European Journal of Human Genetics (2006)14, 1215–1222

www.nature.com/ejhg

ARTICLE

A functional candidate screen for coeliac disease genes

Christine R Curley[1,4], Alienke J Monsuur[2,4], Martin C Wapenaar[2], John D Rioux[1,3] and Cisca Wijmenga*[,2]

[1]*The Broad Institute, Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA;* [2]*Complex Genetics Section, DBG-Department of Medical Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands;* [3]*Université de Montreal and the Montreal Heart Institute/Institut de Cardiologie de Montreal, Montreal, Quebec, Canada*

It is increasingly evident that different inflammatory disorders show some overlap in their pathological features, concurrence in families and individuals, and shared genetic factors. This might also be true for coeliac disease, a chronic inflammatory disorder of the gastrointestinal system, which shares two linkage regions with inflammatory bowel disease: on chromosome 5q31 (CELIAC2 and IBD5) and 19p13 (CELIAC4 and IBD6). We hypothesised that these regions contain genes that contribute to susceptibility to both disorders. The overlapping 5q31 region contains only five positional candidate genes, whereas the overlapping 19p13 region has 141 genes. As the common disease gene probably plays a role in inflammation, we selected five functional candidate genes from the 19p13 region. We studied these 10 positional and functional candidate genes in our Dutch coeliac disease cohort using 44 haplotype tagging single-nucleotide polymorphisms. Two genes from 19p13 showed a small effect on familial clustering: the cytochrome *P*450 F3 gene *CYP4F3* ($P_{nominal}$ 0.0375, odds ratio (OR) 1.77) and *CYP4F2* ($P_{nominal}$ 0.013, OR 1.33). CYP4F3 and CYP4F2 catalyse the inactivation of leukotriene B4 (LTB4), a potent mediator of inflammation responsible for recruitment and activation of neutrophils. The genetic association of LTB4-regulating gene variants connects the innate immune response of neutrophil mobilisation with that of the established Th1 adaptive immunity present in coeliac disease patients. The findings in coeliac disease need to be replicated. Expanding genetic association studies of these cytochrome genes to other inflammatory conditions should reveal whether their causative influence extends beyond coeliac disease.
European Journal of Human Genetics (2006) **14,** 1215–1222. doi:10.1038/sj.ejhg.5201687; published online 12 July 2006

Keywords: coeliac disease; inflammatory bowel disease; *MYO9B*; inflammation; candidate gene study; IBD5

Introduction

Coeliac disease is a chronic disease characterised by an inflammatory response in the gastrointestinal tract and an impaired intestinal epithelial barrier.[1] It is a complex genetic disorder, involving multiple genetic variants. Genetic studies on coeliac disease patients indicate that multiple chromosomal regions predispose to disease susceptibility, confirming the suggestion that common genes contribute to this disorder. Two of the coeliac disease loci, CELIAC2 on chromosome 5q23–q33 and CELIAC4 on 19p13.1,[2,3] coincide with linkage regions for inflammatory bowel disease (IBD),[4,5] giving rise to the hypothesis of common disease susceptibility. The IBD5 and IBD6 loci on 5q31 and 19p13.1, respectively, are among the most significant and consistently

*Correspondence: Professor C Wijmenga, Complex Genetics Section, Stratenum 2.117, DBG-Department of Biomedical Genetics, University Medical Centre Utrecht, PO Box 85060, 3508 AB Utrecht, The Netherlands. Tel: +31 30 253 8427; Fax: +31 30 253 8479;
E-mail: t.n.wijmenga@med.uu.nl
[4]These authors have contributed equally to this work.
Received 14 December 2005; revised 18 May 2006; accepted 30 May 2006; published online 12 July 2006

replicated IBD loci.[6,7] There is ample evidence that (auto)immune-related disorders share some of their genetic susceptibility factors, the best examples being variations in the *CTLA-4* gene[8] and *PTPN22*,[9–11] both of which help regulate T-cell responsiveness.

IBD, clinically classified as Crohn's disease and ulcerative colitis, also shows a chronic inflammation of the gastrointestinal tract.[6] Coeliac disease and IBD co-occur in families and patients, with an approximately five-fold increased prevalence of IBD in coeliac disease patients,[12–15] further suggesting common pathophysiological mechanisms for both diseases.

The sharing of the linkage regions, the concurrence in patients and the pathological commonalities between coeliac disease and IBD led us to hypothesise that genes on chromosomes 5q31 and 19p13.1 might be associated with susceptibility for both disorders, most probably by influencing inflammation in the gut. The CELIAC2 locus on 5q23–q33 overlaps with a 250 kb haplotype on 5q31 associated with Crohn's disease (IBD5).[16] The implicated haplotype contains five genes (*P4HA2*, *IRF1*, *SLC22A5/OCTN2*, *SLC22A4/OCTN1*, *PDLIM3*) (see Table 1), all of which are positional candidate genes for coeliac disease pathogenesis. The CELIAC4 locus ranging from 15.38 to 21.08 Mb (99% confidence interval (CI), Ensembl version 35) lies completely within the IBD6 locus, which spans a large part of chromosome 19p. Of the 141 genes in the overlapping region, five are known to be involved in inflammation and were considered as functional candidate genes: *CYP4F3*, *CYP4F2*, *HSH2D*, *IL12RB1* and *IFI30* (see Table 1). A complementary approach to identifying the susceptibility gene for coeliac disease in the chromosome 19 region involves fine-mapping of the region. We simultaneously undertook such a strategy, which led to

Table 1 Overview of the 10 positional and functional candidate genes studied in relation to the overlapping regions in coeliac disease and IBD

Gene name	*Chromosome*	*Location (bps)*	*Function*	*References*	*No. of tag SNPs*[a]
P4HA2	5	131 556 202–131 590 458	Prolyl 4-hydroxylase α-2 subunit precursor. Catalyses the post-translational formation of 4-hydroxyproline in –Xaa–Pro–Gly– sequences in collagens and other proteins	25	—
PDLIM4	5	131 621 285–131 637 046	PDZ and LIM domain 4. Interacts with the LIM domain with the second and fourth PDZ domains of PTPN13	16	—
SLC22A4	5	131 658 043–131 707 796	Organic cation/carnitine transporter 1 (solute carrier family 22, member 4). Sodium-ion-dependent, low-affinity carnitine transporter. Defects in SLC22A4 may be a cause of susceptibility to Crohn's disease and rheumatoid arthritis	16	5
SLC22A5	5	131 733 343–131 759 202	Organic cation/carnitine transporter 2 (solute carrier family 22). Sodium-ion-dependent, high-affinity carnitine transporter. Involved in the active cellular uptake of carnitine. Transports one sodium ion with one molecule of carnitine Defects are the cause of systemic primary carnitine deficiency and may be a cause of susceptibility to Crohn's disease	16	2
IRF1	5	131 846 678–131 854 333	Interferon regulatory factor 1. Specifically binds to the upstream regulatory region of type I IFN and IFN-inducible MHC class I genes (the interferon consensus sequence) and activates those genes. Deletion or rearrangement of IRF1 are a cause of preleukaemic myelodysplastic syndrome and of acute myelogenous leukaemia	16	—
CYP4F3	19	15 613 226–15 631 234	Member of *P*450 family of cytochromes. Catalyses the inactivation of leukotriene B4 (LTB4), a potent mediator of inflammation	26–28	8
CYP4F2	19	15 849 834–15 869 885	Member of *P*450 family of cytochromes. Catalyses the inactivation of LTB4, a potent mediator of inflammation	24,29	8
HSH2D	19	16 115 501–16 130 375	Target of the two most important signalling pathways of T-cell activation; T-cell receptor antigen signalling and costimulation of the naive T cell	30,31	5
IL12RB1	19	18 030 806–18 104 521	IL-12 receptor β chain 1, which is involved in inducing cell-mediated immunity to intracellular pathogens via the Th1 pathway and interferon production. Deficiency leads to impaired mycobacterial immunity. Large amounts of IL-12 are secreted in mice with an NOD2 mutation in response to TLR2, which facilitates a Th1 response	32–34	3
IFI30	19	18 145 579–18 149 927	Induced by interferon-γ	35	2

[a]Seven of the 16 haploblock tagging SNPs on chromosome 5 are located in the genes, and the others are located outside the genes.

the identification of the myosin IXB gene (*MYO9B*) as a susceptibility gene for coeliac disease.[17] The odds ratio (OR) for this gene is lower than the expected OR for the CELIAC4 locus, leaving room for another susceptibility gene in this locus. In this study, we report our association findings of the five positional candidate genes comprising the 250 kb haplotype on 5q31 and the five functional candidate genes from 19p13.1; all were tested using a haplotype block tag approach for association with coeliac disease in a cohort of Dutch patients.

Materials and methods

Samples

A case–control cohort consisting of 309 independent coeliac disease patients and 358 independent controls, all of Dutch Caucasian origin, was used for the genetic association study. Patients were diagnosed according to the ESPGHAN criteria and approximately 93% of the patients were HLA-DQ2 positive. We included for this study only coeliac disease patients with a biopsy-proven Marsh III lesion, as described by Van Belzen *et al*.[3] Blood samples were collected and DNA was isolated according to the standard laboratory procedures.[3] The study was approved by the Medical Ethics Committee of the University Medical Centre in Utrecht and informed consent was obtained from all individuals.

Candidate gene and single-nucleotide polymorphism (SNP) selection

A total of 16 haplotype tagging SNPs were selected from the 5q31 region, as described before.[16] We observed comparable haplotypes to the ones published by Rioux *et al*.[16] We also selected five functional candidate genes located in the overlapping CELIAC4 and IBD6 locus on chromosome 19 for this study, based on their known function, expression studies and HapMap SNP coverage. We selected SNPs based on block-tagging ability, validation status, allele frequency and SNP density. Previous performance rating (genotyping $>90\%$) from the IBD cohort typing, on the Sequenom platform, was also taken into account. Haplotype tagging SNPs were selected on the basis of HapMap data as of September 2004 (www.hapmap.org) using Haploview to determine the haplotype tagging SNPs. These SNPs were selected at an early stage of the HapMap project when there was less information than available today. If tag SNPs could not be determined from HapMap data, Supplemental data sets (Programs for Genomic Applications (PGA) and previous haplotype analyses by our group (JD Rioux, personal communication)) were used for haplotype tagging SNP selection using Haploview as described above. Supplementary Table 1 lists the selected tagging SNPs per gene that were tested on the coeliac disease cohort.

Genotyping

Genomic DNA extracted from whole-blood samples was used. Genotyping assays were designed using the Sequenom Assay Design program and genotypes were obtained using the Sequenom Mass Array system at the Broad Institute of MIT and Harvard as described by Gabriel *et al*.[18] Genotyping data was analysed for Hardy–Weinberg equilibrium and allele frequency. The quality control criteria used to determine if genotyping results were successful were: a minimum of 75% genotyping success for each SNP, Hardy–Weinberg equilibrium values >0.01 and observed heterozygosity $>0.5\%$. Of 44 SNPs tested in the region, 42 SNPs passed the above criteria (one was monomorphic and the other failed owing to Hardy–Weinberg errors).

Statistical analysis

Allele and haplotype counts in cases *versus* controls were analysed for association. A single-marker and multimarker association study for each gene in the case–control cohort was conducted by a standard χ^2 test (2×2 contingency table). Haplotypes were constructed using Haploview for the unrelated cases and for the control cohorts separately.[19] OR represents maximum-likelihood estimate of odds ratio, and the corresponding 95% CI was approximated using Woolf's method.

The relative risk for coeliac disease associated with the CELIAC4 locus base on the linkage data is calculated to be 2.3.[3] The OR associated with *MYO9B* heterozygosity is estimated to be 1.6 and homozygosity 2.3.[17] We calculated the power for this study based on a relative risk of 1.7, which gives us more than 75% power to detect a disease variant with minor allele frequencies ranging from 0.1 to 0.3, assuming a dominant inheritance, a disease prevalence of 0.1, a D' of 1 and an equal frequency of the tested SNP and the high-risk variant.

Results

Coeliac disease and IBD share linkage regions on chromosome 5q23–q33 (CELIAC2 and IBD5) and 19p13 (CELIAC4 and IBD6). We performed association studies on 10 positional and functional candidate genes to search for association with genes that might play a primary role in both disorders. Forty-four SNPs were selected to tag the haplotype blocks, thereby excluding redundant typing, and were genotyped in a cohort of 309 independent Dutch coeliac disease cases and 358 Dutch controls. Two tag SNPs failed to pass our quality control standards.

In the chromosome 5 region, one SNP showed association ($P_{\text{nominal}} <0.05$): rs7705826 located in SLC22A5 (P_{nominal} 0.033, OR 1.39, 95% CI 1.03–1.88). Owing to the high linkage disequilibrium (LD) in the region, we would have expected to find more than one SNP from this region to be associated. Therefore, this observation is

probably false positive also because this SNP deviates from Hardy–Weinberg equilibrium in the control population.

In the chromosome 19 region, four SNPs showed single SNP association, two of which were located in *CYP4F2* and two in *CYP4F3* (Table 2, Figure 1). These genes are located head-to-head 218 kb apart. SNPs rs7252046 ($P_{nominal}$ 0.0427, OR 1.28, 95% CI 1.01–1.61) and rs3093156 ($P_{nominal}$ 0.013, OR 1.33, 95% CI 1.06–1.66) showed association in *CYP4F2*, and SNPs rs1290622 ($P_{nominal}$ 0.0447, OR 1.58, 95% CI 1.01–2.48) and rs1290625 ($P_{nominal}$ 0.0375, OR 1.77, 95% CI 1.03–3.05) in *CYP4F3*. Heterozygotes for the most associated SNP (rs3093156 in *CYP4F2*) have a 1.6 times higher risk of coeliac disease ($P_{nominal}$ 0.021, 95% CI 1.07–2.4), whereas in homozygotes the risk increases to 1.84 ($P_{nominal}$ 0.01, 95% CI 1.15–2.93). The results obtained for these genes are independent as the r^2 between the SNPs in the genes is <0.06. Haplotype analyses of each of the two SNPs in the *CYP4F2* and *CYP4F3* genes are shown in Table 3. The other seven genes (*IRF1*, *SLC22A4/OCTN1*, *PDLIM3*, *P4HA2*, *IF130*, *IL12RB1* and *HSH2D*) showed no association.

Discussion

In the last decade, much research has been devoted to elucidating the genetic basis of complex traits such as coeliac disease. Although increasingly successful, this work has been complicated by the fact that multiple genes can be associated with a trait, but each has insufficient impact to account for the total genetic susceptibility of the disease under study. Given the extent of linkage regions and the large number of genes they usually encompass, it can be a daunting task to select the most plausible candidate gene, particularly when little or no knowledge is available on gene function or the biological process perturbed. Both the coeliac disease loci on chromosome 5q23–q33 and 19p13.1 coincide with linkage regions for IBD. We therefore hypothesised that genes related to biological processes common to both disorders would make excellent functional candidates.

In this study, we observed nominal association to a single SNP located in SLC22A5 on chromosome 5. Although it is not clear which of the five genes in this region really confer susceptibility to Crohn's disease,[16] changes in the SLC22A4 and SLC22A5 have been observed in relation to Crohn's disease,[20] which potentially have a functional relevance. The effect of the association in coeliac disease is quite modest compared to the original findings in Crohn's disease. Furthermore, the other SNPs that we tested in the region show no association despite being in near-complete LD. Given these results, we feel that the most parsimonious explanation is that this association of SLC22A5 to celiac disease is a false positive, but this will require confirmation by independent groups.

Recently, a study has been published investigating 56 candidate genes from chromosome 19 for their involvement in IBD.[21] All but one (*CYP4F2*) of the genes included in this study overlap with the genes published by Tello-Ruiz *et al*.[21] We observed association to the cytochrome genes, *CYP4F2* and *CYP4F3*, in a cohort of Dutch coeliac disease patients. Although the genes are juxtaposed on chromosome 19, the observed association signals are independent as there is hardly any LD between them (maximum r^2 between the associated SNPs of the two genes is <0.06). The relative risk associated to each of the two genes ranges from 1.6 to 1.8, which is insufficient to fully explain the linkage result observed earlier.[3] It is interesting that variants in the myosin IXB gene (*MYO9B*), located approximately 1.2 Mb proximal to *CYP4F2*, have recently been shown to be associated with increased risk (OR = 1.7) to coeliac disease.[17] The association found in the *CYP4F2* and *CYP4F3* genes is not owing to long-range LD between these genes and *MYO9B* (maximum r^2 <0.0044). Although the observation with the *CYP4F2* and *CYP4F3* genes has not been corrected for multiple testing and needs to be independently replicated, it is tempting to speculate that the original strong linkage signal on 19p13.1[3] resulted from the presence of multiple susceptibility genes.

If we look at their function, the involvement of these two *CYP4F* genes is intriguing. Both CYP4F isoforms are involved in the oxidative degradation of leukotriene B4 (LTB4), the arachidonic acid-derived lipid inflammatory mediator responsible for the recruitment and activation of neutrophils. The genetic association of LTB4-regulating gene variants connects the innate immune response of neutrophil mobilisation with that of the established Th1 adaptive immunity present in coeliac disease. These genetic variants may influence neutrophil migration and thus create an environment in the mucosa that contributes to coeliac disease pathogenesis. It has been reported that activated neutrophils have an effect on epithelial tight junctions that results in enhanced permeability[22] in the intestine, which could facilitate the influx of various commensals from the lumen leading to the recruitment of even more phagocytic neutrophils. This self-sustaining cycle of barrier impairment could also enable gluten to enter the lamina propria, where it could be presented to resident $CD4^+$ T cells to evoke the Th1 response. In turn, activation of the Th1 immune pathway would further undermine barrier integrity through the release of interferon-γ.[23] In a parallel gene expression study, we observed increased neutrophil numbers not only in untreated coeliac patients but also in patients in complete remission, probably reflecting a genetic impairment of the epithelial barrier.[25] This observation is in line with the recently identified *MYO9B* gene, which is expected to confer genetic susceptibility to coeliac disease pathogenesis by also affecting the intestinal barrier.[17] A more detailed assessment of the contribution of *CYP4F2* and *CYP4F3* variants

Table 2 Association results for the SNPs that were polymorphic and in Hardy–Weinberg equilibrium (HWE)

							Frequency[a]		*Allele counts*[b]				
Gene	*rs number*	*Chromosome*	*Base pair position*	*MAJ allele*[a]	*HWE P-value controls*	*HWE P-value cases*	*Case*	*Control*	*Case*	*Control*	χ^2	*P-value*	*OR (95% CI)*[c]
SLC22A4	rs7705189	5	131 651 257	T, T	0.227	0.802	0.552	0.532	328:266	367:323	0.530	0.467	
SLC22A4	rs162887	5	131 657 064	C, C	0.417	0.737	0.690	0.686	422:190	479:219	0.016	0.898	
SLC22A4	IGR3081ms1	5	131 658 751	T, T	0.043	0.764	0.572	0.537	348:260	382:330	1.705	0.192	
SLC22A4	IGR3066ms1	5	131 666 223	A, A	0.217	0.436	0.528	0.520	319:285	366:338	0.089	0.766	
SLC22A4	rs27060	5	131 679 710	A, A	0.392	1.000	0.606	0.615	367:239	429:269	0.111	0.740	
—	rs4705938	5	131 721 976	A, A	0.176	0.638	0.545	0.520	325:271	361:333	0.813	0.367	
—	rs671473	5	131 736 434	G, G	0.449	0.523	0.685	0.686	419:193	480:220	0.002	0.967	
—	rs274567	5	131 742 308	G, G	1.000	0.340	0.562	0.543	326:254	357:301	0.475	0.491	
SLC22A5	rs17622208	5	131 744 949	C, C	0.228	0.469	0.542	0.514	323:273	356:336	0.971	0.324	
SLC22A5	rs7705826	5	131 760 355	C, C	0.022	0.739	0.822	0.865	498:108	595:93	4.550	0.033	1.39 (1.03–1.88)
—	rs11739135	5	131 761 296	C, C	0.272	0.992	0.610	0.567	376:240	397:303	2.528	0.112	
—	rs13179841	5	131 770 719	G, G	0.504	0.502	0.934	0.938	564:40	655:43	0.116	0.734	
—	rs4475253	5	131 804 405	T, T	0.867	0.588	0.698	0.706	420:182	490:204	0.108	0.742	
—	rs4705950	5	131 821 185	G, G	0.095	1.000	0.569	0.552	340:258	384:312	0.370	0.543	
—	rs6894249	5	131 825 446	T, T	0.303	1.000	0.613	0.649	373:235	454:246	1.723	0.189	
—	rs2248116	5	13 183 2246	T, T	0.225	0.896	0.577	0.554	352:258	391:315	0.718	0.397	
CYP4F3	rs4807964	19	15 609 827	G, G	1.000	0.231	0.841	0.811	461:87	555:129	1.873	0.171	
CYP4F3	rs1290617	19	15 612 897	T, T	0.233	0.789	0.591	0.623	357:247	441:267	1.385	0.239	
CYP4F3	rs1290618	19	15 613 137	C, C	0.379	0.075	0.770	0.758	442:132	532:170	0.260	0.610	
CYP4F3	rs1290622	19	15 614 423	C, C	0.838	0.860	0.947	0.918	551:31	652:58	4.029	0.045	1.58 (1.01–2.48)
CYP4F3	rs1290625	19	15 618 156	G, G	0.650	0.122	0.940	0.965	521:33	643:23	4.327	0.038	1.77 (1.03–3.05)
CYP4F3	rs1290626	19	15 618 927	C, C	1.000	0.912	0.587	0.577	338:238	411:301	0.120	0.730	
CYP4F3	rs1543284	19	15 637 785	G, G	0.906	1.000	0.575	0.572	354:262	406:304	0.011	0.917	
CYP4F3	rs1543286	19	15 637 945	C, C	0.987	0.976	0.503	0.524	289:285	373:339	0.529	0.467	
CYP4F2	rs2189784	19	15 820 200	G, G	0.187	0.834	0.610	0.613	376:240	429:271	0.008	0.927	
CYP4F2	rs2079288	19	15 825 203	T, T	0.918	0.970	0.755	0.762	447:145	538:168	0.086	0.770	
CYP4F2	rs7252046	19	15 832 473	T, T	1.000	0.217	0.708	0.655	415:171	468:246	4.107	0.043	1.28 (1.01–1.61)
CYP4F2	rs12610189	19	15 839 641	T, T	0.669	0.568	0.635	0.664	352:202	426:216	1.038	0.308	
CYP4F2	rs2108622	19	15 851 431	C, C	0.054	0.660	0.712	0.732	430:174	521:191	0.641	0.424	
CYP4F2	rs3093156	19	15 861 609	T, A	0.850	0.124	0.544	0.527	309:259	355:319	6.167	0.013	1.33 (1.06–1.66)
CYP4F2	rs3093135	19	15 865 371	T, T	0.452	0.326	0.844	0.880	449:83	523:71	3.165	0.075	
CYP4F2	rs3761014	19	15 872 763	C, C	0.382	0.401	0.832	0.844	506:102	604:112	0.312	0.577	
HSH2	rs2032882	19	16 105 817	A, A	0.151	0.639	0.910	0.879	453:45	564:78	2.824	0.093	
HSH2	rs444053	19	16 113 072	C, C	0.531	1.000	0.719	0.725	433:169	516:196	0.048	0.826	
HSH2	rs285290	19	16 120 460	C, C	0.535	0.541	0.832	0.848	476:96	599:107	0.626	0.429	
HSH2	rs2258476	19	16 126 017	A, A	0.403	0.599	0.833	0.829	498:100	585:121	0.040	0.842	
HSH2	rs681059	19	16 130 507	A, A	1.000	0.686	0.717	0.721	433:171	516:200	0.023	0.879	
IL12RB1	rs404733	19	18 030 997	A, A	0.585	0.850	0.513	0.513	311:295	363:345	0.000	0.986	
IL12RB1	rs375947	19	18 041 451	A, A	0.675	0.454	0.686	0.674	424:194	473:229	0.228	0.633	
IL12RB1	rs436857	19	18 058 635	G, G	0.476	0.525	0.798	0.803	487:123	565:139	0.036	0.849	
IFI30	rs273266	19	18 144 501	T, T	0.932	0.213	0.787	0.772	455:123	545:161	0.429	0.513	
IFI30	rs7125	19	18 149 069	A, G	0.670	0.160	0.502	0.531	306:304	360:318	1.367	0.242	

[a]Major allele in cases and controls, which may be the opposite allele.
[b]Actual allele counts for cases and controls; the major allele is shown first.
[c]Odds ratio (OR) calculated using the not-associated allele as reference.

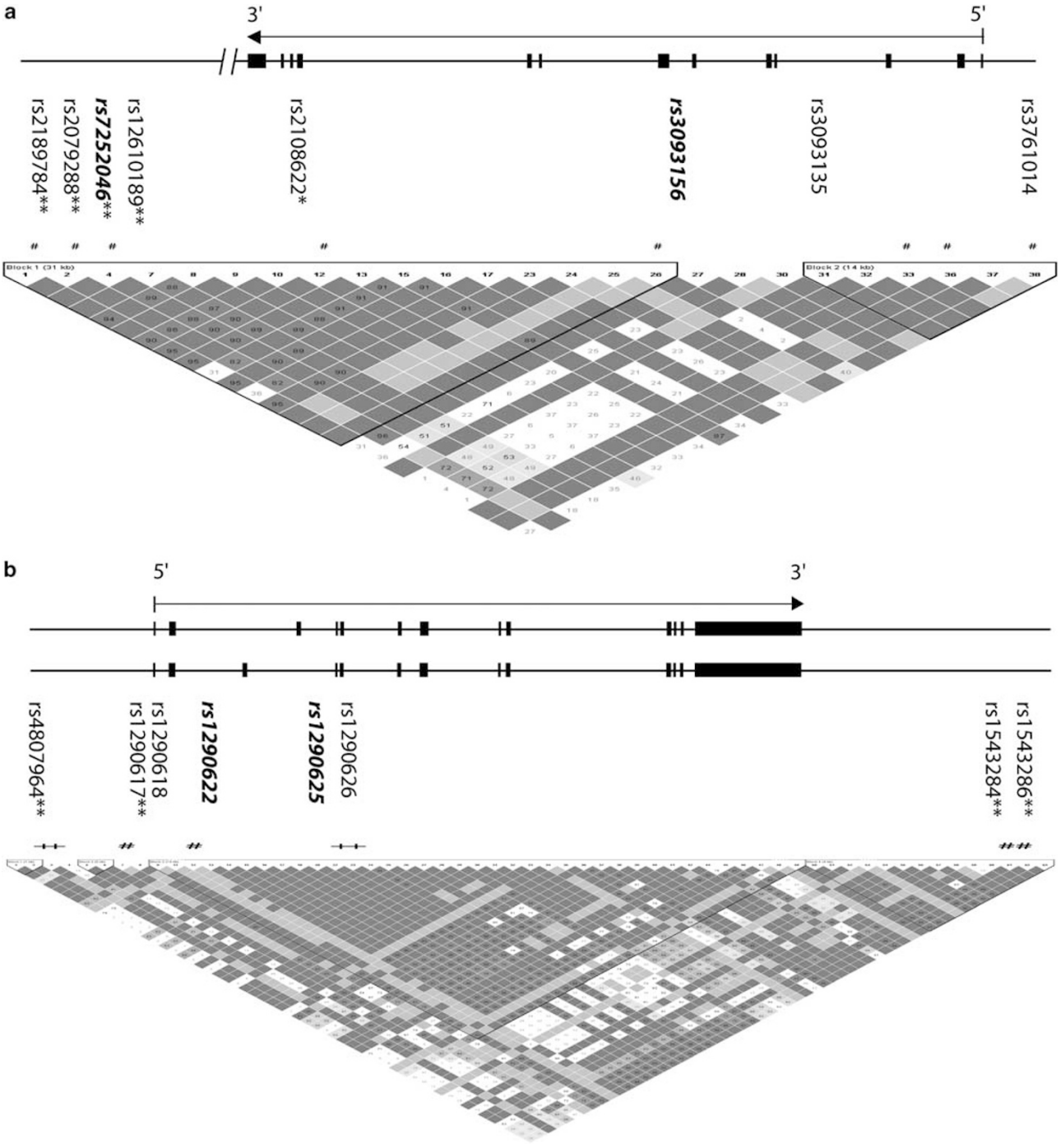

Figure 1 Overview of *CYP4F2* (**a**) and *CYP4F3* (**b**), isoforms a and b, respectively, showing the exons, the locations of the tag SNPs (associated tag SNPs are listed in bold italics) and the observed LD based on the HapMap information. **The locations of the SNPs outside the genes are not true to scale. *rs2108622 is a coding SNP; # shows the position of the SNPs in the LD plot; and + shows for the SNPs that were not typed in the HapMap, the relative position of the SNPs in the LD plot.

to neutrophil recruitment and intestinal permeability will require further functional analysis. Replication in coeliac disease and expanding genetic association studies of these cytochrome genes to other inflammatory conditions should reveal whether their causative influence is true and extends beyond coeliac disease.

Table 3 Haplotype analyses of associated SNPs in CYP4F3 (rs1290622 and rs1290625) and CYP4F2 (rs7252046 and rs3093156)

		Frequency		*Number*			
Gene	*Haplotype*	*Case*	*Control*	*Case*	*Control*	χ^2	*P-value*
CYP4F3	GC	0.888	0.883	539.6	632.2	0.067	0.7960
	GT	0.054	0.082	33	58.4	3.882	0.0488
	AC	0.058	0.035	36	25.4	3.959	0.0466
CYP4F2	TT	0.468	0.417	289	298.3	3.572	0.0587
	AC	0.225	0.288	139	206.5	7.045	0.0079
	AT	0.238	0.239	147.4	171	0.0	0.9861
	TC	0.069	0.056	42.5	40.2	0.922	0.3369

Acknowledgements

We thank all the patients, their physicians, pathologists and the Dutch Coeliac Disease Foundation for participating in this study. We thank Jackie Senior for critically reading the manuscript. This study was supported by grants from the Netherlands Organisation for Scientific Research (Grant 912-02-028), the Dutch Digestive Diseases Foundation (Grants 97-44 and 03-06) and the Coeliac Disease Consortium, an Innovative Cluster approved by the Netherlands Genomics Initiative and partially funded by the Dutch Government (Grant BSIK03009). JDR was supported by grants from the American National Institute of Diabetes and Digestive and Kidney Diseases and the Crohn's and Colitis Foundation of America.

References

1 Macdonald TT, Monteleone G: Immunity, inflammation, and allergy in the gut. *Science* 2005; **307**: 1920–1925.
2 Babron MC, Nilsson S, Adamovic S *et al*: Meta and pooled analysis of European coeliac disease data. *Eur J Hum Genet* 2003; **11**: 828–834.
3 Van Belzen MJ, Meijer JW, Sandkuijl LA *et al*: A major non-HLA locus in celiac disease maps to chromosome 19. *Gastroenterology* 2003; **125**: 1032–1041.
4 Rioux JD, Silverberg MS, Daly MJ *et al*: Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet* 2000; **66**: 1863–1870.
5 van Heel DA, Satsangi J, Carey AH, Jewell DP: Inflammatory bowel disease: progress toward a gene. *Can J Gastroenterol* 2000; **14**: 207–218.
6 Schreiber S, Rosenstiel P, Albrecht M, Hampe J, Krawczak M: Genetics of Crohn disease, an archetypal inflammatory barrier disease. *Nat Rev Genet* 2005; **6**: 376–388.
7 Van Heel DA, Fisher SA, Kirby A, Daly MJ, Rioux JD, Lewis CM: Inflammatory bowel disease susceptibility loci defined by genome scan meta-analysis of 1952 affected relative pairs. *Hum Mol Genet* 2004; **13**: 763–770.
8 Ueda H, Howson JM, Esposito L *et al*: Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; **423**: 506–511.
9 Begovich AB, Carlton VE, Honigberg LA *et al*: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004; **75**: 330–337.
10 Bottini N, Musumeci L, Alonso A *et al*: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004; **36**: 337–338.
11 Zhernakova A, Eerligh P, Wijmenga C, Barrera P, Roep BO, Koeleman BP: Differential association of the PTPN22 coding variant with autoimmune diseases in a Dutch population. *Genes Immun* 2005; **6**: 459–461.
12 Cottone M, Marrone C, Casa A *et al*: Familial occurrence of inflammatory bowel disease in celiac disease. *Inflamm Bowel Dis* 2003; **9**: 321–323.
13 Schedel J, Rockmann F, Bongartz T, Woenckhaus M, Scholmerich J, Kullmann F: Association of Crohn's disease and latent celiac disease: a case report and review of the literature. *Int J Colorectal Dis* 2005; **20**: 376–380.
14 Tursi A, Giorgetti GM, Brandimarte G, Elisei W: High prevalence of celiac disease among patients affected by Crohn's disease. *Inflamm Bowel Dis* 2005; **11**: 662–666.
15 Yang A, Chen Y, Scherl E, Neugut AI, Bhagat G, Green PH: Inflammatory bowel disease in patients with celiac disease. *Inflamm Bowel Dis* 2005; **11**: 528–532.
16 Rioux JD, Daly MJ, Silverberg MS *et al*: Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001; **29**: 223–228.
17 Monsuur AJ, Bakker PI, Alizadeh BZ *et al*: Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. *Nat Genet* 2005; **37**: 1341–1344.
18 Gabriel SB, Schaffner SF, Nguyen H *et al*: The structure of haplotype blocks in the human genome. *Science* 2002; **296**: 2225–2229.
19 Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–265.
20 Peltekova VD, Wintle RF, Rubin LA *et al*: Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004; **36**: 471–475.
21 Tello-Ruiz MK, Curley C, DelMonte T *et al*: Haplotype-based association analysis of 56 functional candidate genes in the IBD6 locus on chromosome 19. *Eur J Hum Genet* 2006; **14**: 780–790.
22 Matter K, Balda MS: Signalling to and from tight junctions. *Nat Rev Mol Cell Biol* 2003; **4**: 225–236.
23 Mankertz J, Tavalali S, Schmitz H *et al*: Expression from the human occludin promoter is affected by tumor necrosis factor alpha and interferon gamma. *J Cell Sci* 2000; **113** (Part 11): 2085–2090.
24 Diosdado B, van Bakel H, Strengman E *et al*: A genomics view on celiac disease points to enhanced neutrophil recruitment and barrier impairment, 2005, (submitted).
25 Helaakoski T, Annunen P, Vuori K, MacNeil IA, Pihlajaniemi T, Kivirikko KI: Cloning, baculovirus expression, and characterization of a second mouse prolyl 4-hydroxylase alpha-subunit isoform: formation of an alpha 2 beta 2 tetramer with the protein disulfide-isomerase/beta subunit. *Proc Natl Acad Sci USA* 1995; **92**: 4427–4431.
26 Christmas P, Jones JP, Patten CJ *et al*: Alternative splicing determines the function of CYP4F3 by switching substrate specificity. *J Biol Chem* 2001; **276**: 38166–38172.

27 Christmas P, Ursino SR, Fox JW, Soberman RJ: Expression of the CYP4F3 gene. Tissue-specific splicing and alternative promoters generate high and low K(m) forms of leukotriene B(4) omega-hydroxylase. *J Biol Chem* 1999; **274**: 21191–21199.
28 Kikuta Y, Kusunose E, Endo K *et al*: A novel form of cytochrome *P*-450 family 4 in human polymorphonuclear leukocytes. cDNA cloning and expression of leukotriene B4 omega-hydroxylase. *J Biol Chem* 1993; **268**: 9376–9380.
29 Kikuta Y, Miyauchi Y, Kusunose E, Kusunose M: Expression and molecular cloning of human liver leukotriene B4 omega-hydroxylase (CYP4F2) gene. *DNA Cell Biol* 1999; **18**: 723–730.
30 Greene TA, Powell P, Nzerem C, Shapiro MJ, Shapiro VS: Cloning and characterization of ALX, an adaptor downstream of CD28. *J Biol Chem* 2003; **278**: 45128–45134.
31 Oda T, Muramatsu MA, Isogai T, Masuho Y, Asano S, Yamashita T: HSH2: a novel SH2 domain-containing adapter protein involved in tyrosine kinase signaling in hematopoietic cells. *Biochem Biophys Res Commun* 2001; **288**: 1078–1086.
32 Altare F, Durandy A, Lammas D *et al*: Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* 1998; **280**: 1432–1435.
33 Chua AO, Chizzonite R, Desai BB *et al*: Expression cloning of a human IL-12 receptor component. A new member of the cytokine receptor superfamily with strong homology to gp130. *J Immunol* 1994; **153**: 128–136.
34 Watanabe T, Kitani A, Murray PJ, Strober W: NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat Immunol* 2004; **5**: 800–808.
35 Arunachalam B, Phan UT, Geuze HJ, Cresswell P: Enzymatic reduction of disulfide bonds in lysosomes: characterization of a gamma-interferon-inducible lysosomal thiol reductase (GILT). *Proc Natl Acad Sci USA* 2000; **97**: 745–750.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)