
rs7791394	10	C	Intron 20	anti-IgG ASCA +ve CD ²	59.4%	50.6%	1.42	1.15 – 1.77	0.001
rs4727608	16	C	Intron 20	anti-IgG ASCA +ve CD ²	71.8%	66.2%	1.30	1.03 – 1.65	0.03
rs3807728	17	G	Intron 20	anti-I2+ve CD ³	49.0%	43.1%	1.27	1.02 – 1.58	0.03
rs10264296	2	C	Intron 2	anti-CBir1 +ve CD ⁴	55.7%	49.1%	1.25	1.02 – 1.54	0.02
rs10808167	2	A	Intron 2	anti-CBir1 +ve CD ⁴	53.0%	47.0%	1.27	1.02 – 1.58	0.03
rs10242163	2	G	Intron 2	anti-CBir1 +ve CD ⁴	59.0%	53.2%	1.27	1.02 – 1.58	0.04
rs10239917	12	T	Intron 3	anti-CBir1 +ve CD ⁴	43.6%	33.5%	1.53	1.22 – 1.92	0.0002
rs10239917/rs1773635	12	CA	Intron 3	anti-CBir1 +ve CD ⁴	35.9%	46.2%	0.65	0.52 – 0.82	0.0001
rs10239917/rs1773635	12	TA	Intron 3	anti-CBir1 +ve CD ⁴	43.6%	33.5%	1.53	1.22 – 1.92	0.0002

MAGI2 SNP or Haplotype	MAGI2 locus	'Risk' allele	Gene location (intron etc)	Phenotype	Case allele Hz	Control allele Hz	OR	95% CI	P value
rs2110630	18	A	Intron 4	anti-CBir1 +ve CD ⁴	36.0%	29.9%	1.31	1.04 – 1.66	0.02
rs1990577	19	A	Intron 5	anti-CBir1 +ve CD ⁴	49.1%	43.0%	1.28	1.03 – 1.59	0.03
rs3735442	20	A	Exon 6	anti-CBir1 +ve CD ⁴	34.7%	28.7%	1.32	1.04 – 1.67	0.02
s759334	21	A	(ns)	anti-CBir1 +ve CD ⁴	52.6%	45.7%	1.32	1.06 – 1.64	0.01
rs6951193/rs759333	15	AA	Intron 6	anti-CBir1 +ve CD ⁴	34.4%	27.5%	1.38	1.09 – 1.75	0.008
rs7801139	22	C	Intron 7	anti-CBir1 +ve CD ⁴	30.0%	24.0%	1.36	1.06 – 1.74	0.02
rs798292	23	A	Intron 9	anti-CBir1 +ve CD ⁴	79.3%	74.6%	1.30	1.01 – 1.69	0.05
rs11773635	13	C	Intron 3	anti-OmpC +ve CD ⁵	25.1%	17.6%	1.56	1.20 – 2.04	0.0009
rs10239917/rs11773635	13	CC	Intron 3	anti-OmpC +ve CD ⁵	25.1%	17.6%	1.55	1.20 – 2.03	0.0009
rs10239917/rs11773635	13	CA	Intron 3	anti-OmpC +ve CD ⁵	36.4%	42.0%	0.79	0.64 – 0.99	0.04
rs725555	24	G	Intron 4	anti-OmpC +ve CD ⁵	25.2%	18.0%	1.53	1.18 – 1.99	0.001
rs759334	21	A	Intron 6	anti-OmpC +ve CD ⁵	54.1%	47.4%	1.30	1.05 – 1.62	0.02
rs798285	25	C	Intron 9	anti-OmpC +ve CD ⁵	45.9%	38.6%	1.35	1.08 – 1.68	0.007
rs798287	26	G	Intron 9	anti-OmpC +ve CD ⁵	45.9%	38.7%	1.35	1.08 – 1.68	0.008
rs798292	23	A	Intron 9	anti-OmpC +ve CD ⁵	81.1%	75.2%	1.41	1.09 – 1.85	0.01
rs798279	27	G	Intron 9	anti-OmpC +ve CD ⁵	80.9%	75.5%	1.37	1.05 – 1.79	0.02

¹ Anti-ASCA +ve CD – allele frequency in CD patients who are anti-ASCA positive compared to those who are anti-ASCA negative.

² anti-IgG ASCA +ve CD – allele frequency in CD patients who are anti-IgG ASCA positive compared to those who are anti-IgG ASCA negative.

³ anti-I2+ve CD – allele frequency in CD patients who are anti-I2 positive compared to those who are anti-I2 negative.

⁴ Anti-CBir1 +ve CD – allele frequency in CD patients who are anti-CBir1 positive compared to those who are anti-CBir1 negative.

⁵ Anti-OmpC +ve CD – allele frequency in CD patients who are anti-OmpC positive compared to those who are anti-OmpC negative. Hz – frequency. OR – odds ratio. CI – confidence intervals.

Table 3
Association between quantitative antibody levels and MAGI2 SNPs

The MAGI2 locus column identifies loci across the gene for ease of cross-referencing.

MAGI2 SNP	MAGI2 locus ^a	Associated allele	Gene position	Antibody level	P value
rs10239917	12	T	Intron 3	anti-IgA ASCA	0.001
rs319872	25	G	intron 3	anti-IgA ASCA	0.034
rs17436052	26	G	Intron 4	anti-IgA ASCA	0.0003
rs1030015	27	G	Intron 4	anti-IgA ASCA	0.039
rs1990577	28	A	Intron 5	anti-IgA ASCA	0.013
rs3735442	20	A	Exon 6	anti-IgA ASCA	0.019
rs6951193	15	G	Intron 6	anti-IgA ASCA	0.004
rs7791394	10	T	Intron 20	anti-IgA ASCA	0.038
rs17454991	29	T	Intron 2	anti-IgG ASCA	0.023
rs10239917	13	T	Intron 3	anti-IgG ASCA	0.0008
rs17436052	26	G	Intron 4	anti-IgG ASCA	0.0002
rs1990577	27	A	Intron 5	anti-IgG ASCA	0.029
rs7803705	8	G	Intron 6	anti-IgG ASCA	0.011
rs6951193	15	G	Intron 6	anti-IgG ASCA	0.007
rs759334	21	A	Intron 6	anti-IgG ASCA	0.011
rs798343	30	T	Intron 9	anti-IgG ASCA	0.044
rs798279	24	A	Intron 9	anti-IgG ASCA	0.050
rs798292	23	G	Intron 9	anti-IgG ASCA	0.041
rs798356	30	T	Intron 10	anti-IgG ASCA	0.038
rs2107992	31	G	Intron 1	anti-I2	0.040
rs17436052	32	G	Intron 4	anti-I2	0.007
rs1030015	33	G	Intron 4	anti-I2	0.022
rs1990577	19	A	Intron 5	anti-I2	0.038
rs3807694	34	A	Intron 16	anti-I2	0.035
rs12668675	35	C	Intron 16	anti- CBir1	0.032

P value calculated by logistic regression across the genotype (see statistical methods for further details)