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AIM: To study whether the glucocorticoid receptor (GR/NR3C1) gene haplotypes influence the steroid therapy outcome in inflammatory bowel disease (IBD).

METHODS: We sequenced all coding exons and flanking intronic sequences of the NR3C1 gene in 181 IBD patients, determined the single nucleotide polymorphisms, and predicted the NR3C1 haplotypes. Furthermore, we investigated whether certain NR3C1 haplotypes are significantly associated with steroid therapy outcomes.

RESULTS: We detected 13 NR3C1 variants, which led to the formation of 17 different haplotypes with a certainty of > 95% in 173 individuals. The three most commonly occurring haplotypes were included in the association analysis of the influence of haplotype on steroid therapy outcome or IBD activity. None of the NR3C1 haplotypes showed statistically significant association with glucocorticoid therapy success.

CONCLUSION: NR3C1 haplotypes are not related to steroid therapy outcome.

Key words: Inflammatory bowel disease; Steroid therapy; Glucocorticoid receptor; Pharmacogenetics; Haplotype analysis

INTRODUCTION

Inflammatory bowel diseases (IBD), Crohn’s disease (CD) and ulcerative colitis (UC), are multifactorial disorders, which are characterized by chronic recurrent inflammation of the gastrointestinal tract[1]. The molecular pathogenesis of IBD is not fully elucidated, although an exaggerated mucosal immune response triggered by intestinal bacteria...
in genetically susceptible individuals appears to play an important role[9]. The combined prevalence of CD and UC is estimated to be 100 to 200 per 100000 individuals in developed countries[9]. IBD shows extensive variation in individual clinical presentation and outcomes, which is likely to be caused by differences in genetic susceptibility, environmental factors, intestinal bacteria and activation of the intestinal immune system[9].

Although great advances have been made in the management and therapy of IBD, curative therapy does not yet exist. The anti-inflammatory agents mesalazine (5-aminosalicylic acid, 5-ASA) and sulfasalazine, in combination with glucocorticoids (GCs), are common first line therapy options in induction and maintenance of UC remission. Severe cases of UC are treated intravenously with GCs or cyclosporine. CD is mainly treated with GCs and/or antibiotics, and azathioprine (AZA), 6-mercaptopurine (6-MP), or the anti-folate methotrexate (MTX) are often added to maintain the state of remission[10]. GC-resistant or -dependent disease courses can be treated with anti-TNF-α antibodies, such as infliximab and adalimumab. GCs are often used in the initial treatment of most cases of moderate to severe active UC or CD. However, 20% of patients develop GC resistance within one year of treatment[11,12]. Non-response to GCs often leads to the need for a surgical intervention as a result of a poor therapy outcome. For example, it has been reported that 38% and 29% of steroid-resistant CD and UC patients, respectively, required surgery within one year after beginning GC treatment[13].

Glucocorticoids are potent inhibitors of T cell activation and cytokine secretion, primarily via binding to the cytoplasmically located glucocorticoid receptor (GR) as ligands. Due to ligand binding, homodimers consisting of two activated GRs are formed that translocate into the nucleus. The complex subsequently binds to specific glucocorticoid response elements (GREs) within the regulatory regions of GR target genes[7]. The mechanisms by which GC resistance develops, are not fully understood. Three possible mechanisms have been proposed. First, decreased plasma levels of GCs through overexpression of the drug efflux system P-glycoprotein (MDR1). Second, an altered function of GR or, third, excessive synthesis of pro-inflammatory cytokines induced by activation of pro-inflammatory transcription factors may reduce the affinity of GR to its ligands and lead to the development of GC resistance[9].

NR3C1 is known to be expressed as several polymorphic variants[8]. Several mutations in the NR3C1 gene have been found to modulate individual GC sensitivity in vitro investigations and in studies with healthy individuals[8,9]. In the present study we evaluated the association between the NR3C1 gene haplotypes and therapeutic outcome of GC administration in a well-sized cohort of 181 patients with IBD. The aim was to comprehensively determine abundant GR variants by sequencing all protein-coding NR3C1 exons (exons 2 to 9) and the first 50 bp of the neighbouring intronic regions in all individuals. We hypothesized that NR3C1 gene polymorphisms may influence GC sensitivity and thus might serve as predictive markers for treatment success with GCs in IBD patients.

MATERIALS AND METHODS

Patients

One hundred and eighty-five clinically diagnosed Swiss IBD patients were recruited at the centers participating in the Swiss Inflammatory Bowel Disease Cohort Study (SIBDCS)[11]. All patients gave their informed consent for inclusion into the study. An ethical approval was obtained from the Medical Ethical Committees of the University Hospital Lausanne, Switzerland, and all local study sites. All patients had been treated with steroids and past steroid therapy outcome had been recorded. The standard employed criteria for the steroid therapy success or failure are available on the website www.epact.ch. Briefly, an insufficient response upon appropriate treatment in terms of doses and duration was considered a steroid therapy failure. EDTA-blood samples were stored at the central tissue repository at the Institute of Pathology, University of Bern, Switzerland. The SIBDCS data center at the University Hospital of Lausanne, Switzerland, provided data on past and current disease characteristics and GC therapy outcome. Diagnosis of IBD (CD or UC) was confirmed by the study investigators based on clinical presentation, endoscopic findings and histology.

Sequencing reactions

DNA was extracted from EDTA-blood using the QIAcube robotic workstation and a standard procedure (QiAamp DNA Mini Kit, QIAGEN, Switzerland). The PCR and sequencing primer design was based on the NCBI reference sequence (GenBank accession number NT_029289). Primers for genomic DNA were designed to span all expressed exons (2 to 9) and at least 50 bp of flanking intronic sequences at both 5' and 3'-ends. The DNA sequences of purified PCR fragments were obtained with an ABI 3730xl sequencing machine. Details of the PCR primers can be found in the Table 1. Optimized PCR conditions, and methods used for subsequent purification and sequencing of the fragments are available upon request.

Haplotype analysis

The PHASE software was used to calculate the haplotypes based on the detected single nucleotide polymorphisms (SNPs) and mutations in the NR3C1 gene. PHASE predicts in silico haplotypes on the basis of a Bayesian inference algorithm[12,13]. Haplotype calculations were performed on 181 individuals, from which sequence data of adequate quality were obtained. To allow referral to specific haplotypes, a frequency-based priority criterion was used to name them (e.g. GR_1 for the most often occurring haplotype, Table 2).

Calculation of linkage disequilibria

Linkage disequilibria (LD) were calculated using the $r^2$ statistics. Calculations were performed using the software package Haploview (www.haploview.com).

Statistical analysis

To detect differences in haplotype distribution between
groups with different GC therapy outcomes, the Chi-Square test or the Fisher’s exact test was used. It was analyzed whether one or two copies of a specific haplotype were associated with a particular therapy outcome compared to the GR wild-type carriers. If the number of subjects per group was large enough, heterozygous carriers with one wild-type allele and homozygous carriers of one distinct haplotype were analyzed together against homozygous wild-type carriers. The latter calculations were only performed for haplotypes which occurred in a reasonably large (> 40) number. The statistical analysis was performed using the software package SPSS 17 (SPSS Inc., Chicago, IL).

RESULTS

NR3C1 sequence variability

DNA samples from 185 IBD patients (CD or UC) were initially sequenced for the NR3C1 coding exons 2 to 9.

Table 1  Oligonucleotides used as polymerase chain reaction primers to amplify the NR3C1 exons

| Primer name | Primer sequence | Nested PCR | Primer name | Primer sequence |
|-------------|-----------------|------------|-------------|-----------------|
| GR_2_F      | CACTTAGTTGCTACCTTCCCTAC | Y          | GR_2_Fa     | TCTTTAAGGCCACCTAATTTC |
| GR_2_R      | GATAGAATCACTTCTTGTTGAAC   | Y          | GR_2_Ra     | CTTGGAGATCAGACCTTGTTG |
| GR_3_F      | CAATTAGTTGCTACCTTCCCTAC  | N          | GR_3_Fa     | CTTGGAGATCAGACCTTGTTG |
| GR_3_R      | GATAGAATCACTTCTTGTTGAAC   | Y          | GR_3_Ra     | CTTGGAGATCAGACCTTGTTG |
| GR_4_F      | TTGGAGTTGTCTACCTTCCCTAC  | Y          | GR_4_Fa     | CTTGGAGATCAGACCTTGTTG |
| GR_4_R      | GATAGAATCACTTCTTGTTGAAC   | Y          | GR_4_Ra     | CTTGGAGATCAGACCTTGTTG |
| GR_5_F      | GCACCACACTCATCATAACTC     | N          | GR_5_Fa     | CTTGGAGATCAGACCTTGTTG |
| GR_5_R      | GCACCACACTCATCATAACTC     | N          | GR_5_Ra     | CTTGGAGATCAGACCTTGTTG |
| GR_6_F      | TCCATAACACTCATCATAACTC    | N          | GR_6_Fa     | CTTGGAGATCAGACCTTGTTG |
| GR_6_R      | TCCATAACACTCATCATAACTC    | N          | GR_6_Ra     | CTTGGAGATCAGACCTTGTTG |
| GR_7_F      | ATCTGTCATTGCACCTTCTTCTT   | Y          | GR_7_Fa     | CTTGGAGATCAGACCTTGTTG |
| GR_7_R      | ATCTGTCATTGCACCTTCTTCTT   | Y          | GR_7_Ra     | CTTGGAGATCAGACCTTGTTG |
| GR_8_F      | CTTGGAGATCAGACCTTCTTCTT   | Y          | GR_8_Fa     | CTTGGAGATCAGACCTTGTTG |
| GR_8_R      | CTTGGAGATCAGACCTTCTTCTT   | Y          | GR_8_Ra     | CTTGGAGATCAGACCTTGTTG |
| GR_9a_F     | ATCTGTCATTGCACCTTCTTCTT   | N          | GR_9a_Fa    | CTTGGAGATCAGACCTTGTTG |
| GR_9a_R     | ATCTGTCATTGCACCTTCTTCTT   | N          | GR_9a_Ra    | CTTGGAGATCAGACCTTGTTG |
| GR_9b_F     | ATCTGTCATTGCACCTTCTTCTT   | N          | GR_9b_Fa    | CTTGGAGATCAGACCTTGTTG |
| GR_9b_R     | ATCTGTCATTGCACCTTCTTCTT   | N          | GR_9b_Ra    | CTTGGAGATCAGACCTTGTTG |

PCR: Polymerase chain reaction; GR: Glucocorticoid receptor.
Table 3  Frequencies of single nucleotide polymorphisms detected in the glucocorticoid receptor gene (NR3C1)

| SNP number | Alternative name1 | Variant number2 | DNA position1 | DNA region | cDNA position1 | Nucleotide reference | Nucleotide variant | Amino acid exchange | Allele frequency (n = 362) | Reported allele frequencies3 |
|------------|-------------------|-----------------|---------------|------------|---------------|---------------------|--------------------|----------------------|--------------------------|-----------------------------|
| 1          | 2.1               | rs6189          | 3942366       | Exon 2     | 558           | G                   | A                  | E22E                 | 0.025                    | 0.002-0.034                |
| 2          | 2.2               | rs6190          | 3942364       | Exon 2     | 560           | G                   | A                  | R23K                 | 0.025                    | 0.002-0.034                |
| 3          | 2.3               | rs72542742      | 3942647       | Exon 2     | 1177          | G                   | A                  | A229T                | 0.003                    | 0.002                      |
| 4          | 2.4               | rs56149945      | 3942244       | Exon 2     | 1580          | A                   | G                  | N363S                | 0.025                    | 0.000-0.046                |
| 5          | 3.1               | rs4986593       | 3856773       | Intron 3   | T             | C                   | C                  | -                    | 0.213                     | 0.008-0.228                |
| 6          | 4.1               | rs61753484      | 3852731       | Intron 4   | G             | C                   | C                  | -                    | 0.006                     | 0.000-0.009                |
| 7          | 5.1               | rs6188          | 3843271       | Intron 5   | G             | T                   | T                  | -                    | 0.290                     | 0.000-0.500                |
| 8          | 6.1               | rs6194          | 3841288       | Intron 6   | T             | C                   | T                  | H588H                | 0.006                     | 0.000-0.091                |
| 9          | 8.1               | rs258751        | 3825207       | Exon 8     | 2526          | C                   | T                  | D678D                | 0.006                     | 0.000-0.149                |
| 10         | 8.2               | rs6189 SNP      | 3824968       | Intron 8   | A             | G                   | G                  | -                    | 0.003                     | NA                         |
| 11         | 8.3               | rs258750        | 3824816       | Intron 8   | G             | T                   | T                  | -                    | 0.307                     | 0.091-0.362                |
| 12         | 8.4               | rs10482704      | 3824969       | Intron 8   | G             | T                   | G                  | -                    | 0.017                     | 0.000-0.027                |
| 13         | 9.1               | rs6196          | 3824417       | Exon 9     | 2790          | T             | C                  | N766N                | 0.022                     | 0.058-0.325                |

1Defines both the exon/intron localization and the single nucleotide polymorphism (SNP) number; 2According to the National Center for Biotechnology Information (NCBI) SNP database; 3According to the NCBI genomic reference sequence NT_029289.11; 4According to the NCBI cDNA reference cDNA Sequence NM_000176.2. NA: Not applicable.

Haplotypes

| Haplotype | Exon | Intron | Amino acid | Allele frequency (n = 362) |
|-----------|------|--------|------------|---------------------------|
| GG G A T  | T    | G      | C           | 45.3%                     |
| GG G A T  | G    | C      | C A G T     | 24.9%                     |
| GG G A G  | T    | G      | C A T G     | 20.1%                     |
| GG G A T  | G    | C      | C A T G     | 2.2%                      |
| AA G A T  | G    | C      | C A G T     | 1.7%                      |
| GG G A G  | T    | G      | C A G C     | 1.1%                      |

Figure 1  Most frequently occurring NR3C1 haplotypes and their single nucleotide polymorphisms composition. The localization of the variant nucleotides in the NR3C1 gene is indicated. All detected non-synonymous single nucleotide polymorphism (SNP) (R23K, A229T, N363S) flank the N-terminal transactivation domain. Only four out of 17 predicted haplotypes occur at a frequency higher than 2%.

and 50 bp of the neighbouring intronic sequences. The sequencing results of 181 individuals were of adequate quality and further used for SNP and haplotype analyses. The sequence data were screened for genetic variations in the NR3C1 gene, using the Basic Local Alignment Search Tool (BLAST; www.ncbi.nlm.nih.gov) and the GenBank entry NT_029289.11 as the reference sequence.

In Table 3 we list the allele frequencies of all detected SNPs within the IBD cohort under study. Thirteen variants were detected, which were-with exception of one mutation (rs6196, P < 0.01)-in Hardy-Weinberg equilibrium. All variants were single nucleotide substitutions.

Six variants were detected within the intronic regions, whereas seven variants were found in exons (Figure 1). Three of the seven variants detected within the coding regions of the NR3C1 gene resulted in non-synonymous amino acid exchanges, while four of them did not lead to changes in the GR amino acid sequence. Eight variants occurred with an allelic frequency of more than one percent (rs56149945, rs6189, rs6190, rs4986593, rs6188, rs258750, rs10482704, rs6196). All non-synonymous amino acid exchanges (R23K, A229T, N363S) were found in the N-terminal half of GR, flanking the N-terminal transactivation domain10. The intronic variant found at
DNA position 3824968 has not been previously listed in the NCBI SNP database.

**Haplotype analysis**

The 13 NR3C1 variants described above were included in the haplotype calculations using the computer program PHASE. All 181 individuals were included in the haplotype prediction analysis (Figure 2). Twenty-five NR3C1 haplotypes were predicted by PHASE to exist in the cohort of 181 inflammatory bowel disease patients. Counter (a to y) for the 25 theoretically arising haplotypes in the inflammatory bowel disease cohort. SNP: Single nucleotide polymorphism.

**Analysis of NR3C1 haplotypes in relation to steroid therapy outcome**

An overview of the demographic data of the 173 subjects included in the association analysis is shown in Table 4 (further patient data on comedication and extraintestinal manifestations are given in Tables 5 and 6), and the haplotype combinations calculated for all patients are shown in Table 7. As the numbers of homozygous carriers of variant NR3C1 haplotypes were low, the subjects were analyzed as carriers of one or two copies of a distinct variant haplotype, irrespective of whether the other allele was determined to be wild-type or variant in the case of heterozygotes (Table 8). Furthermore, haplotypes GR_2 and GR_3 were analyzed by testing the heterozygous allele combinations GR_2 + GR_1 (wt) together with the

**Table 4 Demographic data of 173 inflammatory bowel disease patients included in the association analysis**

| Characteristics | Crohn’s disease | Ulcerative colitis | All |
|-----------------|-----------------|-------------------|-----|
| Patients        | 84 (49%)        | 89 (51%)          | 173 (100%) |
| Age (documented for 171 individuals) | 37.5 (± 13.3) | 41.7 (± 14.2) | 39.7 (± 14.9) |
| mean ± SD       | 35              | 42                | 39  |
| Median          | 16              | 18                | 16  |
| Minimum         | 72              | 82                | 82  |
| Maximum         |                 |                   |     |
| Known GC treatment outcome in the past | 50              | 50                | 100 |
| No. of patients currently treated with GCs | 83              | 60                | 143 |
| Male/female     | 52 (58.4%)/    | 40 (47.6%)/    | 92 (53.2%)/ |
|                 | 37 (41.6%)     | 44 (52.4%)     | 81 (46.8%) |
| Wild-type carriers | 15 (16.6%)   | 20 (23.8%)     | 35 (20.2%) |
| Carriers of one variant haplotype | 48 (53.9%) | 39 (46.4%) | 87 (50.3%) |
| Carriers of two variant haplotypes | 26 (29.2%) | 25 (29.8%) | 51 (29.5%) |

GCs: Glucocorticoids.
Table 5  Past and current additional medication of 173 inflammatory bowel diseases patients included in the association analysis

| Additional medication               | n  |
|------------------------------------|----|
| 5-Aminosalicylic acid              | 142|
| 6-Mercaptopurine                   | 33 |
| Adalimumab                         | 3  |
| Antibiotics                        | 64 |
| Azathioprine                       | 122|
| Bisphosphonates                    | 8  |
| Certolizumab                       | 1  |
| Cholestyramine                     | 8  |
| Cyclosporine                       | 45 |
| Infliximab                         | 27 |
| Methotrexate                       | 12 |
| Sulfasalazine                      | 3  |
| Ursodeoxycholic acid               | 3  |

Table 6  Extraintestinal manifestations

| Extraintestinal manifestations       | n (%) |
|--------------------------------------|-------|
| Peripheral arthritis                 | 46 (27.2) |
| Uveitis/iritis                       | 6 (3.6) |
| Pyoderma gangrenosum                 | 4 (2.4) |
| Erythema nodosum                     | 9 (5.3) |
| Aphthous oral ulcers                 | 10 (5.9) |
| Ankylosing spondylitis               | 7 (4.1) |
| Primary sclerosing cholangitis       | 6 (3.6) |

\(^1\)Documented for 169 patients.

homzygous GR\(_2\) subjects and the allele combination GR\(_3\) + GR\(_1\) (wt) together with homozygous GR\(_3\) carriers against wild-type carriers. For all individuals, prior success of GC therapy was documented, and for patients under GC therapy at the point of study entry the applied dosage was also noted.

No significant associations were observed between haplotype GR\(_2\) and success of GC therapy (Figure 4). Upon stratification of the patient cohort according to gender or disease subgroup (UC or CD), no significant association between therapy success or haplotype GR\(_2\) was observed either. Similarly, when stratifying according to the subgroup of heterozygous GR\(_2\), GR\(_1\) and homozygous GR\(_2\), no statistically significant difference in therapy response compared to wild-type carriers could be observed.

No significant associations were observed between either haplotype GR\(_3\) (Figure 5) or GR\(_4\) (Table 8) and GC therapy outcome, or between individual SNPs and therapy success (Figure 6). Similarly, we observed no significant associations between the severity of disease (active or inactive state of UC or CD) or currently taken GC dosage levels and NR3C1 haplotypes (data not shown).

**DISCUSSION**

Glucocorticoid receptor (GR) plays an important role in many physiological and pathological processes and is the main target of glucocorticoids, widely used as therapeutic agents to treat a variety of autoimmune diseases\(^{[8,17]}\). Two GR isoforms, GR\(_\alpha\) and GR\(_\beta\), generated by alternative mRNA splicing exist\(^{[18]}\). Only GR\(_\alpha\) can be activated by glucocorticoid ligands, while GR\(_\beta\) does not bind glucocorticoids and may in fact act as an inhibitor of glucocorticoid action\(^{[19]}\). Genetic variation in the NR3C1 gene has been shown to affect both disease pathophysiology and response to glucocorticoid therapy\(^{[19,20-22]}\), suggesting that SNPs might play a role in GR function and associated steroid therapy outcome also in IBD patients. GR is known to regulate the intestinal bile acid uptake transporter ASBT\(^{[23,24]}\), the expression of which is altered in IBD patients\(^{[25]}\). While it has been reported that GR mRNA expression levels are not predictors of steroid response in IBD\(^{[26]}\) and that the GR polymorphisms R23K and N363S are not associated with CD in a pediatric Caucasian population\(^{[27]}\), no studies on the role of NR3C1 gene variants in steroid therapy success were previously available. The aim of the current study was to analyze sequence variation and

Table 7  Predicted frequencies of haplotype combinations in 173 inflammatory bowel diseases patients

| Haplotype combination       | n  | Frequency |
|-----------------------------|----|-----------|
| GR\(_1\) + GR\(_2\) or GR\(_2\) hom | 51 | 0.295     |
| GR\(_1\) + GR\(_3\) or GR\(_3\) hom | 41 | 0.237     |
| GR\(_1\) hom (wt)            | 36 | 0.208     |
| GR\(_2\) + GR\(_3\)          | 23 | 0.133     |
| GR\(_1\) + GR\(_4\) or GR\(_4\) hom | 6 | 0.035     |
| GR\(_1\) + GR\(_5\)          | 3  | 0.017     |
| GR\(_3\) + GR\(_6\)          | 2  | 0.012     |
| GR\(_1\) + GR\(_7\) or GR\(_7\) hom | 1 | 0.006     |
| GR\(_1\) + GR\(_16\) or GR\(_16\) hom | 1 | 0.006     |
| GR\(_2\) + GR\(_8\)          | 1  | 0.006     |
| GR\(_2\) + GR\(_9\)          | 1  | 0.006     |
| GR\(_6\) + GR\(_13\)         | 1  | 0.006     |
| GR\(_7\) + GR\(_15\)         | 1  | 0.006     |
| GR\(_2\) + GR\(_4\)          | 1  | 0.006     |
| GR\(_2\) + GR\(_11\)         | 1  | 0.006     |
| GR\(_5\) + GR\(_9\)          | 1  | 0.006     |
| GR\(_9\) + GR\(_17\)         | 1  | 0.006     |

Table 8  Association between glucocorticoids therapy outcome and the haplotype GR\(_4\)

| Haplotype | Cohort composition | Therapy success rate in wt carriers (success/ no success) | Therapy success rate in het/hom variant carriers (success/ no success) | P-value | OR (CI) |
|-----------|--------------------|---------------------------------------------------------|-------------------------------------------------------------------|---------|---------|
| GR\(_4\)  | All                | 0.682 (15/7)                                           | 0.4 (2/3)                                                        | 0.326   | 3.214 (0.434-25.787) |
|           | Male               | 0.733 (11/4)                                           | NA (0/0)                                                         | NA      | NA      |
|           | Female             | 0.571 (4/3)                                            | 0.4 (2/3)                                                        | 1.000   | 2.000 (0.194-20.614) |

NA: Not applicable (at least one cell box was counted as 0, OR and P not calculable).
haplotype structures in the coding parts of the \textit{NR3C1} gene in a cohort of 181 Swiss IBD patients. We investigated whether \textit{NR3C1} genetic variants or haplotypes may influence steroid therapy outcome in IBD patients.

We identified 13 variants in this study, of which 12 had already been previously submitted to the NCBI SNP database. We calculated the corresponding haplotypes in the IBD patient cohort and studied the association of the most prevalent SNP combinations with steroid therapy outcome, disease activity, and age of disease onset. Several \textit{NR3C1} SNPs have been previously associated with altered disease susceptibility or risk of disease progression in other autoimmune diseases, such as Guillain-Barré Syndrome or multiple sclerosis\cite{15,20,21}. Most of these studies only analyzed the impact of a small number of pre-defined SNPs, such as the BclI polymorphism or the E22E/R23K polymorphisms\cite{17,28}. Few reports have been published on the potential influence of \textit{NR3C1} SNPs on sensitivity to endogenous or exogenously given GCs\cite{17,28}, and only one significant association between the polymorphism E22E/R23K and sensitivity to exogenously administered GCs in elderly Dutch people has been reported\cite{21}. So far no large cohort studies have been reported in which the influence of \textit{NR3C1} SNPs on GC therapy outcome in IBD patients...
has been investigated. Here, we describe five \( NR3C1 \) haplotypes occurring at a frequency \( > 1\% \) and analyze the potential association of the three most common haplotypes \( GR_2, GR_3, \) and \( GR_4 \) with GC therapy outcome in IBD patients. While a large number of \( NR3C1 \) variants are already registered in the NCBI SNP database, we observed only eight variants that occurred at a frequency \( > 1\% \), and these were responsible for the composition of a relatively small group of commonly occurring haplotypes. The overall risk for a certain UC and/or CD activity state or for a different steroid therapy outcome was not altered in \( GR_2, GR_3, \) or \( GR_4 \) carriers, in comparison with the wild-type carriers. Furthermore, no significant associations were observed between individual SNPs and GC therapy success. In the case of certain SNPs/haplotypes (e.g. \( GR_4, E22E/R23K \)), a larger cohort would have been preferable in order to obtain more reliable results, as these variants occurred quite rarely in our patient group. Similarly to our observations, Dekker et al.\(^{9} \) could not detect any associations between distinct haplotypes and SNPs in a Guillaum-Barré Syndrome cohort treated with methylprednisolone, although the authors noted that their study group was too small to obtain statistically reliable results. It remains to be seen whether the rare GR variants present in our study cohort will show significant associations in larger cohorts of IBD patients.

In conclusion, we have performed a comprehensive study analyzing the role of genetic variants in the \( NR3C1 \) gene in glucocorticoid sensitivity in a Swiss cohort of IBD patients. We show that \( NR3C1 \) haplotypes are not a general modulating factor in glucocorticoid therapy outcome.

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COMMENTS

Background

Crohn’s disease and ulcerative colitis are two distinct types of inflammatory bowel disease (IBD), which is an increasingly prevalent disease condition worldwide. Wide variation is observed in clinical manifestation and therapy responses in IBD, partly due to individual genetic variation.

Research frontiers

Glucocorticoid therapy is commonly used in treatment of IBD, however the response to therapy varies between individuals. The authors hypothesized that genetic variation in the \( NR3C1 \) gene encoding the glucocorticoid receptor (GR) may affect the response to glucocorticoids in IBD patients.

Innovations and breakthroughs

In this comprehensive genetic analysis, all coding exons and exon-intron junctions of the \( NR3C1 \) gene were sequenced in 181 IBD patients, who had been treated with glucocorticoids and whose past responses to this treatment had been recorded. This is the first published study on the effects of genetic variation in GR on glucocorticoid therapy in IBD patients, in a modestly sized study cohort.

Applications

If significant associations between genetic GR variants and glucocorticoid therapy outcome had been observed, this could have allowed more considered design of the individual therapy options upon prior genotyping of the patients.

Terminology

The transcription factor of the steroid receptor family, GR, is proposed to be a major mediator of anti-inflammatory pathways elicited by therapeutically administered glucocorticoids.

Peer review

The genetic study investigates the predictive value of \( NR3C1 \) gene variants towards the clinical outcome of patients with Crohn’s disease and ulcerative colitis. Although the result of this study was negative, the study was meaningful in that abundant GR variants were determined and analyzed in IBD patients.

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Mwinyi J et al. GR haplotypes and IBD

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