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No association between C-reactive protein gene polymorphisms and decrease of C-reactive protein serum concentration after infliximab treatment in Crohn's disease

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Abstract:

We recently showed an association between the *FCGR3A V/F* polymorphism and the biological response [assessed on the basis of a C-reactive protein (CRP) concentration decrease] to infliximab in Crohn's disease. The *CRP* and *FCGR3A* genes are located on the same 1 q23 locus. The present study aimed: (i) to exclude a linkage disequilibrium (LD) between the two genes and (ii) to study the association between *CRP* polymorphisms and the response to infliximab, particularly the decrease in CRP after treatment, in Crohn's disease patients. *FCGR3A* (V/F) polymorphism and three *CRP* polymorphisms (-717G/A, 1444C/T, *CRP* 4A/G) were determined in 206 healthy blood donors and 189 Crohn's disease patients who had received infliximab for either refractory luminal or fistulizing Crohn's disease. Clinical response was defined as complete, partial or absent according to the same definition as in controlled trials. The biological response was defined on the basis of CRP decrease. There was no LD between *CRP* and *FCGR3A* in healthy donors or Crohn's disease patients. *CRP* polymorphisms had no impact on CRP decrease after infliximab. The proportions of Crohn's disease having a positive clinical or biological response were not statistically different among the various genotypes of *CRP* polymorphisms. There was no LD between CRP and FCGR3A polymorphisms. CRP polymorphisms were not associated with the response to infliximab in Crohn's disease.

Keywords: C-reactive protein, Crohn's disease, infliximab, pharmacogenetics

INTRODUCTION

Infliximab is a monoclonal chimeric antibody [immunoglobulin (Ig)Gl] directed against tumour necrosis factor (TNF)-α [1] and has been used as a treatment for Crohn's disease for several years. Although it is effective in refractory and fistulizing forms of the disease [2,3], there is an inter-individual variability in response to this drug, associated with several clinical or biological factors [4-7]. Particularly, a positive clinical response to infliximab has been associated with an elevated C-reactive protein (CRP) level before treatment [8]. Furthermore, a polymorphism in the *FCGR3A* gene, encoding a receptor for the Fc portion of IgGs, as involved in antibody-dependent cell-mediated cytotoxicity (ADCC), was shown to be associated with the biological response to infliximab in patients with Crohn's disease: *FCGR3A*-158V/V patients had a better response, as assessed by variation in CRP concentrations before and after treatment, compared to F carriers [9]. CRP, a member of the pentraxin family, is a plasma protein that contributed to innate host defence. It is also an acute phase protein that is usually elevated in active Crohn's disease. The gene coding for CRP has been mapped to lq23, approximately 2000 kb downstream of *FCGR3A* in the centromeric position of the *FCGR* gene cluster. Due to this close physical location, a genetic linkage disequilibrium (LD) between these two genes is possible [10], and might explain the better decline in CRP concentrations in *FCGR3A*-158V homozygous patients. If this was the case, the *CRP* gene would not necessarily have a direct impact on the objective response to infliximab, but rather could influence the magnitude of the CRP decrease in the serum after infliximab treatment.

Indeed, several functional polymorphisms have been described in the *CRP* gene, such as the -717G/A substitution located in the promoter or the 1444C/T variant in the 3' untranslated region (UTR). The -717G/A substitution was not associated with variations in CRP concentrations [11] but -717G/G homozygotes had a greater risk of developing type 2 diabetes in Pima Indians [12]. The 1444C/T substitution located in the 3' UTR

was associated with variations in CRP serum concentrations: CRP concentrations were higher in 1444T/T homozygous healthy volunteers both at baseline and after exercise than in 1444C carriers [11]. In patients undergoing coronary artery bypass, mean CRP concentrations after surgery were also higher in 1444T/T homozygotes [12]. More recently, in a cohort of patients suffering from systemic lupus erythematosus (SLE) and their family members, it was also found that the *CRP* haplotype containing the 1444T allele was associated with high basal CRP concentrations. In the same study, a new polymorphism named *CRP* 4, also located in the 3' UTR, was identified and linked with SLE; the *CRP* 4A rare allele was associated with low basal CRP concentrations, with a gene dose effect [13].

The present study aimed: (i) to analyse LD between *CRP* -717G/A, *CRP* 1444C/T, *CRP* 4A/G and *FCGR3A* 4985 G/T (encoding the 158V/F amino acid variants in the protein, respectively) polymorphisms and (ii) to evaluate the association between CRP gene polymorphisms and response to infliximab, particularly the decrease in CRP.

PATIENTS AND METHODS

Study population

Blood samples were obtained from two hundred and six healthy anonymous blood donors from Tours (France) for LD analysis. The second part of our study was based on a cohort of 189 patients with Crohn's disease, largely similar to the one used in a previous study [9]. Briefly, all patients were treated with infliximab for the first time. They had refractory luminal or fistulizing Crohn's disease. Approval from the Ethics Committee of Liège University was obtained and patients provided their written informed consent for both infliximab treatment and pharmacogenetic ancillary studies. The demographic and clinical characteristics of the patients are given in Table 1. There were two subgroups of patients: 137 patients with active luminal (non-fistulizing) disease and 52 patients with fistulizing disease. Luminal Crohn's disease patients were treated with a single infusion of infliximab (5 mg/ kg). Crohn's disease activity index (CDAI) [14] was calculated and CRP measured (by routine procedure using immunoturbidimetry, in local laboratories, CRP values then being normalized according to the upper limit of the normal value in each laboratory) at weeks 0 and 4. Patients with fistulizing disease were treated with three consecutive infusions (5 mg/kg) at weeks 0, 2 and 6. CDAI was calculated, CRP measured and fistulous tracks examined, at weeks 0 and 10.

***Table 1**: Demographic, clinical and biological characteristics of the Crohn's disease patients (n = 189)*

Characteristic	Value
Mean ± SD Crohn's diseases activity index	263.5±121.7
Median C-reactive protein (unormalize id) (range)	6.7 (1-80)
Female/male (%)	116/73 (61.4/38.6)
Fistulizing/refractory (%)	52/137 (27.5/72.5)
Median age (years) (range)	34 (1 6-76)
Disease location	
Ileal (%)	36 (19.0)
Colonic (%)	63 (33.3)
lleocolonic (%)	77 (40.7)
Upper gastrointestinal involvement (%)	13 (6.9)
Anal involvement (%)	74 (39.1)
Concomitant treatment	
5-aminosalicylates (%)	88 (46.7)
Corticosteroids (%)	80 (42.3)
Immunosuppressives (%)	1 20 (63.5)
Smoking (%)	66 (34.9)

Classification of response to infliximab

The response to infliximab was assessed on the basis of both clinical and biological evolution. Clinically, patients were classified as complete responders, partial responders or non-responders according to published controlled clinical trials [2,3]. In non-fistulizing disease, patients were considered complete or partial responders

at week 4, if they had a decrease of CDAI below 150 or a decrease of at least 70 points from baseline, respectively. In fistulizing disease, patients were considered as complete or partial responders at week 10, in the case of complete fistula closure or a decrease of at least 50% in fistula drainage at two consecutive visits. Furthermore, the variation in CDAI between pre-treatment (week 0) and post-treatment periods (week 4 or 10) was calculated for all patients.

Biologically, patients were classified as complete, partial or non-responders at 4 weeks (non-fistulizing disease) or 10 weeks (fistulizing disease) on the basis of CRP variation. This analysis was performed on a subgroup of 148 patients for whom CRP values before and after treatment were available and who had an elevated CRP (more than twice the upper limit of the normal range) before treatment. Patients were classified as complete responders or partial responders in the case of normalization of CRP after treatment or a decrease of at least 25% from baseline level, respectively. Furthermore, for all patients, the variation of CRP (in absolute and relative value) was calculated between pre-treatment (week 0) and post-treatment periods (week 4 or 10).

DNA analysis

For healthy blood donors, genomic DNA was extracted from 2 ml of EDTA-anticoagulated blood with the Qiamp DNA Blood Midi Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's recommendations. DNA concentrations were determined at 260 nm and samples were stored at -20°C until use. For Crohn's patients, genomic DNA was isolated until use as previously described [9] and stored at -20°C.

FCGR3A-158V/F and *CRP* 4A/G genotyping were performed according to previously described allele specific polymerase chain reaction (PCR) [15] and PCR-restric-tion fragment length polymorphism (RFLP) [13] methods, respectively.

For the *CRP* 1444C/T polymorphism, a new PCR-RFLP analysis assay was set up using a reverse primer originally described by Brull *et al.* [11], but with slight modifications including a newly designed forward primer (Table 2). The reverse primer covers nucleotides 1579-1600 of the Genbank M11880 sequence and the forward primer covers nucleotides 1419-1443. The forward primer contains one mismatch (underlined) which creates a 1444T allele-specific restriction site for *Nla*III (6ATG), and is 5'-elongated (italic) to obtain a 30-bp difference between the two PCR products. The 20 µl PCR reaction mixture contained 50 ng genomic DNA, 2mM $MgCl_2$, 200 µM of each dNTP (MBI Fermentas, Mundolsheim, France), 1 µM of each primer and 0.5 U of EurobioTaq DNA polymerase (Eurobio, Les Ulis, France) in its specific buffer. Samples underwent 40 PCR cycles (60 s at 94°C, 60 s at 61.2°C and 60s at 72°C) using an iCycler thermocycler (Biorad, Marnes la coquette, France). After digestion of PCR products with *Nla*III, an electrophoresis in a 3% agarose gel discriminated the two alleles (Table 2).

For the -717G/A *CRP* variant, known as SNP 133552 [12] or RS2794521 [11], PCR was conducted as described above except that the annealing temperature was 65°C and that the reaction mixture contained 1.5 mM $MgCl_2$, with primers defined by Wolford *et al.* [12]. PCR products were digested with *Nla*III and the G allele was visualized as a fragment of 184 bp (five *Nla*III restriction sites) and the A allele as a fragment of 163 bp (six *Nla*III restriction sites) (Table 2).

CRP 1444C/T-CRP 4A/G haplotypes, as well as *CRP 1444C/T-CRP 4A/G-CRP* -717G/A haplotypes, were constructed considering only non-ambiguous patients (i.e. those having no more than one heterozygosity for the three polymorphisms studied).

Table 2 *Primers and enzyme used in polymerase chain reaction-restriction fragment length polymorphism assays*

Polymorphism	Primers	Allele	Size
CRP -717	Forward: ATGCTCCTCCCAGAGC[a]	G	184
	Reverse: GCCGTCATTTAGTGCCAAG[a]	A	163
CRP +1444	Forward: *ATATTAATAA*GGAGCTCGTTAACTATGCTGGGAC<u>C</u>A[b]	C	190
	Reverse: TTCTCAGCTCTTGCCTTATGAG	T	160
CRP 4	Forward: CGAGTGAGACATCTTCTTG	A	227
	Reverse: CTTATAGACCTGGGCAGT	G	130

[a]Defined in Wolford *et al.* [1 2].
[b]Underlined position indicates a mismatch introducing a restriction site on the T allele; italic characters indicate the unspecific 5' tail.

Statistical analysis

An exact test [16] tested departure from Hardy-Weinberg equilibrium using GENEPOP software [17]. Significance of LD between two loci was estimated using a chi-square test between maximum likelihood estimated and expected (under independency hypothesis) haplotypes (EH software) [18]. LD values were calculated by subtracting estimated and expected haplotype frequencies. Subsequently, relative LD values were obtained by dividing LD by maximum LD (product of lower frequency allele frequencies). Allele and genotype proportions in the two different populations were compared using a chi-square test for independency and Fisher's exact test, respectively. In the subgroup of patients with active luminal disease (n = 137), baseline CRP values were compared in various subgroups of patients, who were defined according to CRP genotypes, using Mann-Whitney and Kruskal-Wallis tests. Furthermore, the proportions of various CRP genotypes were compared between patients with elevated or normal baseline CRP using a chi-square test for independence. Proportions of patients having a positive clinical or biological response to infliximab in the different genotype groups were compared using a chi-square or Fisher's exact test, as required. The decreases in CRP or CDAI values according to genotype were compared using Kruskal-Wallis or Mann-Whitney tests, as required, P-values were corrected (P_c) for multiple tests (three gene polymorphisms tested for influence on baseline CRP values, as well as clinical and biological responses to infliximab). $P_c < 0.05$ was considered statistically significant.

RESULTS

CRP genotyping of normal individuals and infliximab-treated Crohn's disease patients

To elucidate a possible role for *FCGR3A-CRP* LD in the previously described association between the biological response to infliximab in Crohn's disease patients and the *FCGR3A-158V/F* polymorphism, three relevant *CRP* polymorphisms were genotyped in our 189 patients and in 206 healthy individuals. All the genotype frequencies were in Hardy-Weinberg equilibrium in both healthy blood donors and Crohn's disease patients. There was no statistically significant difference in allele or genotype proportions between the two different populations. Particularly, frequencies of C/C, C/T and T/T *CRP* + 1444 genotypes were 40.8%, 48.5% and 10.7% versus 41.2%, 48.7% and 10.1% in controls and Crohn's disease patients, respectively (P = 0.99); frequencies of A/A, A/G and G/G *CRP* -717 polymorphisms were 54.4%, 38.8% and 6.8% versus 56.3%, 37.1% and 6.6% in controls and Crohn's disease, respectively (P = 0.93); frequencies of A/A, A/G and G/G *CRPA* genotypes were 10.7%, 42.2% and 47.1% versus 9.1%, 45.7% and 45.2% in controls and Crohn's disease patients, respectively (P = 0.75). As previously published [13], each studied *CRP* gene polymorphism was in LD with each other. This was observed both in healthy donors and in Crohn's disease patients (Fig. 1). By contrast, none of these three polymorphisms proved to be in LD with *FCGR3A,* neither in healthy donors nor in Crohn's disease patients (Fig. 1).

CRP 1444C/T and *CRP* 4A/G polymorphisms produced three main haplotypes: CA, CG and TG, representing 29.3%, 39.1% and 30.1% of haplotypes in healthy controls (184 haplotypes in 92 subjects) and 28.3%, 41.8% and 29.3% in Crohn's disease patients (184 haplotypes in 92 patients), respectively. When adding the *CRP* -717G/A polymorphism, four main haplotypes were produced: CAA, CGA, CGG and TGA representing 27.7%, 21.2%, 17.9% and 29.3% of haplotypes in healthy controls (184 haplotypes in 92 subjects) and 27.7%, 23.4%, 18.5% and 28.3% in Crohn's disease patients (184 haplotypes in 92 patients), respectively.

CRP genotypes and baseline CRP concentrations in Crohn's disease patients

Although Crohn's disease patients overall do not differ from a normal Caucasian population in the *CRP* allele or genotype frequencies, it is possible that *CRP* polymorphisms are associated with particular presentations of the disease, particularly CRP concentrations. The subgroup of Crohn's disease patients with active luminal disease and elevated CRP (more than the upper limit of the normal value) had *CRP* genotype proportions similar to those of Crohn's disease patients with active luminal Crohn's disease and normal CRP (data not shown). No significant differences were found either when looking at haplotype frequencies (data not shown). Furthermore, median baseline CRP was similar in the various genotypic groups (Table 3).

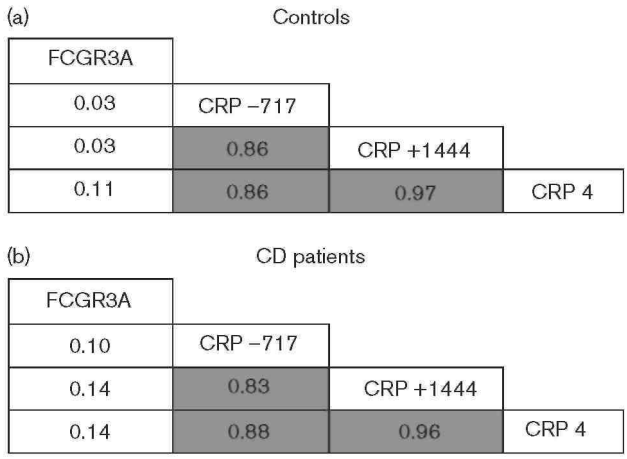
CRP genotypes according to clinical and biological response to infliximab

Mean CDAI before and after infliximab treatment was 263.5 ±121.7 and 151.9 ±119.5, respectively. Clinical response was complete in 97 patients (51.3%), partial in 34 (18%), absent in 44 (23.3%) and not assessable in 14

(7.4%). Median (normalized) CRP before and after infliximab was 6.7 (1-80) and 3.2 (1-52.3), respectively. Biological response (as previously defined [9]) was complete in 68 patients (45.9%), partial in 46 (31.1%) and absent in 34 (23%). Overall, there was a significant correlation between relative decrease in CDAI and relative decrease in CRP (r = 0.298, 95% confidence interval = 0.125-0.454; *P* = 0.0007). However, in a significant number of cases, there was a discrepancy between the clinical and biological response as previously defined (discordance in 34.1% of cases).

There was no significant association between the studied *CRP* gene polymorphisms and the clinical or biological response to infliximab in Crohn's disease. Proportions of patients with a positive clinical or biological response to infliximab, as well as median relative decrease in CRP, are shown in Table 3. There was no significant difference in median absolute CRP decrease. There was no significant difference in CDAI decrease between *CRP* genotypes (data not shown). Finally, no significant difference was found when looking at haplotype frequencies (data not shown).

Fig. 1: *Linkage disequilibrium (LD) between the four studied loci in (a) healthy blood donors and (b) in Crohn's disease patients. The values of relative LD (= 1 when one of the four theoretical haplotypes is not represented) between each biallelic polymorphism are presented in boxes. Grey boxes, Significant LD (P<0.05); empty, non-significant LD (P>0.05).*



Discussion

We have previously shown that a positive response to infliximab in Crohn's disease is associated with elevated CRP serum concentrations before treatment [8]. More recently, we also generated data indicating that the 158V/F polymorphism of *FCGR3A,* a gene coding for an IgG Fc receptor potentially involved in ADCC, is associated with more prominent decrease in CRP concentrations in response to infliximab in Crohn's disease [9]. Some published data suggest that functional polymorphisms in *CRP* gene may influence serum CRP concentration in humans [11,13]. Finally, the *CRP* gene is located in lq23, close to the *FCGR3A* gene. Despite these complex relationships between CRP, *FCGR3A* polymorphism, Crohn's disease and the response to infliximab, the present study shows that there is no LD between the *FCGR3A* and *CRP* studied polymorphisms. Moreover, no association was observed between several *CRP* polymorphisms and CRP serum concentrations in active Crohn's disease before infliximab treatment on the one hand or clinical and biological response to infliximab on the other.

Because the association that we have previously reported between the *FCGR3A* gene and response to infliximab in Crohn's disease was significant for the biological response (defined by the CRP decrease after treatment) but not for the clinical response [9], and because these two genes are located close to each other on lq23, this association could have been a false-positive association due to a LD between these two genes. The true association would then have been with one or several of the described *CRP* gene polymorphisms. Our data show that this is probably not the case because there is no LD between the *CRP* gene polymorphisms studied and the *FCGR3A*-158V/F polymorphism both in controls and in a population of Crohn's disease patients who were treated with infliximab and because no association was observed between CRP polymorphisms and the response

to infliximab, particularly the decrease in serum CRP after treatment.

Similar to previously published studies [11,13], the three *CRP* polymorphisms studied were themselves in LD. This LD allowed Russell *et al.* [13] to define, in a large cohort of SLE families, five major haplotypes based on five genotypic polymorphisms. These haplotypes were associated with different baseline CRP concentrations. Two of these polymorphisms (*CRP* 1444 and *CRP* 4) were selected for use in the present study because they could define three major haplotypes groups among the five Russell's haplotypes, each of them being characterized with a particular pattern of basal CRP production: 1444C and *CRP* 4A alleles define Russell's haplotypes 2 and 5 associated with significantly low CRP basal concentration, 1444T and *CRP* 4 G alleles define haplotype 1 associated with high CRP concentration and 1444C and *CRP* 4 G alleles characterize haplotypes 3 and 4 [13]. Moreover, we have included an analysis of the *CRP* -717G/A polymorphism, which was not included in the Russell's study but which has been associated with type 2 diabetes [12]. A haplotype analysis performed on our cohort of normal individuals demonstrates that *CRP* -717G/A polymorphism brings a further level of complexity to Russell's haplotypes. Indeed, when analysing the three precedently defined groups of haplotypes and the -717G/A polymorphism, we could define four major haplotypes. The first two groups containing haplotypes 1, 2 and 5 are mainly associated with the -717A allele but the third group is associated either with -717A or G allele creating four major haplotypes in our analysis. The three *CRP* polymorphisms presently studied therefore ensure a correct assessment for the *CRP* gene. However, none of these three tag CRP polymorphisms was found to be in LD with *FCGR3A,* either individually or as haplotypes, leading to the conclusion that *FCGR3A* influences the biological response to infliximab by itself, probably by facilitating the killing of TNF-α-expressing inflammatory cells in the gut [9]. The probability that another *CRP* polymorphism would be in LD with *FCGR3A* cannot be totally excluded but is extremely low.

Whatever the risk of LD between *FCGR3A* and *CRP,* which can now be considered as very low, the possible association between *CRP* polymorphisms and the response to infliximab remains a relevant question. Indeed, a positive response to infliximab has been associated with an elevated CRP before treatment [8]. The paradoxical absence of a CRP increase in some patients with clinically active Crohn's disease may be due to a genetic predisposition to produce less CRP. Indeed, family studies have suggested that 30-40% of the variation in plasma CRP concentration is genetically determined [19]. The three variants of the *CRP* gene studied here have been shown to be functional, and either associated with certain diseases [12,13] or with variations in CRP plasma concentrations [11,13]. However, none of the CRP polymorphisms was found to be associated with low

CRP concentrations before treatment. According to the genetic hypothesis, some *CRP* genotypes could also be associated with a suboptimal response to infliximab in Crohn's disease, particularly when assessed by CRP decrease. However, again, this is not the case in the present study because similar response rates were observed, according to both clinical and biological criteria, in the various genotype groups, whatever the *CRP* genotype considered. Thus, *CRP* polymorphisms do not appear to be the basis of the association between CRP concentration before treatment and the response to infliximab in Crohn's disease. Therefore, other explanations, including functional symptoms and particular pathways of inflammation, appear to provide more likely reasons for low CRP concentrations despite active Crohn's disease and suboptimal response to infliximab. The fact that CRP polymorphisms do not have any influence on CRP variations after infliximab treatment is also important because CRP variation is considered as a relevant marker of response to infliximab in addition to clinical scores such as CDAI [2,8,9].

The observation that *CRP* -717, *CRP* 1444 and *CRP* 4 had no influence on serum CRP concentrations before infliximab treatment appears to be in contrast to that observed in coronary disease or in SLE, where *CRP* 1444T allele and *CRP* 4A allele were associated with higher and lower CRP concentrations, respectively [11,13]. However, in a recent study of a cohort of patients with coronary heart disease and a population of controls, this association was not confirmed [20]. One of the main reasons for these discrepancies may be that *CRP* variants influence slight variations in low CRP levels in healthy individuals or diseases that are not associated with a significant elevation of CRP [20]. Furthermore, an association with higher levels of CRP in diseases such as Crohn's disease, which is associated with a pronounced elevation of this protein, would be very difficult to disclose, with the heterogeneity in the degree of inflammatory reaction being a major confounding factor.

In conclusion, *CRP* -717, *CRP* 1444 and *CRP* 4 polymorphisms are not in LD with the *FCGR3A*-158V/F polymorphism previously associated with CRP decrease after infliximab treatment. In addition, these polymorphisms are not associated with the clinical or biological response to infliximab in Crohn's disease and, in particular, are not associated with CRP decrease after infliximab treatment.

Table 3 *Baseline C-reactive protein (CRP), clinical and biological response to infliximab, according to CRP genotypes*

Locus	Genotype	Proportion of patients with positive clinical response to infliximab (%)	Proportion of patients with positive biological response to infliximab (%)	Median baseline CRP (normalized; median-range)	Median relative CRP decrease after infliximab (%)
CRP -717G/A	A/A	74/98 (75.5)	53/75 (70.7)	4.6 (1-80)	-40 (-99-1461)[a]
	A/G	43/60 (71.7)	50/58 (86.2)	6.1 (1-62.2)	-66 (-98-100)[a]
	G/G	9/1 2 (75)	4/8 (50)	5.6 (1-16.6)	-14 (-73-571)[a]
CRP 1444C/T	T/T	1 6/1 9 (84.2)	8/12 (66.7)	5.2 (1-75)	-32.5 (-97-39)
	C/T	65/83 (78.3)	52/68 (76.5)	5.4 (1-67.2)	-60 (-96-1461)
	C/C	50/72 (69.4)	50/64 (78.1)	5.4 (1-80)	-61 (-98-571)
CRP 4A/G	A/A	12/14 (85.7)	9/1 2 (75)	3.8 (1-63)	-39 (-95-51)
	A/G	57/81 (70.4)	53/70 (75.7)	5.2 (1-80)	-62 (-91-1461)
	G/G	59/76 (77.6)	50/64 (78.1)	5.8 (1-75)	-56.5 (-98-571)

[a]*P=0.018; P_o = 0.162 (Kruskal-Wallis).*

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References

1 Knight DM, Trinh H, Le J, Siegel S, Shealy D, McDonough M, *et al.* Construction and initial characterization of a human-mouse chimeric anti-TNF antibody. *Mol Immunol* 1993; 30:1443-1453.

2 Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, *et al.* A short term study of chimeric antibody cA2 to tumour necrosis factor alpha for Crohn's disease. *N Eng J Med* 1 997; 337:1029-1035.

3 Present DH, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, *et al.* Infliximab for the treatment of fistulas in patients with Crohn's disease. W *Eng J Med* 1999; 340:1398-1405.

4 Farrell RJ, Shah SA, Lodhavia PJ, Alsahli M, Falchuk KR, Michetti P, Peppercorn MA. Clinical experience with Infliximab therapy in 100 patients with Crohn's disease. *Am J Gastroenterol* 2000; 95:3490-3497.

5 Vermeire S, Louis E, Carbonez A, Van Assche G, Noman M, Belaiche J, *et al.* Demographic and clinical parameters influencing the short-term outcome of anti-tumour necrosis factor (infliximab) treatment in Crohn's disease. *Am J Gastroenterol* 2002; 97:2357-2363.

6 Arnott ID, McNeill G, Satsangi J. An analysis of factors influencing short-term and sustained response to infliximab treatment for Crohn's disease. *Aliment Pharmacol Ther* 2003; 17:1451-1457.

7 Parsi MA, Achkar JP, Richardson S, Katz J, Hammel JP, Lashner BA, Brzezinski A. Predictors of response to infliximab in patients with Crohn's disease. *Gastroenterology* 2002; 123:707-713.

8 Louis E, Vermeire S, Rutgeerts P, De Vos M, Van Gossum A, Pescatore P, *et al.* A positive response to infliximab in Crohn disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. *Scand J Gastroenterol* 2002; 37:818-824.

9 Louis E, El Ghoul Z, Vermeire S, Dall'Ozzo S, Rutgeerts P, Paintaud G, *et al.* Association between polymorphism in IgG Fc receptor Ilia coding gene and biological response to infliximab in Crohn's disease. *Aliment Pharmacol Ther* 2004; 19:511-519.

10 Van der Pol WL, Jansen MD, Sluiter WJ, van de Sluis B, Leppers-van de Straat FG, Kobayashi T, *et al.* Evidence for non-random distribution of Fcgamma receptor genotype combinations. *Immunogenetics* 2003; 55:240-246.

11 Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, *et al.* Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. *Arterioscler Thromb Vase Biol* 2003; 23:2063-2069.

12 Wolford JK, Gruber JD, Ossowski VM, Vozarova B, Antonio Tataranni P, Bogardus C, *et al.* A C-reactive protein promoter polymorphism is associated with type 2 diabetes mellitus in Pima Indians. *Mol Genet Metab* 2003; 78:136-144.

13 Russell AI, Cunninghame Graham DS, Shepherd C, Roberton CA,Whittaker J, Meeks J, *et al.* Polymorphism at the Creactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. *Hum Mol Genet* 2004; 13:137-147.

14 Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology* 1976; 70:439-444.

15 Dall'Ozzo S, Andres C, Bardos P, Watier H, Thibault G Rapid single-step FCGR3A genotyping based on SYBR Green I fluorescence in real-time multiplex allele-specific PCR. *J Immunol Methods* 2003; 277:1 85-1 92.

16 Rousset F, Raymont M. Testing hétérozygote excess and deficiency. G*enetics* 1995; 140:1413-1419.

17 Raymont M, Rousset F GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 1 995; 86: 248-249.

18 Xie X, Ott J. Testing linkage disequilibrium between a disease gene and marker loci. *Am J Hum Genet* 1993; 53:1107.

19 Pankow JS, Folsom AR, Cushman M, Borecki IB, Hopkins PN, Eckfeldt JH, Tracy RP Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. *Atherosclerosis* 2001 ;154:681-689.

20 Kovacs A, Green F, Hansson LO, Lundman P, Samnegard A, Boquist S, *et al.* A novel common single nucleotide polymorphism in the promoter region of the C-reactive protein gene associated with the plasma concentration of C-reactive protein. *Atherosclerosis* 2005; 178:193-198.