INFLAMMATORY BOWEL DISEASE

Lack of association of MYO9B genetic variants with coeliac disease in a British cohort

K A Hunt, A J Monsuur, W L McArdle, P J Kumar, S P L Travis, J R F Walters, D P Jewell, D P Strachan, R J Playford, C Wijmenga, D A van Heel

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See end of article for authors' affiliations

Correspondence to: Professor D A van Heel, Institute of Cell and Molecular Science, Barts and The London, Queen Mary's School of Medicine and Dentistry, Turner St, London E1 2AD; d.vanheel@qmul.ac.uk

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Background and aims: Development of coeliac disease involves an interaction between environmental factors (especially dietary wheat, rye, and barley antigens) and genetic factors (there is strong inherited disease susceptibility). The known human leucocyte antigen (*HLA*)-*DQ2* and -*DQ8* association explains only a minority of disease heritability. A recent study in the Dutch population suggested that genetic variation in the 3′ region of myosin IXB (*MYO9B*) predisposes to coeliac disease. MYO9B is a Rho family GTPase activating protein involved in epithelial cell cytoskeletal organisation. MYO9B is hypothesised to influence intestinal permeability and hence intestinal antigen presentation.
Methods: Four single nucleotide polymorphisms were chosen to tag all common haplotypes of the *MYO9B* 3′ haplotype block (exons 15–27). We genotyped 375 coeliac disease cases and 1366 controls (371 healthy and 995 population based). All individuals were of White UK Caucasian ethnicity.
Results: UK healthy control and population control allele frequencies were similar for all *MYO9B* variants. Case control analysis showed no significant association of any variant or haplotype with coeliac disease.
Conclusions: Genetic variation in *MYO9B* does not have a major effect on coeliac disease susceptibility in the UK population. Differences between populations, a weaker effect size than originally described, or possibly a type I error in the Dutch study might explain these findings.

Coeliac disease, characterised by small intestinal inflammation induced by dietary wheat, rye, and barley, is common in the UK population, with a prevalence of ~1%. Twin and family studies indicate a high inherited predisposition.[1] The only clearly identified genetic risk factor for disease is the human leucocyte antigen (*HLA*)-*DQ* locus. HLA-DQ is critical to present toxic cereal derived peptides to intestinal T cells. However, although the *HLA-DQ2* variant is present in >90% of coeliacs (or DQ8 in a minority), it is also found commonly in healthy individuals (~30%). This suggests that *HLA-DQ2* is necessary but not sufficient for disease development. The *HLA-DQ* locus accounts for <40% of coeliac disease heritability.[1–3]

A recent study suggested that genetic variation in the *MYO9B* gene (encoding myosin IXB) might predispose to coeliac disease.[4] This gene lies within a region of human chromosome 19 first identified in a genome wide linkage study performed in a Dutch population.[5] A meta-analysis of other European linkage studies provided further (albeit weak) support for this finding.[6] Monsuur *et al* recently studied 291 haplotype tagging single nucleotide polymorphisms (SNPs) covering the 99% confidence interval of this linkage peak, and reported significant association in two independent Dutch case control cohorts with variants in the 3′ region of the *MYO9B* gene (which are part of a large haplotype block between exons 15–27).[4] A non-coding variant (A allele of rs2305764) in intron 28 completely explained the observed association and was considered a marker for disease risk. This allele was reported to confer odds ratios for developing coeliac disease of 1.7 (heterozygotes) and 2.3 (homozygotes).

It is currently unknown how this non-coding variant might influence *MYO9B* expression or function. MYO9B is a single motor protein with a Rho GTPase activating domain.[7 8] Rho-GTPase proteins are involved in epithelial cell tight junction assembly and cytoskeletal remodelling.[9] It has been hypothesised that *MYO9B* variants might alter epithelial permeability, thus allowing toxic wheat, rye, and barley components to be presented to the immune system in coeliac disease patients. We sought to assess the impact of *MYO9B* genetic variation in a large cohort of UK coeliac disease patients.

METHODS

Study cohorts

White Caucasian individuals recruited from the UK were studied. Coeliac disease patients (three sites in South East England) were included. Inclusion criteria were as described previously,[10] based on the presence of villous atrophy at diagnosis and (since test introduction) positive antiendomysial/tissue transglutaminase antibody (table 1). Healthy controls were recruited from clinical and laboratory staff volunteers, and from UK National Blood Transfusion Service donors. Population based controls were analysed from the 1958 British Birth Cohort. Ethics committee and local approval were obtained for all cohorts. Genomic DNA was extracted from peripheral blood or from immortalised peripheral blood lymphocyte cell lines (1958 British Birth Cohort).

Genotyping

Three variants (rs2305767, rs2305765, rs2305764) were genotyped using the Sequenom platform (Sequenom, San Diego California, USA). Primers for the multiplex assay were designed using the Sequenom SpectroDesigner software. The Taqman platform (Applied Biosystems, Warrington, UK) was used for rs1457092 genotyping. We also genotyped 342 DNA samples for rs2305767, rs2305765, and rs2305764 with both Taqman and Sequenom methods and obtained identical results. All primer sequences and conditions are available on request. Genotyping on both platforms had >96% success rate. The 384 well plates contained multiple negative controls

Abbreviations: HLA, human leucocyte antigen; SNP, single nucleotide polymorphism; MYO9B, gene encoding myosin IXB

Table 1 Demographics of the UK cohorts and comparison with previously reported Dutch cohorts

	UK coeliac cases	Dutch coeliac cases*	UK healthy controls	UK population controls	Dutch controls†
No of individuals	375	463	371	995	686
Female (%)	73%	69%	48%‡	50%	49%
Age (y) (at Dec 2005) (median (range))	55 (17–86)	44 (3–93)	na (all >16)	48 (48–48)	48 (6–93)
Age at diagnosis (y) (median (range))	42 (0–78)	35 (1–83)			
HLA-DQ2 positive (%) (possession of DQA1*05 + DQB1*02 heterodimer)	94%§	94%	na	na	na
Small intestinal villous atrophy (%) (at diagnosis, Marsh III lesion)	100%	100%			
IgA antiendomysial/tTG antibody status (%)	¶				
Known positive (when untreated)	70.6%	na			
Not performed when untreated	28.2%				
Known IgA deficiency	1.2%				

*Combined cases set 1 and 2 from Dutch study.[4]
†Combined controls set 1 and 2 from Dutch study.[4]
‡Data available on 134 of 371 UK healthy controls, others mostly anonymous blood donors.
§Data available on 249 of 375 UK cases.
¶Serology data available for 344 of 375 UK coeliac cases.
na, not available; tTG, tissue transglutaminase; HLA, human leucocyte antigen.

(no DNA). The 1958 British Birth Cohort samples contained 12 blinded duplicates across genotyping plates. No genotype discrepancies were observed.

A previous study demonstrated that three of these variants (rs2305767, rs2305764, rs1457092) were sufficient to tag 97% of all observed Caucasian haplotypes for the 19 kb block, spanning exons 15–27 of *MYO9B* (this block contains all of the variants showing association in the Dutch population[4]). We genotyped rs2305765 to provide an independent test of the most associated SNP in the Dutch population (rs2305764). We confirmed these two markers to be in almost complete linkage disequilibrium in the UK population, as reported in the Dutch population.[4]

Statistics

Case control association analyses were performed using the Haploview program (version 3.2).[11] Haplotypes were estimated from the unphased individual data using an accelerated expectation-maximisation algorithm.[12] Association was tested using frequency counts in a two tailed χ^2 test for both individual SNPs and common (>1%) haplotypes. Significance was assumed at $p<0.05$. All p values are presented uncorrected for multiple statistical testing. Power calculations were performed using the genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/).[13] Genotype data for the four variants in both control groups was tested (Haploview) and found to be in Hardy-Weinberg equilibrium ($p>0.01$).

RESULTS

We first estimated that our study had 94% power at $p<0.001$ to detect association in the UK coeliac disease and population control cohorts (assuming similar results as reported in the Dutch population for rs2305764 with an allele frequency of 0.379, odds ratio 1.66 for AG genotype, and odds ratio 2.27 for AA genotype). In a ''worst case'' scenario, assuming the lower 95% confidence intervals of genotypic odds ratios reported in the Dutch population, our study had 72% power at $p<0.05$ to detect association.

Table 2 summarises the genotyping results and case control association analyses at the single SNP level. Allele frequencies for all four *MYO9B* variants studied were similar in the UK healthy control and population control populations, as expected. No significant difference in allele frequencies was observed between males (n = 500) and females (n = 495) from the population control cohort (data not shown). No significant association (uncorrected $p<0.05$) was seen with coeliac disease cases compared with healthy or population controls. No significant association was seen between coeliac disease cases and combined control cohorts.

Modest differences in allele frequencies of rs2305764 (reported to be the strongest associated *MYO9B* variant in Dutch coeliac disease[4]) were observed between populations: Dutch healthy controls (37.9%), UK healthy controls (42.3%), and UK population controls (41.1%); Dutch coeliac disease (46.5%) and UK coeliac disease (41.8%).

Table 3 summarises the results of haplotype estimation and haplotype based case control association analyses. Haplotype

Table 2 Case control association analysis of *MYO9B* (gene encoding myosin IXB) variants by allele

Cohort	SNP	Minor/major allele	n with genotypes	Homozygous (minor allele)	Heterozygous	Homozygous (major allele)	Minor allele frequency (%)	p Value *v* healthy controls	p Value *v* population controls
Coeliac disease	rs2305767	G/A	348	65	161	122	41.8	0.61	0.71
(n = 375)	rs1457092	A/C	356	41	170	145	35.4	0.28	0.31
	rs2305765	T/C	371	55	178	138	38.8	0.25	0.22
	rs2305764	A/G	373	55	179	139	38.7	0.16	0.26
Healthy controls	rs2305767	G/A	352	51	183	118	40.5	–	0.82
(n = 371)	rs1457092	A/C	347	46	173	128	38.2	–	0.76
	rs2305765	T/C	364	58	188	118	41.8	–	0.88
	rs2305764	A/G	366	60	190	116	42.3	–	0.57
Population controls	rs2305767	G/A	971	181	434	356	41.0	–	–
(n = 995)	rs1457092	A/C	971	146	437	388	37.5	–	–
	rs2305765	T/C	939	175	428	336	41.4	–	–
	rs2305764	A/G	977	177	450	350	41.1	–	–

All p values are presented without correction for multiple testing.
SNP, single nucleotide polymorphism.

Table 3 Case control association analysis of *MYO9B* (gene encoding myosin IXB) variants by haplotype in UK cohorts

MYO9B SNP haplotype				Cohort*		
rs2305767	rs1457092	rs2305765	rs2305764	Coeliac disease	Healthy controls	Population controls
G	C	C	G	41.7%	39.0% p=0.51	40.3% p=0.31
A	A	T	A	34.7%	37.6% p=0.34	36.7% p=0.21
A	C	C	G	19.3%	17.9% p=0.42	18.0% p=0.52
A	C	T	A	3.8%	3.5% p=0.96	3.8% p=0.81

Rare (<1%) haplotypes are not shown.
All p values are presented without correction for multiple testing.

frequencies for all four *MYO9B* variants studied were similar in the UK healthy control and population control populations. No significant association (uncorrected $p<0.05$, individual values not shown) was seen for coeliac disease cases compared with healthy or population controls.

DISCUSSION

Our understanding of the pathogenesis of coeliac disease is now better than for most other human autoimmune disorders. Recent advances include identification of immunologically dominant wheat T cell epitopes, the role of tissue transglutaminase, and how HLA-DQ2 binds wheat peptides.[14] However, the primary genetic causes outside of the HLA remain largely unknown. Several factors suggest that identification of disease predisposing susceptibility genes should be possible: the disease shows strong heritability, the disease is common (1% prevalence in UK population), and there are highly sensitive and specific diagnostic tests. The HLA locus was recognised as being involved in coeliac disease pathogenesis in the early 1970s but identification of other disease susceptibility genes has been elusive.

A recent study in the Dutch population[4] identified genetic variation in the 3′ region of *MYO9B* as a risk factor for coeliac disease. This study reported significant association with multiple variants in the 3′ region of the *MYO9B* gene, specifically found within a haplotype block between exons 15–27.[4] The association observed in the *MYO9B* gene could be pinpointed to a single SNP (rs2305764, a non-coding variant in intron 28), which could be considered a marker for coeliac disease risk. Although part of the function of MYO9B is understood, it is not clear how this genetic variant might alter MYO9B activity. Indeed, it is possible that the actual disease causing mutation lies elsewhere within *MYO9B* or other close at hand genes, and has not been located by initial resequencing efforts.

We have analysed a large cohort of coeliac patient samples and two sets of control samples to assess the role of *MYO9B* variants in the British population. Our study found no evidence to support the recent findings in the Dutch population.[4] Indeed, the Dutch study found a higher frequency of the rs2305764 A allele in coeliac disease compared with controls whereas the frequency of the A allele was (non-significantly) lower in UK coeliac disease cases compared with controls. We did not comprehensively assess common genetic variation in the entire *MYO9B* gene, which spans 111 575 base pairs and contains five haplotype blocks.[4]

There are a number of potential reasons for the discrepancy between the UK and Dutch studies which are worthy of specific discussion in context here (although most are well recognised in complex genetic trait studies[15]).

- *Explanation 1: The Dutch report is a false positive*. The study by Monsuur and colleagues[4] involved multiple association testing of a large number of several hundred genetic markers, with a second independent case control cohort used to replicate initial findings and so minimise type I error. The association signal (greatest at $p = 2.1\times10^{-6}$ for rs2305764) withstood conservative Bonferroni correction. Nevertheless, despite these strategies, only reports testing *MYO9B* variants in multiple independent populations by other independent investigators will resolve the possibility of type I error.
- *Explanation 2: The current UK report is a false negative*. We examined the statistical power of the current study using genotypic odds ratio results, as published in the Dutch population, and concluded that our study was highly powered. Our study had similar numbers of coeliac cases (375 versus 463 in the Dutch study) and twice as many controls (1346 versus 686 in the Dutch study). If, however, the actual effect size of *MYO9B* was at the lower confidence limits of that reported in the Dutch population, our study was considerably weaker although power was just within accepted bounds. The effect size observed in a initial genetic study is commonly stronger than subsequent reports, as illustrated by the studies of the *NOD2* and *IBD5/5q31* variants in Crohn's disease.[16–19]
- *Explanation 3: There is heterogeneity between British and Dutch populations*. It is possible, although highly unlikely, that the reported coeliac disease association in the Dutch study is due to a founder effect in the Dutch population. Coeliac disease is reported to have similar clinical features, immunopathogenesis (for example, toxic T cell epitopes, serological responses) and prevalence in the UK and Dutch populations. Furthermore, similar frequencies of *NOD2* mutations have been found in Crohn's disease patients from Caucasian populations in the UK, across Europe, the USA, and Australia. We specifically compared demographic and diagnostic data between the British and previously reported Dutch cohorts (table 1). There were minimal differences between the control populations. The coeliac cohorts were very similar in terms of male/female ratio, intestinal biopsy features at diagnosis, and *HLA-DQ* status. Data were not available to assess the proportion of cases with positive family history, a group in which the genetic contribution to disease susceptibility might be higher. Dutch coeliac cases had a lower median age at diagnosis (35 *v* 42 years) but overall there were only small differences in demographics between the populations.

Further genetic studies in different populations are now necessary to resolve whether *MYO9B* variants truly predispose to coeliac disease and, if so, obtain an accurate estimate of

effect size. Our large study provides a definitive result for the UK population. Functional studies may also illuminate the role of MYO9B and its putative role in influencing intestinal barrier function. Knowledge of *MYO9B* (and *HLA-DQ* variants) and analysis of potential gene-gene interactions might simplify identification of other coeliac disease susceptibility genes. The availability of new technologies enabling comparison of common human variation at a genome wide level makes this a realistic prospect.[20] Our understanding of coeliac disease pathogenesis seems set to increase rapidly.

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Authors' affiliations

K A Hunt, R J Playford, D A van Heel, Institute of Cell and Molecular Science, Barts and The London, Queen Mary's School of Medicine and Dentistry, London, UK
J R F Walters, Division of Medicine, Imperial College London (Hammersmith Campus), London, UK
A J Monsuur, C Wijmenga, Complex Genetics Section, University Medical Centre Utrecht, the Netherlands
W L McArdle, Avon Longitudinal Study of Parents and Children, University of Bristol, Bristol, UK
P J Kumar, Department of Gastroenterology, Barts and the London NHS Trust, London, UK
S P L Travis, D P Jewell, Department of Gastroenterology, John Radcliffe Hospital, Oxford, UK
D P Strachan, Division of Community Health Sciences, St George's, University of London, London, UK

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