Contribution of the MHC region to the familial risk of coeliac disease

S Bevan, S Popat, C P Braegger, A Busch, D O'Donoghue, K Falth-Magnusson, A Ferguson, A Godkin, L Hogberg, G Holmes, K B Hosie, P D Howdle, H Jenkins, D Jewell, S Johnston, N P Kennedy, G Kerr, P Kumar, R F A Logan, A H G Love, M Marsh, C J J Mulder, K Sjoberg, L Stenhammer, J Walker-Smith, A M Marossy, R S Houlston

Section of Cancer Genetics, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK
S Bevan
S Popat
A M Marossy
R S Houlston

University Children's Hospital, Steinwiesstrasse 75, Zurich, Switzerland
C P Braegger

University Children's Hospital, Eberhard-Karls-Universitat, Tubingen, Germany
A Busch

Gastroenterology and Liver Unit, St Vincent's Hospital, Dublin, Ireland
D O'Donoghue

Department of Paediatrics, Linkoping University, Sweden
K Falth-Magnusson

Department of Medicine, Western General Hospital, Edinburgh, UK
A Ferguson

Gastroenterology Unit, The Radcliffe Infirmary, Oxford, UK
A Godkin
D Jewell

Department of Gastroenterology, Norrkoping Hospital, Sweden
L Hogberg
L Stenhammer

Department of Medicine, Derbyshire Royal Infirmary, Derby, UK
G Holmes

University Surgical Unit, Northern General Hospital, Sheffield, UK
K B Hosie

Division of Medicine, St James's University Hospital, Leeds, UK
P D Howdle

Abstract

Susceptibility to coeliac disease is genetically determined by possession of specific HLA-DQ alleles, acting in concert with one or more non-HLA linked genes. The pattern of risk seen in sibs and twins in coeliac disease is most parsimonious with a multiplicative model for the interaction between the two classes of genes. Based on a sib recurrence risk for coeliac disease of 10% and a population prevalence of 0.0033, the sib relative risk is 30. To evaluate the contribution of the MHC region to the familial risk of coeliac disease, we have examined haplotype sharing probabilities across this region in 55 coeliac disease families. Based on these probabilities the sib relative risk of coeliac disease associated with the MHC region is 3.7. Combining these results with published data on allele sharing at HLA, the estimated sib relative risk associated with the MHC region is 3.3. Therefore, the MHC genes contribute no more than 40% of the sib familial risk of coeliac disease and the non-HLA linked gene (or genes) are likely to be the stronger determinant of coeliac disease susceptibility.
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Gluten sensitivity, or coeliac disease, is the result of T cell sensitisation and includes a range of mucosal abnormalities that may lead to malabsorption.[1] Studies of small bowel biopsies from first degree relatives of coeliac patients provide compelling evidence that genetic factors influence susceptibility to the disease.[2] Different studies have reported varying risks of coeliac disease among relatives. Reported estimates vary from under 5% to over 20% with most being 10%.[2] This variation probably results not only from genetic and environmental heterogeneity among populations, but from differing diagnostic criteria between studies. Further support for an inherited predisposition to develop coeliac disease comes from twin studies. The concordance rate of coeliac disease in monozygotic twins is around 70%.[2] Incomplete concordance between monozygotic twin pairs suggests that additional factors might be involved, although not all the twin pairs studied had proven monozygosity, and some of the twin pairs had insufficient long term follow up to preclude the future development of coeliac disease.

Coeliac disease shows a strong HLA association. The DQ2 molecule encoded by the alleles β1*0201 and α1*0501 is possessed by 95% of coeliac patients compared to 20-30% of controls.[3] This DQ(α1*0501,β1*0201) heterodimer can be encoded in cis or in trans configuration. The possibility that the DQ2 molecule conferring susceptibility to coeliac disease might be unique has been excluded by showing that the DQβ1*0201 and DQα1*0501 alleles do not show any disease specific sequences in affected subjects.[3] The difference in concordance rates between monozygotic twins and HLA identical sibs (70% *v* 30%) implicates non-HLA genes in the genetic predisposition to coeliac disease.[2 3] One caveat of this is that studies which have been reliant on establishing HLA identity in the past depended largely on serological testing from markers at one or two HLA loci and did not examine HLA identity across the entire HLA class D region. Therefore, subjects assumed to be HLA class II identical may not be identical.

Although the principal association between coeliac disease and the HLA system is determined by the DQα1*0501/DQβ1*0201 heterodimer, this does not preclude involvement of other genes in the MHC acting as susceptibility genes. In order to evaluate the contribution of the MHC gene region to the familial risk of coeliac disease, we have studied the haplotype sharing probabilities across this region in 55 coeliac disease families.

Material and methods

PATIENTS AND FAMILIES

Fifty five coeliac disease families containing 84 affected sibships were studied. All were of northern European ancestry. The diagnosis of coeliac disease was established in all cases by demonstration of severe enteropathy with villous atrophy on small bowel biopsy. All made a symptomatic recovery and the majority of patients had a follow up biopsy showing mucosal recovery on a gluten free diet. All affected family members were typed for HLA-DQ alleles by PCR-SSP (details from O Olerup, personal communication).

MARKER TYPING

DNA was salt extracted from EDTA venous blood samples. Subjects were genotyped at the following loci: D6S260, D6S273, and D6S426. A total of 12.5 ng of genomic DNA was ampli-

Department of Child Health, University Hospital of Wales, Cardiff, UK
H Jenkins

Department of Medicine, The Queen's University of Belfast, Belfast, UK
S Johnston
A H G Love

Department of Clinical Medicine, St James's Hospital, Dublin, Ireland
N P Kennedy

Gastroenterology Unit, Royal Shrewsbury Hospital, Shrewsbury, UK
G Kerr

Department of Gastroenterology, St Bartholomew's Hospital, London, UK
P Kumar

Department of Public Health and Epidemiology, University Hospital, Nottingham, UK
R F A Logan

University Department of Medicine, Hope Hospital, Salford, Manchester, UK
M Marsh

Hepatogastroenterology, Rijnstate Hospital, Arnhem, The Netherlands
C J J Mulder

Department of Medicine, University of Lund, Malmo, Sweden
K Sjoberg

University Department of Paediatric Gastroenterology, Royal Free Hospital, London, UK
J Walker-Smith

Correspondence to:
Dr Houlston.

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fied using PCR in 10 µl of 75 mmol/l Tris-HCL (pH 9), 0.01% (v/v) Tween, 1.5 mmol/l $MgCl_2$, 0.25 U *Taq* polymerase, 20 mmol/l $(NH_4)_2SO_4$, 0.1% (w/v) BSA, 0.2 mmol/l each dNTP, and each fluorescently labelled primer at 5 ng μl^{-1}. Thirty cycles of the PCR were carried out at 94°C for one minute, 55-50°C for one minute, and 72°C for one minute with a final extension step at 72°C for 10 minutes. The products were electrophoresed through 6% denaturing polyacrylamide gels and were sized by an automatic ABI 377 (PRE-ABI), using the programs GENESCAN and GENOTYPER to allocate alleles.

Marker allele frequencies were estimated from observed relative frequencies in founder subjects. (These were identical to the relative frequencies seen across all typed subjects.) Distances between markers were obtained from the Genethon (http://www.cephb.fr/ceph-genethon-map.html) database.

STATISTICAL ANALYSIS

Linkage analysis of the data was undertaken in accordance with the maximum likelihood score (MLS) approach implemented in the program MAPMAKER-SIBS.[4] This program calculates, for a sample of affected sib pairs, the MLS and the corresponding maximum likelihood estimate of the IBD vector. To assess the contribution of the MHC to sibs with coeliac disease, haplotype sharing among affected sib pairs was assessed with the "all pairs" option. The allele sharing probabilities of affected sib pairs (ASPs) depend on the contribution any gene makes to the genetic variation of the trait. The 2, 1, and 0 sharing probabilities are given by: $Z_0=\alpha_0(1/\lambda_s)$, $Z_1=\alpha_1(\lambda_o/\lambda_s)$, $Z_2=\alpha_2(\lambda_{mz}/\lambda s)$,[5] where Z_i=P (sibs share i marker alleles IBD | ASP) and α_i=P (sibs share i marker alleles IBD). For i=0, 1, 2, α_i equals 1/4, 1/2, and 1/4 respectively. When a marker is unlinked, $Z_i=\alpha_i$, $\lambda_s=K_s/K$, $\lambda_o=K_o/K$, and $\lambda_{mz}=K_{mz}/K$ where K_s, K_o, and K_{mz} are the sib, offspring, and monozygotic recurrence risks respectively, and K is the population risk of the disease. These formulae hold true irrespective of the mode of inheritance at the disease locus, the number of alleles, and their frequencies, penetrance, and population prevalence.[5] Using the allele sharing probabilities among ASPs the relative risks of coeliac disease associated with the MHC can be determined. To compare the haplotype sharing probabilities observed in this study with published data, values of Z_2 and Z_1 were combined. Heterogeneity is then given by: $X^2_{overall}-(\Sigma t_i)^2/n$, where i refers to individual study ASP probabilities, n the number of studies in which ASP probabilities have been determined, and t_i is X_i^2.

Results

All of the affected subjects in the families studied had HLA class II genotypes compatible with possession of the DQ(α1*0501,β1*0201) alleles. The marker D6S273 lies within the HLA system and D6S260 and D6S426 lie 15 cM upstream and downstream of D6S273 respectively. Table 1 shows the lod scores and allele sharing probabilities for the three markers for all the families studied. As expected, there is evidence for excess sharing at HLA and at the two close flanking markers. Significant linkage was seen across the whole region, with a maximum lod score of 3.06 between markers D6S273 and D6S426. Based upon the allele sharing probabilities at D6S273, the sib relative risk attributable to the MHC region is 3.6.

Table 1 Lod scores and allele sharing probabilities across the MHC region

Locus	*Distance (cM)**	*Lod score*	Z_2	Z_1	Z_0
D6S260	29.93	0.62	0.33	0.5	0.17
D6S273	44.96	2.42	0.43	0.5	0.07
D6S426	60.44	2.31	0.41	0.5	0.09

*Derived from http://www.cephb.fr/ceph-genethon-map.html

Estimates of the prevalence of coeliac disease in Europe ranged from a high of 1 in 300 in western Ireland to between 1 in 1000 and 1 in 2000 in other regions.[6–8] The true prevalence of coeliac disease is likely to have been underestimated because a substantial number of subjects are asymptomatic or have only mild symptoms. Studies using antibodies for screening have shown that the prevalence of coeliac disease among some symptom free subjects is as high as 1 in 200.[9–13] Based on a prevalence of 0.005, a 10% sib recurrence risk equates to a relative risk of 20.

The contribution of the MHC region to this relative risk depends on the model of interaction between the HLA linked and unlinked loci. They could interact either additively (that is, the penetrance of the disease is represented by the sum of the penetrances contributed by two or more loci) or multiplicatively (that is, the penetrance of the disease is the product of the penetrances contributed by two or more loci). The familial risks seen in sibs and monozygotic twins are most parsimonious with a multiplicative model, since a simple additive model would violate the mathematical relationship between sib, parent-offspring, and monozygotic twin relative risks. (The relative risk in monozygotic twins, λmz, is given by: 4λs-2λpo-1[5].) Under the assumption of a multiplicative model, the contribution of the MHC region to the overall familial risk of coeliac disease (given by: $\ln\lambda_{MHC}/\ln\lambda_s$) will be 44%. A number of other studies have also examined the sharing across the HLA region in sibships with coeliac disease.[14–25] In all but one of these, sharing between ASPs has been based on determination of HLA status. Table 2 details the allele sharing probabilities and derived sib relative risks associated with the HLA linked locus in the published studies. There is no evidence for heterogeneity between these studies (χ^2=11.1, 16 df, p>0.1) so allowing data to be combined for a more precise estimate of the familial risk of coeliac disease associated with the MHC. Using the pooled data, the estimated sib relative risk of coeliac disease associated with the MHC is 3.3 (95% CI 2.3–4.9). Based on this estimate, the relative risk associated with the MHC region does not account for more than 39% of the familial risk of coeliac disease.

Table 2 ASP haplotype/allele sharing probabilities in this study and published reports. Also shown are the derived sib relative risks

Reference	Determination of haplotype sharing	No of sib pairs	Z_2	Z_1	Z_0	λ_S
14	HLA typing	18	0.56	0.33	0.11	—
15	HLA typing	3	1.0	0.0	0.0	—
16	HLA typing	14	0.57	0.43	0.0	—
17	HLA typing	5	0.4	0.6	0.0	—
18	HLA typing	13	0.54	0.38	0.08	—
19	HLA typing	5	0.6	0.4	0.0	—
20	HLA typing	4	0.5	0.25	0.25	—
21	HLA typing	14	0.57	0.36	0.07	—
22	HLA typing	3	0.67	0.33	0.0	—
23	HLA typing	28	0.57	0.36	0.07	—
24	HLA typing	1	0.0	1.0	0.0	—
25	HLA typing	10	0.4	0.4	0.2	—
26	HLA typing	8	0.5	0.5	0.0	—
27	HLA typing	28	0.36	0.57	0.07	—
28	HLA typing	18	0.56	0.33	0.11	—
All HLA studies		158	0.51	0.42	0.07	3.6
29	Microsatellite markers	110	0.5	0.41	0.09	2.8
Present study	Microsatellite markers	84	0.43	0.5	0.07	3.7
All studies						3.3

Discussion

The mode of inheritance of coeliac disease is unknown. Using family data Pena *et al*[30] proposed the involvement of two distinct unlinked genes in the aetiology of coeliac disease. A prerequisite for developing coeliac disease was homozygosity at the HLA unlinked locus and participation of an independently inherited gene or genes located in or tightly linked to the major histocompatibility system acting in a dominant fashion. This proposal was supported by some but not all other studies.[27 31] Most agreed on recessivity at the HLA unlinked locus but differed with respect to dominance or recessivity at the HLA linked disease susceptibility locus. It is clear, however, that since expression of the DQ(α1*0501,β1*0201) heterodimer is responsible for the HLA association, constitutive haplotypes will not behave in a simple mendelian recessive or dominant fashion. It is possible that part of the familial risk may be environmentally determined. The concordance rates between monozygotic twins and HLA identical sibs provide considerable support for the hypothesis that the familial risk of coeliac disease is genetically determined. The only caveat of this is the assumption that the environmental correlation between these two types of sibs is similar.

On the assumption that the familial risk of coeliac disease is genetically determined, we previously estimated the sib relative risk associated with the HLA linked genes to be 1.8 under a dominant model, 4.6 under a recessive model, and 4.0 under a codominant model.[2] These estimates are, however, indirect ones derived from the frequency and genotypic risk of the DQ(α1*0501,β1*0201) heterodimer. In this present study the impact of the MHC region on the familial risk of coeliac disease was derived from the haplotype sharing probabilities. Based on these probabilities and those published in other studies, the familial risk associated with the MHC region is less than 4. Given that the risk of coeliac disease in sibs is increased by around 20-fold, the MHC region will not account for more than two fifths of the familial risk and the second gene or genes, unlinked to HLA, are likely to be the stronger determinant of disease susceptibility than the HLA linked locus.

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