Coeliac disease (CD) is an autoimmune enteropathy triggered by ingestion of wheat gluten or related protein from rye and barley, and is one of the most frequently occurring, treatable, lifelong disorders. Undetected or untreated CD may cause other, more severe, complications later, such as autoimmune diseases, osteoporosis, neurological disorders, and infertility.

Several studies have shown CD clusters in families with a sibling relative risk of 20–60 and high concordance between monozygotic twins (75%). This indicates a strong genetic component to coeliac disease. As expected in complex autoimmune diseases, human leukocyte antigen (HLA) linked genes are the main genetic factor involved in CD. More than 90% of patients suffering from CD share the major histocompatibility complex II class HLA-DQ2 haplotype. Most of the remainder present HLA-DQ8. However, there are patients who present neither HLA-DQ8 nor HLA-DQ2 on their antigen-presenting cells. These genetic findings strongly indicate the involvement of other genes in the pathogenesis of coeliac disease. So far, various genome-wide linkage analyses have been performed in order to identify non-HLA linked genes responsible for CD. A recent study by Greco et al. on Italian coeliac families demonstrated the existence of a genetic risk factor on chromosome 5 (5q31–33), which has been reported in other linkage studies in different populations. This genomic region contains several candidate genes for CD, including the interleukin (IL12B) gene, the polymorphisms of which do not seem to be associated with CD, and the gene encoding for CD14.

CD14 is a multifunctional receptor involved in the innate immune response. In fact, it is thought to be one of the most important receptors for lipopolysaccharide and other bacterial wall-derived components. However, it is well known that CD14 has several other functions, including the clearance of apoptotic cells. It is constitutively expressed, primarily on the surface of monocytes, macrophages, and neutrophils (mCD14); a soluble form, sCD14, has been isolated from serum and is derived both from secretion of CD14 and from enzymatically cleaved glycosyl-phosphatidylinositol anchored mCD14.

Promoter polymorphisms in three selected populations in order to explore the influence of CD14 in this common autoimmune disorder.

In our study the allelic and genotype frequencies of C1–159TT did not differ among 115 coeliac patients carrying the typical HLA haplotype (DQ2 or DQ8), 37 coeliac patients with an atypical HLA haplotype, and 180 selected healthy controls of the same ethnic origin. There was a significant skewing in coeliac patients with for the genotype frequencies at position –1359 compared with healthy controls. This difference is mainly due to the higher frequency of (−1359)TT subjects in coeliac patients carrying the atypical HLA haplotype.

Several hypotheses could explain the involvement of CD14 in coeliac disease; our findings show the importance of apoptosis regulation. Furthermore, they indicate that the genes encoding proteins, which play a key role in this process, are potential candidates for CD.

**METHODS**

**Patients and controls**

For this study 115 Italian coeliac patients with typical HLA haplotypes (either DQ2 or DQ8), 37 Italian coeliac patients with atypical HLA haplotypes, and 180 pan-ethnic controls (healthy blood donors with no history of either gastrointestinal or autoimmune diseases) were enrolled. Diagnosis of coeliac disease was performed following the ESPGHAN...
CD14 genotyping
DNA was extracted from peripheral whole blood using standard laboratory protocols. The detection of the bi-allelic polymorphism at position −159 was performed following the PCR-RFLP technique already described by Baldini et al.\textsuperscript{11} The detection of the bi-allelic polymorphism at position −1359 was performed with a modified version of the PCR-RFLP technique described by Holla et al.\textsuperscript{13} Briefly, PCR was performed with the forward primer 5′-AGCACCTTGGAGGCCAGG-3′ and the reverse primer 5′-CCACATCCTGACTCTTGGG-3′, both designed on the basis of the published CD14 human sequence with the software Primer Express 1.5 (Applied Biosystems, Foster City, CA, USA). After an initial denaturation step at 95°C for 10 min, 35 cycles with a denaturation step at 95°C for 30 s, an annealing step at 62°C for 30 s, and an elongation step at 72°C for 30 s were carried out. The PCR reaction mix was 1.5 mMol MgCl\textsubscript{2}, 800 μmol/dl NTPs, 200 μmol/l for each primer and contained 1 U of AmpliT\textregistered Tag Gold (Applied Biosystems). PCR products were digested for 2 h at 37°C with the endonuclease Fok\textsubscript{I} (New England Biolabs, Beverly, MA, USA) and subsequently separated on a 2% agarose gel. The G allele showed one fragment of 303 bp, while the T allele showed two fragments of 215 and 88 bp.

Statistical analysis
Gene frequencies were calculated from the observed number of genotypes. The calculation of differences in allele frequencies was calculated by Fisher’s exact test. To test the significance of differences in genotype frequencies a $\chi^2$ test was used. Holm’s correction for multiple tests was performed and only corrected p values ($P_{\text{corr}} < 0.05$) were considered to be significant. All the statistical analyses were carried out with SPSS software (Version 6.1.3; SPSS Inc., Chicago, IL, USA).

RESULTS
Allelic frequencies and genotype frequencies for the position −159 in the three populations studied are shown in Table 1. The frequency of the (−159)C allele was 53%, 46%, and 50% in typical HLA coeliac patients, atypical HLA coeliac patients, and healthy controls, respectively. No differences were observed in genotype frequencies between the three populations.

Table 1
<table>
<thead>
<tr>
<th>Allele and genotype frequencies for the G to T transversion at position −159 in the promoter region of the CD14 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coeliac patients</strong></td>
</tr>
<tr>
<td><strong>Base</strong></td>
</tr>
<tr>
<td>T</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>G/C</td>
</tr>
<tr>
<td>T/T</td>
</tr>
<tr>
<td>C/T</td>
</tr>
<tr>
<td>G/T</td>
</tr>
</tbody>
</table>

*Allelic frequency (Fisher’s exact test) typical v. atypical HLA coeliac patients: $p = 0.302$; typical HLA coeliac patients v. healthy controls: $p = 0.500$; atypical HLA coeliac patients v. healthy controls: $p = 0.581$.

Table 2
<table>
<thead>
<tr>
<th>Allele and genotype frequencies for the G to T transversion at position −1359 in the promoter region of the CD14 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coeliac patients</strong></td>
</tr>
<tr>
<td><strong>Base</strong></td>
</tr>
<tr>
<td>T</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>G/T</td>
</tr>
<tr>
<td>C/T</td>
</tr>
<tr>
<td>G/T</td>
</tr>
</tbody>
</table>

*Allelic frequency (Fisher’s exact test) typical v. atypical HLA coeliac patients: $p = 0.631$; typical HLA coeliac patients v. healthy controls: $p = 0.262$; atypical HLA coeliac patients v. healthy controls: $p = 0.0123$.

DISCUSSION
Coeliac disease is one of the most common multifactorial autoimmune disorders in the white population (1 in 200 individuals). The principal genetic (HLA-linked genes) and environmental (wheat gluten) factors responsible for CD are well known, but few data are available for non-HLA-linked genes.
In this case-control study we have assessed the frequency of two polymorphisms in the promoter region of the CD14 gene, which regulate the expression of the protein in three selected populations.

LeVan et al. demonstrated that −159 TT allele promotes CD14 gene transcription and that homozygous subjects for this allele have increased CD14 serum levels. In our study the allelic and genotype frequencies of C (−159)T did not differ between the 115 coeliac patients carrying the typical HLA haplotype (DQ2 or DQ8), the 37 coeliac patients with an atypical HLA haplotype, and the 180 selected healthy controls of the same ethnic origin. Interestingly, there was a significant skewing in coeliac patients with atypical HLA for the genotype frequencies at position −1359 compared with healthy controls. This difference is mainly due to the higher frequency of (−1359)TT subjects in coeliac patients carrying the atypical HLA haplotype. The frequency of (−1359)TT patients was also greater in coeliac patients with typical HLA, even though our results do not reach statistical significance. As demonstrated by Vercelli et al., (−1359)TT subjects have lower serum levels of CD14. The odds ratio of developing coeliac disease for (−1359)TT atypical HLA coeliac patients is 9.22 (95% CI 2.10 to 40.50).

Several hypotheses could explain the involvement of CD14 in coeliac disease. Firstly, individuals with low levels of sCD14, and thus mCD14, might be more prone to Gram-negative bacterial infections. These infections may disrupt the permeability of intestinal epithelia, leading to an increased concentration of gluten in the lamina propria, where most of the gluten-reactive lymphocytes are localised. Such individuals may be more susceptible to developing coeliac disease. Unfortunately, no studies linking the G(−1359)T polymorphism to a risk of infection have been carried out. Moreover, a recent study by Agnese et al. showed a significant correlation between TLR4 polymorphisms and an augmented risk of gram negative bacterial infection: no association was found between CD14 C (−159)T polymorphism and the incidence of infection or patients’ outcome.

Another possibility, and in our opinion the most interesting, may derive from the role of CD14 in apoptosis regulation. Indeed, the CD14 monocyte differentiation factor 2 (MRF2) protein, which plays a key role in this process, are potential candidates for CD. A larger cohort of typical HLA coeliac patients should probably be screened for CD. Finally, our findings demonstrate the importance of apoptosis regulation. They indicate that the genes encoding proteins, which play a key role in this process, are potential candidates for CD. A larger cohort of typical HLA coeliac patients should probably be screened for CD.

ACKNOWLEDGEMENTS
This work was supported by a grant (PRIN 2002) from the Italian Ministry of Research. Michele Boniotto is the recipient of a long-term fellowship from Trieste University. The authors wish to thank Dr Kevin Danton for his help in revising the manuscript.

Authors’ affiliations
M Boniotto, L Braida, A Ventura, A Amoroso, S Crovella, Dipartimento di Scienze della Riproduzione e dello Sviluppo, Università di Trieste, Via dell’Istria 65/1, 34137 Trieste, Italy
S Peracca, Dipartimento di Pediatria, Universita’ di Napoli Federico II, Via Pansini 5, 80131 Naples, Italy
A Amoroso, S Crovella, Dipartimento di Genetica, IRCCS Burlo Garofolo, Via dell’Istria 65/1, 34137 Trieste, Italy
Correspondence to: Sergio Crovella, Dipartimento di Scienze della Riproduzione e dello Sviluppo, Università di Trieste, Via dell’Istria 65/1, 34137 Trieste, Italy, crovella@burlo.trieste.it
A polymorphism in the CD14 gene is associated with circulating soluble CD14 levels.

Gastroenterology and Nutrition.

A polymorphism in the 5'-flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E.


Promoter polymorphisms of the CD14 gene in Italian patients with coeliac disease

M Boniotto, L Braida, A Ventura, S Percopo, A Amoroso and S Crovella

doi: 10.1136/jmg.40.9.e108

Updated information and services can be found at:
http://jmg.bmj.com/content/40/9/e108

These include:

References
This article cites 38 articles, 17 of which you can access for free at:
http://jmg.bmj.com/content/40/9/e108#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Immunology (including allergy) (604)
- Coeliac disease (12)
- Metabolic disorders (329)
- Calcium and bone (307)
- Drugs: endocrine system (107)
- Inflammatory bowel disease (16)
- Osteoporosis (17)
- Reproductive medicine (519)
- JMG Online mutation reports (168)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/