The 14-bp deletion polymorphism in the HLA-G gene displays significant differences between ulcerative colitis and Crohn’s disease and is associated with ileocecal resection in Crohn’s disease

Jürgen Glas¹,², Helga-Paula Török¹, Laurian Tonenchi¹,², Martin Wetzke¹,², Vanessa Beynon¹,², Molla Y. Teshome¹,², Sebastian Cotofana¹, Uwe Schiemann³, Thomas Griga⁴, Wolfram Klein⁵, Joerg T. Epplen⁵, Christian Folwaczny¹, Matthias Folwaczny², Thomas Mussack¹ and Elisabeth H. Weiss⁶

¹Chirurgische Klinik und Poliklinik–Innenstadt and ²Poliklinik für Zahnerhaltung und Parodontologie, Klinikum der Universität München, Munich, Germany
³Klinik für Allgemeine Innere Medizin, Inselspital Bern, Bern, Switzerland
⁴Medizinische Klinik–Innere Medizin, Knappschaftskrankenhaus Dortmund, Dortmund, Germany
⁵Abteilung für Humangenetik, Ruhr-Universität Bochum, Bochum, Germany
⁶Department Biologie II, Anthropologie und Humangenetik, Ludwig-Maximilians-Universität München, Munich, Germany

Keywords: Crohn’s disease, HLA-G, inflammatory bowel disease, major histocompatibility complex, ulcerative colitis

Abstract

HLA-G is a non-classical MHC class Ib molecule predominantly expressed in cytotrophoblasts and under pathological conditions also in chronically inflamed and in malignant tissues. Recently an increased expression of HLA-G was found in ulcerative colitis (UC), but not in Crohn’s disease (CD). The HLA-G gene is located in IBD3, a linkage region for inflammatory bowel disease (IBD). A 14-bp deletion polymorphism (Del¹/Del²) within exon 8 of the HLA-G gene might influence transcription activity and is therefore of potential functional relevance. To investigate whether the 14-bp deletion polymorphism is associated with IBD, 371 patients with CD, 257 patients with UC and 739 controls were genotyped. The heterozygous genotype (P = 0.031) and the Del¹ phenotype (P = 0.038) were significantly increased, whereas the homozygous Del² phenotype (P = 0.038) was significantly decreased in UC when compared with CD. Thus, the 14-bp deletion polymorphism within the HLA-G gene displayed significant differences between UC and CD. Moreover, a significant increase of the Del¹ allele (P = 0.002) and the Del¹/Del¹ genotype (P = 0.013) and a consecutive decrease of the Del¹/Del² genotype (P = 0.024) were observed in those CD cases positive for ileocecal resection. Thus, a potential effect of the HLA-G gene in IBD may affect both UC and CD. Other polymorphisms linked to the 14-bp deletion polymorphism might also contribute to immunopathogenesis. As there are several partly functional polymorphisms within the promoter region potentially influencing HLA-G expression, further studies in IBD are necessary in the context of differential expression of HLA-G between UC and CD.

Introduction

Chronic inflammatory bowel disease (IBD) comprises the two disease entities Crohn’s disease (CD) and ulcerative colitis (UC) (1, 2). CD is characterized by a segmental chronic granulomatous inflammation of the whole intestinal wall predominantly located in the terminal ileum and potentially affecting every part of the gastrointestinal tract (3). In contrast, the inflammation in UC is more continuous displaying crypt abscesses and ulcers, being restricted to the intestinal mucosa and the disorder occurs within the colorectum (4). The etiology of IBD remains unclear, but environmental factors, e.g. the microbial flora, as well as genetic factors play important roles for disease pathogenesis, resulting in dysregulation of the intestinal mucosal immune system (3, 4). Linkage analyses revealed several linkage regions on different chromosomes (5, 6). The first linkage region for IBD was IBD1 on chromosome 16p containing the NOD2/...
HLA-G polymorphism in inflammatory bowel disease

CARD15 gene, which has been the first gene, in which mutations reproducibly were associated with CD in Caucasians (7, 8). The IBDD region has been linked to chromosome 6p21, a region containing the MHC (9). The MHC is a region of extremely high abundance of genes comprising the MHC class I and class II genes and further genes, named MHC class III, exhibiting functions in immunological processes such as the cytokines tumor necrosis factor (TNF)-α, TNF-β and the heat shock proteins. It is not clear so far, which genes within the MHC family contribute to the pathogenesis of IBD, and thus, diverse genes in this region can be considered as functional and positional candidate genes for IBD.

HLA-G is a non-classical MHC class I molecule displaying restricted tissue expression and low polymorphism (10, 11). Under normal conditions expression of the HLA-G protein is restricted to the feto-maternal interface on the extravillous cytotrophoblast protecting the fetal semi-allograft against the maternal immune system (11, 12) and to the thymus in adults (11, 13). HLA-G expression is up-regulated under pathological conditions in inflammatory diseases such as psoriasis and atopic dermatitis (14, 15), in viral infection (16), malignancies (17) and organ transplants (18). The main function of HLA-G is suppression of several immune processes carrying out inhibitory effects on cytolyis by NK cells and CTL, T cell proliferative responses and maturation of dendritic cells (11). These inhibitory effects are mediated by ligation of HLA-G and inhibitory receptors such as ITIL, ITL4 and KIR2DL4 on the surface of immunocompetent cells (11, 19). HLA-G exhibits its low allelic polymorphism in comparison with the classical MHC class I molecules. Currently, 15 HLA-G alleles are known at the nucleotide level resulting in six different proteins (11, 20, 21). Seven protein isoforms, four membrane bound (HLA-G1, -G2, -G3 and -G4) and three soluble (HLA-G5, -G6 and -G7), are generated by alternative splicing (22-24). Further nucleotide polymorphisms have been described within the non-coding region of the HLA-G gene, 18 single-nucleotide polymorphisms (SNPs) in the promoter region and a 14-bp deletion polymorphism in exon 8 (rs16375) encoding for the 3’ untranslated region (25, 26). The latter polymorphism is potentially functional influencing transcript levels and splicing (11, 27). Recently, a differential pattern of HLA-G expression in CD and UC has been shown. By immunohistochemistry, increased HLA-G surface expression has been only detected in UC, whereas HLA-G expression was absent in CD and also in healthy controls (28). These findings suggest a role of HLA-G in the immunopathogenesis of IBD, and analysis of HLA-G expression possibly can be used for diagnostic purposes to distinguish between CD and UC in cases of indeterminate colitis. Considering the localization in the linkage region IBD and its immunosuppressive function, HLA-G is an excellent candidate gene for IBD. Thus, the present study aimed to investigate whether the 14-bp deletion polymorphism within the HLA-G gene is associated with IBD.

Methods

Study population

The study population comprised 371 patients with CD, 257 patients with UC and 739 healthy unrelated blood donors serving as controls. The patients were recruited from two tertiary referral centers (226 CD and 155 UC cases from the university hospital of the University of Munich and 145 CD and 102 UC cases from the university hospital of the University of Bochum). All study participants were Caucasians of German origin and gave their written informed consent. CD or UC was diagnosed by conventional clinical, radiological, endoscopic and histological criteria. Cases with indeterminate colitis were excluded. There were no significant differences between CD or UC, respectively, and controls with respect to age or gender. Clinical characteristics were available from 176 CD and 119 UC patients recruited from the university hospital of the University of Munich. Hundred and thirty-nine patients with CD displayed a fibrostenotic (fistulae, stenoses or both) and 37 patients an inflammatory phenotype (no fistulae and no stenoses). In 132 CD patients, the disease was located in both the ileum and the colon, whereas in 44 only either the ileum or the colon was affected. Of 176 CD patients, 66 had undergone ileocecal resection. In the group of UC patients, 68 displayed a pancolitis ulcerosa, whereas in 51 cases, the inflammatory process was either restricted to the rectosigmoid or to the left hemicolon.

Genotyping and statistical analysis

DNA was obtained from peripheral blood anti-coagulated with EDTA using in part the QiAamp® DNA Blood Mini kit (Qiagen, Hilden, Germany) and in part the salting out procedure. The 14-bp deletion polymorphism within the HLA-G gene was genotyped using PCR and agarose gel electrophoresis. The total volume of the PCR was 10 µl containing 50 ng of genomic DNA, 1× PCR buffer (Qiagen), 0.5 mM of a deoxynucleoside triphosphate mix (Sigma, Steinheim, Germany), 0.5 U of HotStarTaq™ DNA polymerase (Qiagen) and 5 pmol of each primer (TIB MOLBIOL, Berlin, Germany). The final concentration of MgCl₂ was 1.5 mM. The PCR comprised an initial denaturation step (95°C for 15 min), 35 cycles (94°C for 30 s, 60°C for 30 s and 72°C for 30 s) and a final extension step (72°C for 10 min). PCR amplificates were analyzed by electrophoresis in 4% NuSieve® 3:1 (Cambrex, Rockland, ME, USA) agarose gels stained with ethidium bromide. The genotyping results were additionally confirmed by sequencing several DNA samples of individuals displaying the three possible genotypes using identical PCR conditions. The HLA-G allele lacking the 14 bp in exon 8 is named Del+, whereas the allele containing this sequence is called Del−.

Allele frequencies were tested for Hardy–Weinberg equilibrium. Statistical analysis was performed using Parson’s χ² test with Yates correction (SPSS version 13.0, SPSS Inc., Chicago, IL, USA). P values < 0.05 were considered significant.

Results

Allele, genotype and phenotype frequencies of the 14-bp deletion polymorphism within the HLA-G gene in UC and CD

The frequencies of the 14-bp deletion polymorphism within the HLA-G gene in UC and CD, respectively, displayed no significant associations when compared with the control
Results of the three common mutations R702W, G908R and L1007insC within the NOD2/CARD15 gene associated with CD were available in 367 (98.9%) of the 371 CD patients. Hundred and forty-two (38.7%) of the CD cases displayed at least one mutated NOD2/CARD15 allele. These cases were classified as positive for NOD2/CARD15 mutations (NOD2/CARD15+) and compared with the remaining 225 cases containing only wild-type NOD2/CARD15 alleles classified as negative for NOD2/CARD15 mutations (NOD2/CARD15−). The allele frequencies of the 14-bp deletion polymorphism within the HLA-G gene showed no significant differences in the CD group according to the NOD2/CARD15 mutation status, and the Del− allele was observed in 62.0% of the NOD2/CARD15+ cases and in 59.8% of the NOD2/CARD15− cases. Moreover, genotype and phenotype frequencies of the HLA-G 14-bp deletion polymorphism displayed no significant differences depending on the NOD2/CARD15 mutation phenotype. The allele, genotype and phenotype frequencies of the HLA-G 14-bp deletion polymorphism in CD stratified for the NOD2/CARD15 mutation status are depicted in Table 3.

Allele, genotype and phenotype frequencies of the 14-bp deletion polymorphism within the HLA-G gene in CD as stratified for disease-associated mutations within the NOD2/CARD15 gene

Table 1. Allele frequencies of the 14-bp deletion polymorphism (Del+/Del−) within the HLA-G gene in UC and CD

<table>
<thead>
<tr>
<th></th>
<th>UC, n = 514 (%)</th>
<th>CD, n = 742 (%)</th>
<th>Controls, n = 1478 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del+</td>
<td>321 (62.5)</td>
<td>451 (60.8)</td>
<td>905 (61.2)</td>
</tr>
<tr>
<td>Del−</td>
<td>193 (37.5)</td>
<td>291 (39.2)</td>
<td>573 (38.8)</td>
</tr>
</tbody>
</table>

*P = 0.038, **P = 0.038, ***P = 0.038 (UC versus CD).

Table 2. Genotype and phenotype frequencies of the 14-bp deletion polymorphism (Del+/Del−) within the HLA-G gene in UC and CD

<table>
<thead>
<tr>
<th></th>
<th>UC, n = 257 (%)</th>
<th>CD, n = 371 (%)</th>
<th>Controls, n = 739 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del+/Del+</td>
<td>91 (35.4)</td>
<td>142 (38.3)</td>
<td>266 (36.0)</td>
</tr>
<tr>
<td>Del+/Del−</td>
<td>139 (54.1)</td>
<td>167 (45.0)</td>
<td>373 (50.5)</td>
</tr>
<tr>
<td>Del−/Del−</td>
<td>27 (10.5)</td>
<td>62 (16.7)</td>
<td>100 (13.5)</td>
</tr>
<tr>
<td>Del+***</td>
<td>230 (89.5)</td>
<td>309 (83.3)</td>
<td>639 (86.5)</td>
</tr>
<tr>
<td>Del−</td>
<td>166 (64.6)</td>
<td>229 (61.7)</td>
<td>473 (64.0)</td>
</tr>
</tbody>
</table>

*P = 0.031, **P = 0.038, ***P = 0.038 (UC versus CD).

Table 3. Allele, genotype and phenotype frequencies of the 14-bp deletion polymorphism (Del+/Del−) within the HLA-G gene in CD after stratification for presence of mutations (R702W, G908R and L1007insC) within the CARD15/NOD2 gene (CARD15/NOD2+)

<table>
<thead>
<tr>
<th></th>
<th>CARD15/NOD2+ (%)</th>
<th>CARD15/NOD2− (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Del+</td>
<td>n = 284</td>
<td>n = 450</td>
</tr>
<tr>
<td>Del−</td>
<td>176 (62.0)</td>
<td>269 (59.8)</td>
</tr>
<tr>
<td>Genotype/phenotype</td>
<td>n = 142</td>
<td>n = 225</td>
</tr>
<tr>
<td>Del+/Del+</td>
<td>55 (38.7)</td>
<td>83 (36.9)</td>
</tr>
<tr>
<td>Del+/Del−</td>
<td>66 (46.5)</td>
<td>103 (45.8)</td>
</tr>
<tr>
<td>Del−/Del−</td>
<td>21 (14.8)</td>
<td>39 (17.3)</td>
</tr>
<tr>
<td>Del+</td>
<td>121 (85.2)</td>
<td>186 (82.7)</td>
</tr>
<tr>
<td>Del−</td>
<td>87 (61.3)</td>
<td>142 (63.1)</td>
</tr>
</tbody>
</table>

The group of patients with CD was stratified for disease behavior (fibrostenotic versus inflammatory phenotype), for disease location and the need for ileocecal resection. In the subgroup of CD patients with the fibrostenotic phenotype, the frequency of the Del+ allele was 59.7%, whereas in the subgroup with an inflammatory phenotype, the frequency of this allele was 54.1%. Considering disease location/extent in the CD patients, the Del+ allele was found in 59.5% of those cases displaying an involvement of both ileum and colon and in 58.0% of cases displaying an involvement of either the ileum only or the colon only. Thus, the allele and also the genotype frequencies did not significantly differ after stratification for disease behavior or for disease location/extent in CD. Furthermore, the CD patients were stratified for the need of ileocecal resection. In the subgroup of patients with ileocecal resection compared with those cases without ileocecal resection, the frequencies of the Del+/Del+ genotype (47.0 versus 27.3%, P = 0.013) and of the Del+ allele (69.7 versus 52.7%, P = 0.002) were significantly increased, whereas the frequency of the Del−/Del− genotype (7.6 versus 21.8%, P = 0.024) was significantly decreased. In the patients with UC cases with a disease extent restricted to the rectosigmoid or the left hemicolon were compared with...
Discussion
In the present study, a deletion polymorphism (rs16375) within the HLA-G gene has been investigated in IBD for the first time. As the HLA-G gene is located in the linkage region IBD3 and it is involved in immunological processes, it represents an excellent positional and functional candidate gene for IBD. Recently, Torres et al. (28) found high immunohistochemical expression of HLA-G in all segments of the gastrointestinal tract in 100% of cases with UC, whereas HLA-G expression was completely absent in all cases of CD as well as in healthy controls. This differential expression pattern of HLA-G is possibly influenced by genetic variations within the HLA-G gene. In the present study, the 14-bp deletion polymorphism within the HLA-G gene (Del+/Del—), a variation potentially influencing transcription activity (27), has been investigated in 253 patients with UC and 371 patients with CD. The allele, genotype and phenotype frequencies of the 14-bp deletion polymorphism in UC or CD, respectively, displayed no significant differences when compared with a group of 739 healthy controls. The allele frequencies in the control group found herein were similar to those detected in the control groups of other studies (25, 29, 30). Yet, when the UC and CD groups were compared among each other, the Del+ phenotype and the heterozygous Del+/Del— genotype were significantly increased in UC, whereas the frequency of the homozygous genotype Del—/Del— was significantly lower in UC than in CD. These findings are consistent with the differential expression pattern of HLA-G in UC compared with CD as shown by Torres et al. (28) as well as by O’Brien et al. (27), where decreased HLA-G expression was observed in placenta samples of cases with homozygous Del— genotype. It has to be taken into account that no genotyping data were included in the study of Torres et al. (28) and it is therefore unknown, whether different HLA-G expression in the intestine is influenced by genetic variations. It has also to be considered that the present study and the study of Torres et al. (28) have been performed in two different populations, a German population versus a population of Spanish origin. Even though both are of Caucasian origin, the genetic background potentially could differ to some extent. Moreover, though the data of Torres et al. (28) offer an interesting finding toward a different immunopathogenesis in UC and CD, these results have to be confirmed in further independent studies and should be correlated with functional polymorphisms within the HLA-G gene.

The differences of the 14-bp deletion HLA-G polymorphism between UC and CD found herein gives evidence for a contribution of the HLA-G gene in the pathogenesis of IBD; however, this genetic effect might be rather weak and the power of the present study might be too low to detect significant differences in comparison to the control group. Due to its weak observed genetic effect, the 14-bp deletion polymorphism itself cannot exclusively be responsible for the observed gastrointestinal HLA-G expression in UC patients, but other polymorphisms and factors contribute to the pathogenesis of IBD. In the promoter region of the HLA-G gene, several SNPs partly of functional relevance and partly associated with increased miscarriage rates have been described (26). As strong linkage disequilibria exist between the 14-bp deletion polymorphism and SNPs within the HLA-G promoter, the SNP alleles or SNP haplotypes of the promoter region are potentially associated with the differential expression of HLA-G between UC and CD and further studies are indicated to clarify this issue. Moreover, variations in other genes, which could influence the transcription of HLA-G or HLA-G protein synthesis also might account for differential HLA-G expression. Further candidate genes within this context might be those genes encoding receptors recognizing HLA-G. The genes coding for the receptorsILT2, ILT4 and KIR2DL4 are located within a linkage region for IBD on chromosome 19 and particularly the KIR genes display substantial polymorphism (31–33).

As the NOD2/CARD15 gene has been the first gene associated with IBD, in particular with CD (7, 8), the CD group has been stratified for the presence of at least one of the three common CD-associated NOD2/CARD15 mutations (R702W, G908R and L1007fsinsC). However, the distribution of the 14-bp deletion polymorphism in the HLA-G gene displayed no significant differences between cases positive and those negative for NOD2/CARD15 mutations and thus, no evidence for an interaction between HLA-G and NOD2/CARD15

Table 4. Genotype and allele frequencies of the 14-bp deletion polymorphism (Del+/Del—) within the HLA-G gene in CD after stratification for clinical phenotypes (disease behavior, disease location and ileocecal resection)

<table>
<thead>
<tr>
<th>Genotype/allele</th>
<th>Disease behavior</th>
<th>Disease location/extent</th>
<th>Ileocecal resection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fibrostenotic phenotype, n = 139/278 (%)</td>
<td>Inflammatory phenotype, n = 37/74 (%)</td>
<td>Location in both ileum and colon, n = 132/264 (%)</td>
</tr>
<tr>
<td>Del+/Del+***</td>
<td>48 (35.5)</td>
<td>12 (32.4)</td>
<td>46 (34.9)</td>
</tr>
<tr>
<td>Del+/Del—</td>
<td>70 (50.4)</td>
<td>16 (43.2)</td>
<td>65 (49.2)</td>
</tr>
<tr>
<td>Del—/Del—***</td>
<td>21 (15.1)</td>
<td>9 (24.3)</td>
<td>21 (15.9)</td>
</tr>
<tr>
<td>Del**</td>
<td>166 (59.7)</td>
<td>40 (54.1)</td>
<td>157 (59.5)</td>
</tr>
<tr>
<td>Del—</td>
<td>112 (40.3)</td>
<td>34 (45.9)</td>
<td>107 (40.5)</td>
</tr>
</tbody>
</table>

*P = 0.013, **P = 0.024, ***P = 0.002 (ileocecal resection versus no ileocecal resection).
genes could be found. Both the CD and the UC patients were additionally stratified for specific clinical phenotypes. In the UC patients, no association could be detected after stratification for disease location and extent. The frequencies of genotypes and alleles of the 14-bp deletion HLA-G polymorphism were comparable in the groups displaying a disease extent confined to the ileum or to the colon only and in the group with a pancolitis ulcerosa. The CD patients were stratified for disease behavior (fibrostenotic versus inflammatory subtype), for disease location/extent (ileal disease only or colonic disease only versus ileocolonic disease) and for the need of ileocolonic resection. While after stratification for disease behavior and for disease extent no association could be found, a significant association of the 14-bp deletion polymorphism was observed after stratification for the need of ileocolonic resection. In the subgroup of CD patients with ileocolonic resection, the frequency of the Del+/Del+ genotype was 47.0% compared with 27.3% in patients without ileocolonic resection, whereas the two other genotypes were consecutively decreased. The frequency of the Del+ allele was significantly increased in CD patients with in relation to those patients without ileocolonic resection (69.7 versus 52.7%). Since the frequency of the Del+ allele only displayed a significant difference when comparing the subgroups after stratification of CD patients for ileal resection to each other, but not in the comparison of the whole group of CD patients to the group of healthy controls, HLA-G may play a role in modulating the course of CD rather than determining overall susceptibility.

The relative contribution of the MHC to the overall genetic risk was estimated as 64–100% in UC by linkage and epide miologic studies, whereas in CD the values were only 10–33% (5, 9, 34, 35). Considering the findings of the study of Torres et al. (28), the results of the present study suggest a potential role of HLA-G as a disease susceptibility gene rather in UC than in CD, as in UC the tendency toward an association of the HLA-G gene is more pronounced compared with the total population of CD patients. HLA-G potentially functions as a shield against inflammatory aggression (16). It might contribute to tissue protection by inhibiting NK cell activity and tissue infiltration by T cells and monocytes shifting the balance between Tr1 and Tr2 cells toward the Tr2 pathway (11, 16, 36). A potential role of HLA-G in the pathogenesis of UC is in line with the Tr2 pathway rather characteristic for UC (4). Further evidence for a role of HLA-G in chronic Tr2-driven inflammation is given by the identification of HLA-G as a susceptibility gene for asthma includ ing an association of the 14-bp deletion polymorphism (37). In UC, the increased expression of HLA-G might reduce clearing of intestinal micro-organisms and therefore promote a secondary chronic inflammation.

In conclusion, the present study found a significant difference of the distribution of the 14-bp deletion polymorphism within the HLA-G gene between UC and CD. Thus, HLA-G possibly plays a role in the immunopathogenesis of IBD. However, as the genetic effect of the 14-bp deletion polymorphism observed in the present study is rather weak in the populations of CD and UC patients as a whole, probably further genetic factors interacting with HLA-G contribute to IBD. Moreover, the association of this polymorphism with ileocolonic resection in CD suggests a role of HLA-G as a disease-modulating gene.

### Abbreviations

- CD: Crohn's disease
- IBD: inflammatory bowel disease
- SNP: single-nucleotide polymorphism
- TNF: tumor necrosis factor
- UC: ulcerative colitis

### References


