LETTER TO JMG

Genome screening of coeliac disease


Coeeliac disease is caused by T cell sensitisation of the intestine to cereal prolamins, which results in a range of mucosal abnormalities that may lead to malabsorption.1 The population prevalence in western countries is ∼1 in 200.2-4 Evidence for an inherited predisposition to coeliac disease comes from studies of first degree relatives of patients and studies of twins.5-6 A strong association is seen between coeliac disease and the HLA-DQ (α1*05, β1*02) heterodimer (DQ2) which is present in approximately 95% of patients,7-9 compared with 20-30% of healthy subjects.10 11 The difference in concordance rates between monozygotic twins and HLA identical sibs (80-100% v 25%) implicates non-HLA genes in the genetic predisposition to coeliac disease.11 The overall relative risk in sibs is at least 20 and is therefore four-fold higher than that attributable to HLA alone under model of inheritance.7 Genome linkage searches carried out on Irish,12 Italian,13, and UK14 coeliac disease families have identified a number of potential sites for the location of non-HLA linked genes. The putative candidate loci detected in the three studies are, however, largely inconsistent and the findings have not been replicated in other populations.15-19 Here we report the results of a genome screen of 24 multiplex families with coeliac disease and discuss the findings of this study in relation to previously published analyses.

METHODS AND RESULTS

Twenty-four families with two or more members affected with coeliac disease were recruited for this study (fig 1). Nine of these families (Nos 1, 3, 4, 6, 30, 32, 39, 43, and 44) have been used in a previous study of candidate regions.16 All the families were of northern European ancestry. Twelve of the families were recruited from the UK, nine from Sweden, two from Switzerland, and one from The Netherlands. All affected family members had symptomatic disease and were diagnosed according to the revised criteria formulated by the European Society for Paediatric Gastroenterology. Hepatology, and

Figure 1  Pedigree structures of the families studied.
D19S894, nominal p value 0.02).

and at chromosome 19p13.3 (maximal between D19S424 and chromosome 6p21 the strongest linkage was obtained at

there was evidence of excess sharing at both HLA and close

strongest region of linkage was observed at chromosome

shows the distribution of NPL scores by chromosome. The

jects is greater than expected under random segregation. Fig 2

which marker allele sharing by descent between affected sub-

model independent and effectively measure the extent to

approval. All family members were typed for the HLA-DQ

with a mean of 25 years. Blood samples and clinical data were

subjects, 36 male and 52 female, giving a male to female ratio

of 1:1.5. The age at diagnosis ranged from 9 months to 74 years

percent of affected subjects in the families studied had HLA

class II genotypes compatible with possession of the DQA1*05,

Nutrition (ESPGHAN).\textsuperscript{23} Families consisted of 88 affected

DISCUSSION

Three genome wide linkage searches of coeliac disease have

been published,\textsuperscript{12–14} but no regions of significant linkage have

been reported consistently. The first of these was reported by

Zhong \textit{et al}.\textsuperscript{12} Linkage of coeliac disease to five chromosome

regions outside HLA was detected: 6p23 (telomeric to HLA),

7q31.3, 11p11, 15q26, and 22cen. In addition to these regions,

there was also some evidence of linkage to chromosome

19p13.3 (p<0.05) as seen in our study. The second study was

reported by Greco \textit{et al}.\textsuperscript{13} In addition to HLA, there was some

evidence for linkage to 5qter and 11qter, but no evidence to

support the notion that there is a locus on chromosome 6p

distinct from HLA as proposed by Zhong \textit{et al}.\textsuperscript{12} A follow up

study of candidate regions reported by Greco \textit{et al}" failed to

confirm linkage to chromosome 11qter, but gave some support

for linkage to chromosome 5q (maximum MLS 2.9). We were

unable to find any evidence of linkage to chromosome 5q

region in our dataset. The genome wide study recently

reported by King \textit{et al}" was only based on analysis of 16 UK

multiplex coeliac disease families. Two regions, chromosomes

10q23.1 and 16q23.3, provided evidence of linkage in

multipoint analyses using five markers (nominal p values of

0.006). There was limited evidence for linkage to HLA in the

study (lod score <1.2).

In addition to a failure to confirm linkage to chromosome

6p23, neither our study nor that of Greco \textit{et al}" and King \textit{et al}" found evidence for linkage to chromosome 11p11, as

originally proposed by Zhong \textit{et al}.\textsuperscript{12} The proposition of an

additional chromosome 6p locus made by Zhong \textit{et al}" was

based on typing 30 of 45 affected sib pairs at D6S259, and

flanking markers within 1 cM showed no significant evidence

for linkage. In our study, there was no evidence for linkage

to this region. Similarly, there is no support for linkage of

celiac disease to a locus on chromosome 6 telomeric to HLA in

the studies reported by Greco \textit{et al}" and Brett \textit{et al}".

Although we found no significant evidence for linkage to

chromosomes 5qter or 11qter as initially suggested by Greco \textit{et

al},\textsuperscript{13} we cannot preclude these loci as sites of a non-HLA linked

locus. We did, however, find some evidence for linkage at

chromosome 19p13.3, raising the possibility that this region

may define an additional locus.

While coeliac disease is oligogenic, the simple HLA

association coupled with the fact that the environmental trig-
gger is identical in all subjects theoretically makes genetic

analysis of the trait more amenable to dissection than other

complex disorders, such as non-insulin dependent diabetes

mellitus or asthma. As expected, our study confirms HLA as a

risk factor for coeliac disease. Two other regions showed

evidence for comparable linkage, chromosomes 19p13.3 and

4p14. However, neither attains the level of statistical

significance desired for genome wide linkage searches and

hence require confirmation.

The genetic analysis of coeliac disease potentially has many

advantages over other complex traits, both from the perspec-
tive of the magnitude of the genetic trait and the ease of

defining affected status. However, the studies reported to date

suggest that identifying the non-HLA linked component may

not be straightforward. It is possible that the discrepancies in

letters

Figure 2 NPL scores by chromosome.
findings between the four studies are because of differences in the contribution of genetic factors to coeliac disease in the different populations analysed. Alternatively and specifically related to the plethora of significant findings reported by Zhong et al., this might reflect in part the structure of the families studied. Specifically, 31 of the ASPs belonged to three families. The inclusion of large sibships in which no typing information is available may well have led to artificially inflated support for specific regions.

If disease susceptibility is the result of more than one gene, the power to detect linkage depends on the relative contribution to the overall familial risk made by each locus and how the different loci interact. The power of this and previously published studies to detect linkage and the effect of varying heterogeneity between zero and 75% was assessed by non-parametric means using the program ALLEGRO. In the absence of a definitive model for the mode of inheritance of coeliac disease, genotypes were simulated under models chosen on the basis that the sib relative risk conferred by the disease gene was ~3.3. This follows from the assumptions that the overall disease prevalence is 0.005 and that the HLA linked developing coeliac disease. homozygosity at an HLA unlinked locus is a perquisite for sensing the overall familial risk made by each locus and how the power to detect linkage falls markedly in the presence of heterogeneity, with a power of less than 20% to detect linkage under heterogeneity, are required to support or refute the possible location of putative non-HLA linked genes.

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REFERENCES


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**ECHO**

**Covert operations**

Gene therapy holds promise for treating liver diseases. A review in *Gut* by Schmitz et al describes significant progress in search of new options based on genetic techniques for treating intractable diseases such as chronic hepatitis B and C infection and primary and secondary liver cancers.

For example, in viral hepatitis gene transfer of IFNα by adenovirus vector prevents the disease in mice, and combined administration of one adenovirus vector containing selected sequences of hepatitis C virus and another containing interleukin 12 genes potentiated a cellular immune response.

In liver cancer drug sensitisation and genetic immunotherapy look promising. Drug sensitisation—transferring a gene for a foreign enzyme to convert a non-toxic prodrug into an anticancer drug—a suicide gene—is best exemplified by the HSV tk gene. The encoded enzyme converts the prodrug gancyclovir into a toxic compound which halts DNA synthesis. Added to this is an appreciable bystander effect—when the metabolite diffuses into the surrounding cells, stimulating local inflammation and antitumour immunity, owing to the death of cancer cells. Unwanted effects on healthy tissue are reduced by injecting the vector locally or limiting gene expression with tumour specific promoters.

Genetic immunotherapy offers ways of stimulating the immune system against cancer cells. Injecting a virus vector encoding interleukin 12 into rats with hepatocellular carcinoma in the liver and into mice with metastatic colon cancer achieved complete eradication of tumours and induced antitumour immunity. Introducing this vector through the intrahepatic artery increased antitumour activity in an aggressive hepatocellular carcinoma model induced by a carcinogen.

Therapeutic approaches with HSV tk and interleukin 12 are sufficiently promising for trials in humans to be under way.