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Lack of association of the pregnane X receptor (PXR/NR1I2) gene with inflammatory bowel disease: parallel allelic association study and gene wide haplotype analysis

The pregnane X receptor gene (PXR/NR1I2) regulates an array of genes involved in the response to xenobiotics.^{1,2} Dysregulation of

this gene may critically influence intestinal barrier defence and susceptibility to inflammatory bowel disease (IBD).³ Recent data from Ireland have suggested strong associations between polymorphisms within the PXR/NR1I2 gene and IBD. Dring *et al* performed a case control study involving 422 patients with IBD (185 ulcerative colitis (UC) and 237 (Crohn's disease (CD)) and 350 healthy controls, using eight candidate polymorphisms in this gene.⁴ Highly significant associations were demonstrated with UC, CD, and IBD as a whole. This effect was most significant for the two individual single nucleotide polymorphisms (SNPs) in the promoter region of this gene; compared between the IBD cohort and controls, rs3814055/-23585 ($p = 0.000008$; odds ratio (OR) 1.62 (95% confidence interval (CI) 1.31-2.00)) and rs1523127/-24381 ($p = 0.0002$; OR 1.50 (95% CI 1.21-1.84)).

We have critically re-evaluated the contribution of these allelic variants of rs1523127/-24381 of the PXR/NR1I2 gene as determinants of disease susceptibility and phenotype in the Scottish population. In addition, we also performed a gene wide association study using a haplotype tagging strategy to assess in detail the overall contribution of this gene to disease susceptibility. A total of 387 UC and 328 CD patients, together with 338 healthy controls (HC), were studied. This study was designed to have 98% power to replicate the previous association with the rs1523127/-24381 variant ($p < 0.05$). In the haplotype analysis, five tagging SNPs (tSNPs) were selected using a multimarker criterion, haplotype $r^2 > 0.80$ to predict all SNPs/haplotypes. This approach was described by Weale and Goldstein and was successfully applied in our previous study of the ABCB1/MDR1 gene.⁵⁻⁸ The exons,

Table 1 Allelic and genotype frequencies of the selected five tagging single nucleotide polymorphisms (tSNPs)

dbSNP ID position	Allele (1/2)	UC	CD	IBD	HC	UC v HC	UC v HC	CD v HC	CD v HC	IBD v HC	IBD v HC
						1 v 2	1/1 v 2/2	1 v 2	1/1 v 2/2	1 v 2	1/1 v 2/2
						p value	p value	p value	p value	p value	p value
						odds ratio	odds ratio	odds ratio	odds ratio	odds ratio	odds ratio
						95% CI	95% CI	95% CI	95% CI	95% CI	95% CI
rs1523127	AA	139 (35.9%)	102 (31.1%)	241 (33.7%)	119 (35.6%)	0.96	0.91	0.69	1.00	0.82	1.00
120983729	AG	190 (49.1%)	186 (56.7%)	376 (52.6%)	167 (50.0%)	0.99	0.97	0.95	1.03	0.97	0.99
	GG	58 (15.0%)	40 (12.2%)	98 (13.7%)	48 (14.4%)	0.80-1.22	0.61-1.52	0.76-1.19	0.62-1.69	0.81-1.17	0.66-1.49
	A	468 (60.5%)	390 (59.5%)	858 (60.0%)	405 (60.6%)						
	G	306 (39.5%)	266 (40.5%)	572 (40.0%)	263 (39.4%)						
rs2461823	CC	152 (40.9%)	132 (40.2%)	284 (40.6%)	119 (35.8%)	0.38	0.71	0.46	0.80	0.38	0.66
121002815	CT	175 (47.0%)	157 (47.9%)	332 (47.4%)	174 (52.4%)	1.11	1.11	1.09	1.10	1.09	1.10
	TT	45 (12.1%)	39 (11.9%)	84 (12.0%)	39 (11.7%)	0.88-1.37	0.68-1.81	0.87-1.37	0.66-1.82	0.90-1.32	0.71-1.70
	C	479 (64.4%)	421 (64.2%)	900 (64.3%)	412 (62.0%)						
	T	265 (35.6%)	235 (35.8%)	500 (35.7%)	252 (38.0%)						
rs7643645	TT	143 (39.7%)	128 (43.2%)	271 (41.3%)	128 (38.5%)	0.79	0.90	0.82	0.62	0.73	0.91
121008187	TC	172 (47.8%)	120 (40.5%)	292 (44.5%)	168 (50.6%)	1.04	1.04	1.04	0.87	1.04	0.96
	CC	45 (12.5%)	48 (16.2%)	93 (14.2%)	42 (12.6%)	0.84-1.29	0.64-1.69	0.82-1.30	0.54-1.42	0.86-1.26	0.63-1.46
	T	458 (63.6%)	376 (63.5%)	834 (63.6%)	424 (62.7%)						
	C	262 (36.4%)	216 (36.5%)	478 (36.4%)	252 (37.3%)						
rs1464603	AA	172 (45.9%)	153 (46.6%)	325 (46.3%)	167 (49.4%)	0.61	1.00	0.72	1.00	0.62	1.00
121009039	AG	159 (42.5%)	138 (42.1%)	297 (42.3%)	130 (38.5%)	0.94	0.98	0.96	1.01	0.95	0.99
	GG	43 (11.5%)	37 (11.3%)	80 (11.4%)	41 (12.1%)	0.75-1.17	0.61-1.58	0.76-1.21	0.62-1.67	0.78-1.15	0.65-1.52
	A	503 (67.2%)	444 (67.7%)	947 (67.5%)	464 (68.6%)						
	G	245 (32.8%)	212 (32.3%)	457 (32.5%)	212 (31.4%)						
rs2472682	TT	42 (11.0%)	35 (11.1%)	77 (11.0%)	39 (11.7%)	0.65	1.00	0.63	0.70	0.97	0.82
121015342	TG	177 (46.2%)	129 (40.8%)	306 (43.8%)	140 (42.2%)	1.06	1.00	0.94	0.90	1.00	0.96
	GG	164 (42.8%)	152 (48.1%)	316 (45.2%)	153 (46.1%)	0.84-1.32	0.62-1.64	0.74-1.19	0.54-1.50	0.82-1.22	0.62-1.47
	T	261 (34.1%)	199 (31.5%)	460 (32.9%)	218 (32.8%)						
	G	505 (65.9%)	433 (68.5%)	938 (67.1%)	446 (67.2%)						

UC, ulcerative colitis; CD, Crohn's disease; HC, healthy controls; 95% CI, 95% confidence interval.

promoter region, and intronic boundaries were resequenced in 16 unrelated Centre d'Etude du Polymorphisme Humain (CEPH) individuals using 10 randomly spaced amplicons across the PXR/NRII2 gene. Haplotypes and their respective frequencies were constructed by further genotyping in 32 CEPH trios.

No association was seen between rs1523127/-24381 ($r^2 = 0.96$ with rs3814055/-23585) SNP and UC, CD, or IBD (A-allelic frequency: 60.5% UC, 59.5% CD, 60.0% IBD, and 60.6% HC; $p = 0.96, 0.69,$ and $0.82,$ respectively) (table 1). The significant rs6785049 (-7635) variant used in Dring's study was in strong linkage with one of the tSNPs used in this study (rs2472682, $r^2 = 0.86$). In the Scottish dataset, this SNP (rs2472682) was not associated with IBD (T-allele: $p = 0.97,$ OR 1.00 (95% CI 0.82-1.22); TT genotype: $p = 0.82,$ OR 0.96 (95% CI 0.62-1.47)). Log likelihood analyses comparing overall haplotypic distribution of the five tSNPs demonstrated no associations with UC, CD, or IBD ($p = 0.90, 0.90,$ and $0.79,$ respectively). There were no associations observed between each of the five tSNPs and the common constructed haplotypes with UC, CD, and IBD, respectively. Genotype-phenotype analyses using the Montreal classification did not show any associations with the studied variants or haplotypes (full data available on request).

These negative data, based on a true candidate gene approach, combine the use of the single marker and multimarker haplotype tagging approach and set a statistical limit to the importance of the contribution of this gene to disease susceptibility in our population. Discordance between the findings of this study and those data presented in Dring's study require clarification and explanation. When the allelic frequencies and homozygosity rates of the rs1523127/-24381 SNP in these two studies are compared, it is of interest to note that differences in the conclusions between these studies may be driven primarily by differences in allelic and genotypic frequencies in healthy controls studied in these datasets (Scottish v Irish controls: A-allele frequencies: 60.6% v 54.8%, respectively, $p = 0.03,$ OR 1.27 (95% CI 1.02-1.58); AA-genotype frequencies: 35.6% v 32.2%, respectively, $p = 0.01,$ odds ratio 1.75 (95% CI 1.12-2.72)). Differences in frequencies in the Scottish and Irish patient populations were less marked (Scottish v Irish cases: A-allele frequencies: 60.0% v 64.3%, $p = 0.05,$ OR 1.20 (95% CI 1.00-1.44); AA-genotype frequencies: 33.7% v 41.1%, $p = 0.17,$ odds ratio 1.33 (95% CI 0.90-1.98)). Thus potentially a number of explanations exist, including cryptic population stratification, genetic heterogeneity, or a type I error in the initial dataset, each of which may explain the divergent findings in these two populations, in whom previous data had suggested consistent founder effects.⁹ Further studies in these and other Northern European populations may help to resolve this issue formally.

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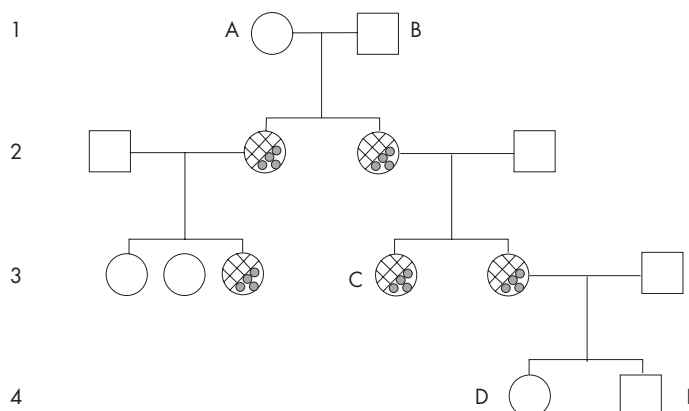
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Genetic association between factor V Leiden and coeliac disease

We identified a family in which a previously unknown genetic association between coeliac disease (CD) and mutation of factor V (factor V Leiden) occurred. The index case was a young woman who abruptly presented with severe ascites due to Budd-Chiari syndrome. At endoscopy, she presented with a scalloped duodenal mucosa suggestive of CD, further confirmed by histology and serological tests. Haematological assessment unveiled the mutation of factor V due to the Arg⁵⁰⁶→Gln mutation at position 506.¹ As shown in fig 1, five siblings were also found to be affected by CD (histology performed in all cases) and were heterozygous carriers of factor V Leiden for the same mutation. The two diseases segregated as one in all cases while no sibling was affected by only one of the two diseases. This pattern of inheritance strongly suggests that the genetic mutation responsible for the onset of CD in this family occurs in a gene which is in high linkage disequilibrium with factor V gene, which is sited on the long arm of chromosome 1. The penetrance of such a gene in our family was found to be 100% as all of the carriers demonstrated coeliac disease at endoscopy.

Although susceptibility to CD is strongly determined by environmental gluten, it is clearly a common genetic disorder. HLA-DQ2 and HLA-DQ8 located in the MHC region on chromosome 6 have been related to a genetic predisposition to CD, although these genes are also present in those not affected by CD.²



A = Refused screening
 B = Dead from cerebral cancer
 C = Index case
 D = 2 and 4 years old not screened

Circle with diagonal lines = Coeliac disease
 Circle with dots = Factor V mutation

Figure 1 Genealogical tree of the family in which an association between factor V Leiden and coeliac disease was unveiled.