Inflammatory bowel disease (IBD) comprises mainly ulcerative colitis (UC) and Crohn’s disease (CD) characterised by long term intestinal inflammation attributable to an exaggerated immune response. The aetiology is still unknown. Genetic susceptibility has been shown by epidemiological and genetic investigations. Recent genome-wide linkage studies and case-control association studies have shown several susceptibility regions for UC and identified the CARD15 (NOD2), the first susceptibility gene for CD. The mutation of TLR4 in mice was shown to abolish responses to endotoxin, and to be insensitive to the LPS. Cario et al identified in another study, but was referred to as a signal transducer for Gram positive bacterial components of the lipopolysaccharide receptor complex. The aim of this study was to investigate the associations between polymorphisms TLR4 Asp299Gly and CD14 C-260T and Chinese patients with ulcerative colitis (UC).

Background and aims: Toll-like receptor 4 and CD14 are the components of the lipopolysaccharide receptor complex. The aim of this study was to investigate the associations between polymorphisms TLR4 Asp299Gly and CD14 C-260T and Chinese patients with ulcerative colitis (UC).

Methods: Using a polymerase chain reaction based restriction fragment length polymorphism, the study genotyped polymorphisms TLR4 Asp299Gly and CD14 C-260T in 114 patients with UC and 160 healthy controls in the Chinese Han population. Moreover a comparison was made with 170 healthy Dutch white subjects.

Results: No TLR4 Asp299Gly mutation was detected in any patients or healthy controls in the Chinese Han population. Moreover a comparison was made with 170 healthy Dutch white subjects. There were no significant differences of CD14 genotypes between healthy controls and the patients with UC.

Abbreviations: UC, ulcerative colitis; CD, Crohn’s disease; IBD, inflammatory bowel disease; TLR4, toll-like receptor 4; LBP, LPS binding protein

Inflammatory bowel disease (IBD) comprises mainly ulcerative colitis (UC) and Crohn’s disease (CD) characterised by long term intestinal inflammation attributable to an exaggerated immune response. The aetiology is still unknown. Genetic susceptibility has been shown by epidemiological and genetic investigations. Recent genome-wide linkage studies and case-control association studies have shown several susceptibility regions for UC and identified the CARD15 (NOD2), the first susceptibility gene for CD. The mutation of TLR4 in mice was shown to abolish responses to endotoxin, and to be insensitive to the LPS. Cario et al identified in another study, but was referred to as a signal transducer for Gram positive bacterial components of the lipopolysaccharide receptor complex. The aim of this study was to investigate the associations between polymorphisms TLR4 Asp299Gly and CD14 C-260T and Chinese patients with ulcerative colitis (UC).

Background and aims: Toll-like receptor 4 and CD14 are the components of the lipopolysaccharide receptor complex. The aim of this study was to investigate the associations between polymorphisms TLR4 Asp299Gly and CD14 C-260T and Chinese patients with ulcerative colitis (UC).

Methods: Using a polymerase chain reaction based restriction fragment length polymorphism, the study genotyped polymorphisms TLR4 Asp299Gly and CD14 C-260T in 114 patients with UC and 160 healthy controls in the Chinese Han population. Moreover a comparison was made with 170 healthy Dutch white subjects.

Results: No TLR4 Asp299Gly mutation was detected in any patients or healthy controls in the Chinese Han population. Moreover a comparison was made with 170 healthy Dutch white subjects. There were no significant differences of CD14 genotypes between healthy controls and the patients with UC.

Abbreviations: UC, ulcerative colitis; CD, Crohn’s disease; IBD, inflammatory bowel disease; TLR4, toll-like receptor 4; LBP, LPS binding protein

Inflammatory bowel disease (IBD) comprises mainly ulcerative colitis (UC) and Crohn’s disease (CD) characterised by long term intestinal inflammation attributable to an exaggerated immune response. The aetiology is still unknown. Genetic susceptibility has been shown by epidemiological and genetic investigations. Recent genome-wide linkage studies and case-control association studies have shown several susceptibility regions for UC and identified the CARD15 (NOD2), the first susceptibility gene for CD. The mutation of TLR4 in mice was shown to abolish responses to endotoxin, and to be insensitive to the LPS. Cario et al identified in another study, but was referred to as a signal transducer for Gram positive bacterial components of the lipopolysaccharide receptor complex. The aim of this study was to investigate the associations between polymorphisms TLR4 Asp299Gly and CD14 C-260T and Chinese patients with ulcerative colitis (UC).

Background and aims: Toll-like receptor 4 and CD14 are the components of the lipopolysaccharide receptor complex. The aim of this study was to investigate the associations between polymorphisms TLR4 Asp299Gly and CD14 C-260T and Chinese patients with ulcerative colitis (UC).

Methods: Using a polymerase chain reaction based restriction fragment length polymorphism, the study genotyped polymorphisms TLR4 Asp299Gly and CD14 C-260T in 114 patients with UC and 160 healthy controls in the Chinese Han population. Moreover a comparison was made with 170 healthy Dutch white subjects.

Results: No TLR4 Asp299Gly mutation was detected in any patients or healthy controls in the Chinese Han population. Moreover a comparison was made with 170 healthy Dutch white subjects. There were no significant differences of CD14 genotypes between healthy controls and the patients with UC.

Abbreviations: UC, ulcerative colitis; CD, Crohn’s disease; IBD, inflammatory bowel disease; TLR4, toll-like receptor 4; LBP, LPS binding protein
allele.\textsuperscript{24} Two papers\textsuperscript{25,26} have reported on the association between IBD and the polymorphism CD14 C-260T, but one is associated with UC while the other is associated with CD. Moreover, Torok \textit{et al}\textsuperscript{16} have reported that they did not find the association between the CD14 promoter polymorphism and UC. Different results might be interpreted by genetic heterogeneity of races.

These studies investigated the possible role of TLR4/CD14 genes in LPS binding and signalling. We hypothesised that polymorphisms of the TLR4 and the CD14 gene may be important factors for determining susceptibility to UC and may be predictive for the disease outcome. To evaluate whether these two polymorphisms contribute to the predisposition to UC, we detected TLR4 and CD14 gene polymorphisms in Chinese patients with UC.

METHODS

Subjects

One hundred and fourteen patients with UC from Zhongnan Hospital of Wuhan University and other central hospitals in Wuhan city were studied. Diagnosis of UC was made by conventional clinical, radiological, endoscopic, and histological criteria.\textsuperscript{27} The patients with UC were classified according to sex (male and female) and location (left sided colitis, proctitis, and extensive colitis). Ethnically matched 160 Chinese healthy controls and 170 Dutch white healthy controls were recruited from staff and students in Wuhan University Zhongnan Hospital and Vrije Universiteit Medical Centre, Amsterdam, Netherlands (VUmc), respectively. All Chinese subjects were unrelated Han Chinese, and Dutch participants were the unrelated white subjects. All the subjects gave informed consents. The study protocol was approved by the ethics committee of Wuhan University Medical School.

DNA isolation

Genomic DNA was isolated from 5 ml EDTA anticoagulated venous blood by conventional proteinase K digestion and phenol/chloroform extraction method. The positive control DNA samples of TLR4 and CD14 genes were provided by the laboratory of immunogenetics of VUmc.

TLR4 Asp299Gly genotyping

The two primer sets were designed from the TLR4 coding sequence (GenBank accession no U88880) referenced to Okayama \textit{et al}\textsuperscript{19} (F1: 5’-TTTAGAATGAGAGGAACCTTGGAAAA AG-3’, R1: 5’-TTTGCACAACATTTAATAAGTGATTAAAT-3’, F2: 5’-AGGATACCTAGACTAACCCTCGATG-3’, R2: 5’-GTTCGCCATCGGAAATTATAAGGAG-3’). The PCR conditions were as follows: an initial denaturation at 94 ˚C for three minutes, followed by 35 cycles of denaturing at 94 ˚C for 45 seconds, annealing at 51 ˚C for 45 seconds, and extension at 72 ˚C for 45 seconds. The PCR product was amplified by F1 and R1, and the 131 bp fragment was amplified by F2 and R2. When digested by BsaBI, the fragments (139 bp for allele G and 112 bp +27 bp for allele A respectively) were obtained. When digested by BstXI, the fragments (108 bp +23 bp for allele G and 131 bp for allele A) were obtained. The products were separated by electrophoresis in non-denaturing polyacrylamide gels containing 8% acrylamide-bisacrylamide (29:1), 0.5x TRIS-borate-EDTA (TBE), 10% ammonium persulphate, and TEMED at 150 V for 1.5 hours. The gels were then subjected to silver staining.

CD14 genotyping

Primers of CD14 P1 (5’-CCTGCGAAGATCCTTGCGT-3’) and CD14 P2 (5’-TACCTCCCACTCCCTGCT-3’) were used to amplify a 107 bp fragment of the CD14 promoter (GenBank accession no U00699). A 25 μl PCR amplification mixture containing 50 pmol of each primer, 2.5 μl of 10×buffer, 1.5 μl of 25 mM MgCl\textsubscript{2}, 0.5 μl of 10 mM dNTPs, 1 μl of template DNA, 1 μl of AmpliTaq DNA polymerase, and 17 μl ddH\textsubscript{2}O, was run in a Perkin-Elmer Thermal cycler. After an initial denaturation at 94˚C for five minutes, 35 cycles of denaturing at 94˚C for 30 seconds, annealing at 55˚C for 30 seconds, and extension at 72˚C for one minute followed. The final extension was continued at 72˚C for seven minutes and kept in 4˚C. PCR products were analysed by electrophoresis in 2% agarose gel with ethidium bromide staining under ultraviolet transillumination. The C allele of the polymorphism of CD14 was determined by digestion with Hae III and the resulting fragments (107 bp for the T allele and 83 bp and 24 bp for the C allele, respectively) were separated on non-denaturing polyacrylamide gels containing 8% acrylamide-bisacrylamide and then silver staining.

Statistics analysis

The data were analysed using SPSS software (version 11.0, SPSS, Chicago, IL). Genotypes and allele frequencies were calculated by direct counting. Significance of the association was determined by χ\textsuperscript{2} test and Fisher’s exact test. Departures from Hardy-Weinberg equilibrium were tested by the χ\textsuperscript{2} method. A value of p<0.05 was considered significant. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated according to Woolf’s method.

RESULTS

In our study, the functional mutation Asp299Gly of the TLR4 gene was not detected in any Chinese subject. All the PCR products were sensitive to BsaBI and resistant to BstXI, they were all wild-type homozygotes. Therefore, significant association was not seen in patients with UC and healthy controls in the Chinese subjects, which is similar to the results in Japanese.\textsuperscript{14} However, mutations TLR4 Asp299Gly occurred in 10% of the Dutch subjects.

The distributions of genotype and allele frequencies of CD14 C-260T in both the patients and the healthy controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number (%)</th>
<th>Number (%)</th>
<th>Genotype</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/T</td>
<td>58 (36.3)</td>
<td>42 (36.8)</td>
<td>T/T</td>
<td>60.3</td>
</tr>
<tr>
<td>C/T</td>
<td>77 (48.1)</td>
<td>62 (54.4)</td>
<td>C/T</td>
<td>64.0</td>
</tr>
<tr>
<td>C/C</td>
<td>25 (15.6)</td>
<td>30 (8.8)</td>
<td>C/C</td>
<td>36.0</td>
</tr>
</tbody>
</table>

Table 1 Genotypes and allele frequencies of CD14 C-260T in healthy controls and patients with ulcerative colitis (UC) in the Chinese Han population
Table 2 Genotype and allele frequencies of CD14 C-260T classified by sex and location of ulcerative colitis in Chinese patients

<table>
<thead>
<tr>
<th>Genotype frequency (%)</th>
<th>Allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T/T</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male [n = 64]</td>
<td>23 (35.9)</td>
</tr>
<tr>
<td>Female [n = 50]</td>
<td>19 (38.0)</td>
</tr>
<tr>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>Left sided colitis [n = 51]</td>
<td>20 (39.2)</td>
</tr>
<tr>
<td>Proctitis [n = 19]</td>
<td>10 (52.6)</td>
</tr>
<tr>
<td>Extensive colitis [n = 44]</td>
<td>2 (4.5)</td>
</tr>
</tbody>
</table>

DISCUSSION

This study has shown that the G allele of TLR4 gene Asp299Gly was not present in the Chinese population tested. Therefore, we could not find any association between the TLR4 Asp299Gly mutated genotype and UC, similar to a study in the Japanese. They genotyped 108 healthy volunteers and 50 patients with UC and did not detect any mutation of the TLR4 Asp299Gly. Comparing the genotype and allele frequencies of the TLR4 Asp299Gly polymorphism among Chinese, Japanese, and Dutch white subjects, we found significant differences between Asians and Dutch white subjects. In Chinese and Japanese, the mutation of the TLR4 Asp299Gly did not occur, while in Dutch white subjects, the combination of AG and GG genotypes occurred in 10%. Our study showed that the distribution of the TLR4 Asp299Gly polymorphism was significantly different between Asia and this western population.

CD14, the co-receptor of TLR4 for LPS, is present in two forms, mCD14 and sCD14. Membrane CD14 is expressed on the surface of monocytes and macrophages and activates through TLR4, while sCD14 facilitates the LPS binding to the cells that do not express mCD14. CD14 gene is located on the long arm of chromosome 5 (5q31–33) and consists of a single base substitution (C→T) at position –260 in the promoter of the CD14 gene. This polymorphism has been reported to influence the density of CD14 expression on monocytes for the activation of monocytes to secrete inflammatory cytokines by LPS. The T variant of the –260 polymorphism can promote the transcription of CD14 gene and cause higher expression of CD14 on monocytes, which may lead to an increased inflammatory response. Furthermore, subjects carrying the T allele have higher soluble CD14 levels than carriers of the C allele. Therefore, the CD14 polymorphism may be a genetic factor responsible for individual differences in the expression of CD14 and inflammatory response to luminal bacterial infections.

Zareie et al found that intestinal lamina propria mononuclear cells (LPMCs) of patients with CD were spontaneously activated by LPS. Active intestinal inflammation in IBD patients has shown increased expression of CD14 in LPMCs. Therefore, activation of the CD14 pathway in LPMCs or intestinal epithelium may be a trigger in the inflammatory response leading to increased production of proinflammatory cytokines and mucosal damage. Two clinical studies have shown an association between polymorphism in the promoter of the CD14 gene and myocardial infarction. One Japanese research group confirmed that the C-260T polymorphism in the promoter of the CD14 monocyte receptor gene was associated with acute myocardial infarction rather than with coronary atherosclerosis. Eng et al found an association between Chlamydia pneumoniae infection and CD14 polymorphism in the Chinese population in Taiwan. Linkage between CD14 polymorphism and IBD has also been studied. In a German population, an association of CD with allele T and the T/T genotype of CD14 gene has been found significant, which may be interpreted by a changed immune response to LPS contributing to the genetic predisposition to CD but not to UC. However, it has been shown that the CD14 gene in 5q31–33 region had a significant linkage with UC in the Japanese population and in the Canadian population.

Our study has shown that CD14 C-260T polymorphism was not associated with UC in the Chinese population. The frequency of the C/C genotype in healthy controls (15.6%) was higher than in UC (8.8%), the C/T genotype in UC (54.4%) was higher than in healthy controls (48.1%), but neither of them is statistically significant. We did not find differences of CD14 C-260T between subgroups of UC as shown in table 2. Furthermore, the distributions of CD14 C-260T genotypes were significantly different Chinese and Japanese, and between Chinese and Dutch white subjects (table 3), which demonstrated ethnic genetic differences. The study implicates that the polymorphism of CD14 C-260T does not confer a genetic predisposition to UC. The lack of association of CD14 gene with UC in the Chinese population may be explained by genetic heterogeneity, and possibly a type 2 statistical error.

In summary, both the polymorphism of CD14 C-260T and TLR4 Asp299Gly were not associated with UC in the Chinese population, but the distributions of these two gene polymorphisms have ethnic differences.
ACKNOWLEDGEMENTS

We thank Professor H S Luo and Dr Y J Ding of the People’s Hospital of Wuhan University, Professor X H Hou and K F Zhou of Union Hospital and Professor N Z Liu and Associate Professor Q Zhao of Tongji Hospital of Huazhong Science and Technology University for assistance with collecting DNA samples for IBD patients. We also thank Ms Jolein Pleijster for assistance with typing most of the Dutch control cohort both CD14 and TLR4 genes.

Authors’ affiliations

Q S Guo, B Xia, Y Jiang, J Cheng, J Li, Department of Internal Medicine and Geriatrics, Research Centre of Digestive Diseases of Zhongnan Hospital; Laboratory of Inflammation and Autoimmune Diseases of the Institute of Allergy and Immune Related Diseases, Wuhan University Medical School, Wuhan, PR of China

S A Morre, J B A Cruysius, A S Peña, Laboratory of Immunogenetics, VU University Medical Centre, Amsterdam, Netherlands

Funding: the study was supported by grants from the State Natural University Medical Centre, Amsterdam, Netherlands

....................

Control cohort both CD14 and TLR4 genes.

thank Ms Jolein Pleijster for assistance with typing most of the Dutch public health bureau (JXIA14).

Conflicts of interest: none.

Ethics approval: all the subjects gave informed consent and the study protocol was approved by the ethics committee of Wuhan University Medical School.

REFERENCES


www.postgradmedj.com

Downloaded from http://pmj.bmj.com/ on July 5, 2017 - Published by group.bmj.com
Polymorphisms of CD14 gene and TLR4 gene are not associated with ulcerative colitis in Chinese patients

Q S Guo, B Xia, Y Jiang, S A Morré, L Cheng, J Li, J B A Crusius and A S Peña

*Postgrad Med J* 2005 81: 526-529
doi: 10.1136/pgmj.2004.030825

Updated information and services can be found at:
http://pmj.bmj.com/content/81/958/526

These include:

**References**
This article cites 38 articles, 17 of which you can access for free at:
http://pmj.bmj.com/content/81/958/526#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.

**Errata**
An erratum has been published regarding this article. Please see next page or:

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

- Immunology (including allergy) (396)
- Inflammatory bowel disease (37)
- Genetics (135)

**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/