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Signal transducer and activator of transcription 6 gene *G2964A* polymorphism and inflammatory bowel disease

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SUMMARY

Signal transducer and activator of transcription 6 (STAT6) is a key transcription factor involved in interleukin 4 (IL-4) and IL-13-mediated Th2 response. The *STAT6* gene is located on chromosome 12q13.3–14·1 (*IBD2* region) and is therefore a positional and functional candidate gene for study in inflammatory bowel disease. We investigated the *G2964A* polymorphism in the 3′ untranslated region of the *STAT6* gene in Dutch patients with inflammatory bowel disease and healthy controls. The *G2964A* polymorphism in the *STAT6* gene was genotyped in 141 unrelated Dutch Caucasian patients with ulcerative colitis, 183 patients with Crohn's disease and 173 healthy individuals by PCR and the amplification-created restriction site method. Patients with Crohn's disease were classified according to the Vienna classification and the patients with ulcerative colitis were classified with the age at onset, extent of disease and colectomy. We did not find significant differences in genotype and allele frequencies of the *G2964A* polymorphism in the *STAT6* gene between ulcerative colitis, Crohn's disease and healthy controls. Subgroups of the patients with Crohn's disease classified according to the Vienna classification and those with ulcerative colitis classified according to age of onset, disease extension and colectomy did not differ in the distribution of this polymorphism. The *STAT6 G2964A* gene polymorphism is not involved in the overall susceptibility or in determining the phenotype of IBD.

Keywords Crohn's disease inflammatory bowel disease STAT6 gene polymorphism ulcerative colitis

INTRODUCTION

Inflammatory bowel diseases (IBD) are clinically classified as ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis. The aetiology of IBD is still unclear and remains under intensive study. IBD manifests as chronic intestinal inflammation due to an exaggerated immune response. An imbalance in the activation of Th1 and Th2 lymphocytes has been found in the intestinal mucosa in IBD [1]. Th1 cytokines including IL-1, IL-2, TNF-α, IL-6, IL-8 and IL-12 promote inflammation while Th2 cytokines down-regulate Th1 responses by secretion of IL-4, IL-10 and IL-13 [2]. IL-4 has multiple functions involved in regulation of CD23 and MHC class II gene expression, B and T cell growth and B cell differentiation into IgE producers and Th0 cell differentiation into Th2 cells [3,4]. The IL-4 receptor (IL-4R) and IL-13R share the IL-4 receptor α-chain encoded by the *IL4RA* gene and result in the activation of signal transducer and activator of transcription 6 (STAT6) through phosphorylation by Janus kinases 1 and 3 [5,6]. STAT6 forms homodimers, translocates to the nucleus and binds to promoter regions for regulation of transcription. STAT6-binding sites are present in the promoter regions of IL-4 inducible genes [7,8]. STAT6-deficient mice lack a Th2 cytokine response and IgE production, demonstrating that STAT6 is a key transcription factor involved in IL-4 and IL-13-mediated Th2 response [4,9].

The *STAT6* gene is located on chromosome 12q13.3–14·1 just within the *IBD2* locus, as defined by recent genome-wide linkage studies [10–13]. These studies identified this region to harbour a susceptibility locus for UC and CD. Later studies found some evidence that this region contributes to the susceptibility to UC, but not to CD [14–16]. However, these results were not confirmed in all linkage studies [17–20]. It is interesting that in this region a *Taq*I polymorphism at codon 352 in exon 8 of the vitamin D-receptor gene was found to be associated with CD [21] while negative results were found for the beta-7 integrin and interferon-γ

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genes also in this region [22,23]. Two groups of investigators found associations of *G2964A* genotype in the 3′ untranslated region of the *STAT6* gene with asthma and mild-type atopy [24] and nut allergy [25]. Another group reported that a dinucleotide repeat polymorphism in exon 1 of the *STAT6* gene was associated with allergic disease [26]. Therefore, *STAT6* is a positional and functional candidate gene for studies of susceptibility to IBD.

In the present study, we investigated the *STAT6* gene *G2964A* polymorphism in Dutch Caucasian patients with IBD and healthy controls, and we studied the association between this single nucleotide polymorphism (SNP) and clinical subgroups of the patients.

SUBJECTS AND METHODS

Subjects

One hundred and forty-one patients with UC (male 68, female 73), 183 patients with CD (male 58, female 125) attending the Department of Gastroenterology at the Vrije Universiteit medical centre (VUmc) and 173 healthy controls (male 79, female 94) were studied. The control population consisted of healthy staff and students of the VUmc. All subjects were unrelated Dutch Caucasians. The mean age at onset was 32 ± 14 years for UC and 28 ± 12 years for CD. The age of the control population was 36 ± 12 years. The mean course of the disease was 5·2 ± 6·6 years for UC and 5·4 ± 6·6 for CD. The diagnosis of UC and CD was determined by conventional clinical, radiological, endoscopic and histological criteria as described by Lennard-Jones [27]. Patients with CD were classified according to the Vienna classification [28], briefly according to age at onset (A1: < 40 years, A2: ≥40 years), disease location (L1: terminal ileum, L2: colon, L3: ileocolon, L4: upper gastrointestinal tract) and disease behaviour (B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating). The patients with UC were classified according to the age at onset (<40 years, ≥40 years), location of the disease (proctitis, left-sided colitis and pancolitis) and colectomy as indicator of severity. This study was carried out with the approval of the Medical Ethical Committee of the VUmc, and informed consent was obtained from all subjects.

METHODS

Genomic DNA was isolated from 10 ml venous blood by a routine method. The *G2964A* polymorphism in the 3′ untranslated region of the *STAT6* gene (NCBI SNP CLUSTER ID: rs324015) was determined by PCR and the amplification-created restriction site method according to Amoli *et al*. [29]. The primers used were 5′-GAA GTT CAG GCT CTG AGA GAC-3′ and 5′-CCA TCA CCC TCA GAG AGC-3′. PCR reaction was performed in the GeneAmp PCR system 9700 (PE Biosystems, CA, USA). The amplification was accomplished by an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 45 s and annealing at 55°C for 45 s and extension at 72°C for 45 s, with a final extension at 72°C for 2 min. After polymerase chain reaction (PCR) amplification, electrophoresis of the reaction products was in 2% agarose gels prestained with ethidium bromide in 0·5 × Tris-borate-EDTA (TBE) buffer. Four units of *Bsa*HI (New England Biolabs Inc., Beverly, MA, USA) were used for digestion of 7 μl PCR product at 37°C for 10 h. The digested products were visualized on 4% agarose gels stained with ethidium bromide. Individuals homozygous for the rare allele (allele *2964 A*) yielded one uncut band (93 bp). The allele *2964G* yielded two bands of 74 bp and 19 bp.

Statistical analysis

χ^2 or Fisher's exact test were used by multiple contingency table or 2 × 2 table. When 20% of cells in the multiple contingency tables have an expected count less than 5, a necessary cell combination was performed. A value of $P < 0{\cdot}05$ was considered statistically significant. For multiple comparisons *P*-values were corrected by multiplying the *P*-value by the number of comparisons (Bonferroni correction). Odds ratios (OR) were calculated according to Woolf's formula.

RESULTS

The results of the genotype and allele frequencies of the *STAT6* gene *G2964A* polymorphism of Dutch Caucasian patients with IBD and ethnically matched healthy controls are shown in

Table 1. *STAT6* gene *G2964A* genotype and allele frequencies in Dutch patients with UC, CD and healthy controls (HC)

		Genotype			Allele	
Population	No.	AA *n* (%)	AG *n* (%)	GG *n* (%)	A *N* (%)	G *N* (%)
HC	173	6 (3)	57 (33)	110 (64)	69 (20)	277 (80)
CD	183	13 (7)	65 (36)	105 (57)	91 (25)	275 (75)
UC	141	6 (4)	51 (36)	84 (60)	63 (22)	219 (78)
Male						
HC	79	4 (5)	28 (35)	47 (60)	36 (23)	122 (77)
CD	58	6 (10)	14 (24)	38 (66)	26 (22)	90 (78)
UC	68	3 (4)	31 (46)	34 (50)	37 (27)	99 (73)
Female						
HC	94	2 (2)	29 (31)	63 (67)	33 (18)	155 (82)
CD	125	7 (6)	51 (41)	67 (53)	65 (26)*	185(74)*
UC	73	3 (4)	20 (27)	50 (69)	26 (18)	120 (82)

n: number of individuals; *N*: number of alleles; percentages are given in parentheses. *: CD *versus* HC: Fisher's exact $P = 0{\cdot}0376$, OR = 1·650, 95% CI = 1·031–2·641; Pc = non-significant.

Table 1. The genotypes of the healthy controls did not deviate from the expected value by Hardy–Weinberg equilibrium. No significant associations were observed in the genotype and allele frequencies between UC and CD when compared with healthy controls in Dutch Caucasians. However, a significant association between CD and healthy controls was observed in women. Here, the frequency of allele *2964 A* was 0·26 in CD *versus* 0·18 in healthy controls, $P = 0{\cdot}037$, OR = 1·65, 95% (CI = 1·03–2·64). However, this association did not hold after Bonferroni correction.

As shown in Table 2 and Table 3, clinical subgroups of the patients with CD and with UC did not differ in the distribution of the *STAT6* genotype and allele frequencies.

DISCUSSION

In the present study, we found no significant association of the *G2964A* polymorphism in the *STAT6* gene with Dutch Caucasian patients with IBD. The frequency of allele *2964 A* was slightly increased in female CD patients when compared with female healthy controls. As shown in this study the *2964 A* allele is the less frequent allele in the Dutch Caucasian population. This has also been found previously in a UK Caucasian population [24,29]. In contrast, Gao *et al*. showed the *2964G* allele to be the minor allele in Japanese subjects [24]. Furthermore, we did not find an association of this polymorphism with subgroups of CD patients clinically classified according to the Vienna classification, and with

Table 2. *STAT6* gene *G2964A* genotype and allele frequencies in Dutch patients with CD classified according to the Vienna classification

		Genotype			Allele	
Classification	No.	AA *n* (%)	AG *n* (%)	GG *n* (%)	A *N* (%)	G *N* (%)
CD all	183	13 (7)	65 (36)	105 (57)	91 (25)	275 (75)
Age at onset						
A1	143	9 (6)	56 (39)	78 (55)	74 (26)	212 (74)
A2	40	4 (10)	9 (23)	27 (67)	17 (21)	63 (79)
Location						
L1	63	4 (6)	20 (32)	39 (62)	28 (22)	98 (78)
L2	43	2 (5)	16 (37)	25 (58)	20 (23)	66 (77)
L3	74	6 (8)	29 (39)	39 (53)	41 (28)	107 (72)
L4	3	1 (33)	–	2 (67)	2 (33)	4 (67)
Behaviour						
B1	69	4 (6)	28 (40)	37 (54)	36 (26)	102 (74)
B2	78	6 (8)	25 (32)	47 (60)	37 (24)	119 (76)
B3	36	3 (8)	12 (33)	21 (59)	18 (25)	54 (75)

n: number of individuals; *N*: number of alleles; percentages are given in parentheses.

Table 3. *STAT6* gene *G2964A* genotype and allele frequencies in Dutch patients with UC classified according to the age, location and colectomy

		Genotype			Allele	
Classification	No.	AA *n* (%)	AG *n* (%)	GG *n* (%)	A *N* (%)	G *N* (%)
UC all	141	6 (4)	51 (36)	84 (60)	63 (22)	219 (78)
Age at onset						
A1	107	5 (5)	41 (38)	61 (57)	51 (24)	163 (76)
A2	34	1 (3)	10 (29)	23 (67)	12 (18)	56 (82)
Location						
Distal colitis	77	3 (4)	31 (40)	43 (56)	37 (24)	117 (76)
Pancolitis	64	3 (5)	20 (31)	41 (64)	26 (20)	102 (80)
Colectomy						
Yes	46	3 (6)	17 (37)	26 (57)	23 (25)	69 (75)
No	95	3 (3)	34 (36)	58 (61)	40 (21)	150 (79)

n: number of individuals; *N*: number of alleles; percentages are given in parentheses.

subgroups of UC classified with age, disease location and colectomy performed. Our results have shown that the *STAT6* gene *G2964A* polymorphism does not predispose to CD or UC in Dutch Caucasian patients and does not influence the course of the disease.

Recent genome-wide linkage studies have shown that *IBD2* susceptibility locus is mapped on chromosome 12q [10–14]. The *STAT6* gene is located well within this region. Since STAT6 is a component involved in the IL-4 signalling pathway, it is interesting to know that the *IL-4RA* gene is located in chromosome 16p12 within the *IBD1* locus and the *IL-4* gene has been mapped to the *IBD5* susceptibility region for CD on chromosome 5q31–33 [30]. Even though the *IL-4RA* gene polymorphism *576R* alone [31] has been found not to be associated with IBD, the combination of *IL-4RA* gene *Q576R* and *IL-4–34T* alleles were found to be a risk factor for CD in a study from the UK [32]. A German study did also show evidence for a weak association between the *IL-4–590T* gene polymorphism and CD [33]. The study of Rioux *et al*. [30] showed an association between CD and a specific common haplotype of the cytokine region in 5q31 including the *IL-4* gene. In the Dutch population, genotypes for both the *IL-4RA S478P* (in strong linkage disequilibrium with Q576R) and a –1111 promoter SNP in the *IL-13* gene revealed synergy in asthma susceptibility [34].

In this study, we found no significant association between the *STAT6 G2964A* gene polymorphism and IBD in Dutch Caucasians. Significant interaction between genes in the same pathway is not unprecedented. Further studies will focus on other polymorphisms located in regulatory and coding regions and at exon/intron boundaries of the *STAT6* gene on chromosome 12q for susceptibility to IBD and explore interactions with *IL-4RA* and *IL-13*, particularly in relation to UC that has features compatible with Th2 dysregulation as opposed to CD where a Th1 response predominates.

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REFERENCES

1 Van Damme N, De Keyser F, Demetter P *et al.* The proportion of Th1 cells, which prevail in gut mucosa, is decreased in inflammatory bowel syndrome. Clin Exp Immunol 2001; **125**:383–90.
2 Xia B, Crusius JBA, Meuwissen SGM, Peña AS. Inflammatory bowel disease. definition, epidemiology, etiology, and immunogenetic studies. World J Gastroenterol 1998; **4**:446–58.
3 Schnare M, Blum H, Jüttner S, Röllinghoff M, Gessner A. Specific antagonism of type I IL-4 receptor with a mutated form of murine IL-4. J Immunol 1998; **161**:3484–92.
4 Takeda K, Tanaka T, Shi W *et al.* Essential role of Stat6 in IL-4 signalling. Nature 1996; **380**:627–30.
5 Lai SY, Molden J, Liu KD, Puck JM, White MD, Goldsmith MA. Interleukin-4-specific signal transduction events are driven by homotypic interactions of the interleukin-4 receptor alpha subunit. EMBO J 1996; **15**:4506–14.
6 Daniel C, Salvekar A, Schindler U. A gain-of-function mutation in STAT6. J Biol Chem 2000; **275**:14255–9.
7 Kraus J, Börner C, Giannini E *et al.* Regulation of mu-opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. J Biol Chem 2001; **276**:43901–8.
8 Kotanides H, Reich NC. Interleukin-4-induced STAT6 recognizes and activates a target site in the promoter of the interleukin-4 receptor gene. J Biol Chem 1996; **271**:25555–61.
9 Shimoda K, van Deursen J, Sangster MY *et al.* Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 1996; **380**:630–3.
10 Satsangi J, Parkes M, Louis E *et al.* Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. Nat Genet 1996; **14**:199–202.
11 Curran ME, Lau KF, Hampe J *et al.* Genetic analysis of inflammatory bowel disease in a large European cohort supports linkage to chromosomes 12 and 16. Gastroenterology 1998; **115**:1066–71.
12 Duerr RH, Barmada MM, Zhang L *et al.* Linkage and association between inflammatory bowel disease and a locus on chromosome 12. Am J Hum Genet 1998; **63**:95–100.
13 Hampe J, Schreiber S, Shaw SH *et al.* A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. Am J Hum Genet 1999; **64**:808–16.
14 Parkes M, Barmada MM, Satsangi J, Weeks DE, Jewell DP, Duerr RH. The *IBD2* locus shows linkage heterogeneity between ulcerative colitis and Crohn's disease. Am J Hum Genet 2000; **67**:1605–10.
15 Lesage S, Zouali H, Colombel JF *et al.* Genetic analyses of chromosome 12 loci in Crohn's disease. Gut 2000; **47**:787–91.
16 Vermeire S, Peeters M, Vlietinck R *et al.* Exclusion of linkage of Crohn's disease to previously reported regions on chromosomes 12, 7, and 3 in the Belgian population indicates genetic heterogeneity. Inflamm Bowel Dis 2000; **6**:165–70.
17 Annese V, Latiano A, Bovio P *et al.* Genetic analysis in Italian families with inflammatory bowel disease supports linkage to the IBD1 locus – a GISC study. Eur J Hum Genet 1999; **7**:567–73.
18 Rioux JD, Daly MJ, Green T *et al.* Absence of linkage between inflammatory bowel disease and selected loci on chromosomes 3, 7, 12, and 16. Gastroenterology 1998; **115**:1062–5.
19 Brant SR, Fu Y, Fields CT *et al.* American families with Crohn's disease have strong evidence for linkage to chromosome 16 but not chromosome 12. Gastroenterology 1998; **115**:1056–61.
20 Cavanaugh J. International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set: Crohn disease and chromosome 16. Am J Hum Genet 2001; **68**:1165–71.
21 Simmons JD, Mullighan C, Welsh KI, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. Gut 2000; **47**:211–4.
22 van Heel DA, Carey AH, Jewell DP. Identification of novel polymorphisms in the beta7 integrin gene: family–based association studies in inflammatory bowel disease. Genes Immun 2001; **2**:455–60.
23 Hampe J, Hermann B, Bridger S, MacPherson AJ, Mathew CG, Schreiber S. The interferon-gamma gene as a positional and functional candidate gene for inflammatory bowel disease. Int J Colorectal Dis 1998; **13**:260–3.
24 Gao PS, Mao XQ, Roberts MH *et al.* Variants of STAT6 (signal transducer and activator of transcription 6) in atopic asthma. J Med Genet 2000; **37**:380–2.
25 Amoli MM, Hand S, Hajeer AH *et al.* Polymorphism in the STAT6 gene encodes risk for nut allergy. Genes Immun 2002; **3**:220–4.
26 Tamura K, Arakawa H, Suzuki M *et al.* Novel dinucleotide repeat polymorphism in the first exon of the STAT-6 gene is associated with allergic diseases. Clin Exp Allergy 2001; **31**:1509–14.
27 Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol Suppl 1989; **170**:2–6.

28 Gasche C, Scholmerich J, Brynskov J *et al.* A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. Inflamm Bowel Dis 2000; **6**:8–15.
29 Amoli M, Ollier WER, Hajeer AH. A novel PCR-RFLP assay for the detection of a polymorphism in the 3′ of STAT6 gene. Genes Immun 2000; **1**:349–50.
30 Rioux JD, Daly MJ, Silverberg MS *et al.* Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 2001; **29**:223–8.
31 Olavesen MG, Hampe J, Mirza MM *et al.* Analysis of single-nucleotide polymorphisms in the interleukin-4 receptor gene for association with inflammatory bowel disease. Immunogenetics 2000; **51**:1–7.
32 Aithal GP, Day CP, Leathart J, Daly AK, Hudson M. Association of single nucleotide polymorphisms in the interleukin-4 gene and interleukin-4 receptor gene with Crohn's disease in a British population. Genes Immun 2001; **2**:44–7.
33 Klein W, Tromm A, Griga T *et al.* Interleukin-4 and interleukin-4 receptor gene polymorphisms in inflammatory bowel diseases. Genes Immun 2001; **2**:287–9.
34 Howard TD, Koppelman GH, Xu J *et al.* Gene–gene interaction in asthma. IL4RA and IL13 in a Dutch population with asthma. Am J Hum Genet 2002; **70**:230–6.