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RAPID COMMUNICATION

No association of the cytotoxic T-lymphocyte associated gene CTLA4 +49A/G polymorphisms with Crohn's disease and ulcerative colitis in Hungarian population samples

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Abstract

AIM: The goal of the current work was to analyse the prevalence of the +49A/G variant of the cytotoxic T-lymphocyte antigen 4 gene (CTLA4) in Hungarian patients with Crohn's disease (CD) and ulcerative colitis (UC).

METHODS: A total of 130 unrelated subjects with CD and 150 with UC, and 170 matched controls were genotyped for the single nucleotide polymorphism (SNP). The genotypes were determined by using PCR/RFLP test.

RESULTS: The G allele frequency and the prevalence of the GG genotype were 38.1% and 12.3% in the CD group, 40.6% and 18.6% in the UC patients, and 37.4% and 15.9% in the control group, respectively.

CONCLUSION: The results of the current study show that carriage of the +49G SNP in heterozygous or in homozygous form does not confer risk either for CD or for UC in the Hungarian population.

Key words: Cytotoxic T-lymphocyte antigen 4; Crohn's disease; Ulcerative colitis; Inflammatory bowel disease

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INTRODUCTION

Inflammatory bowel disease (IBD) is a multifactorial disorder characterized by non-specific inflammation of the digestive tract, causing intestinal malabsorption and immune defense abnormalities, including exaggergated T cell response[1,2]. The cytotoxic T-lymphocyte antigen 4 (EC. number 3.4.21.) gene is a T cell receptor that binds to B7-1 (CD80) and B7-2 (CD86) ligands and plays a key role in the down-regulation of T cell activation[2,3]. The CTLA4 gene +49A/G polymorphism has been suggested to associate with IBD[2].

Two major forms of chronic IBD are Crohn's disease and ulcerative colitis[4,5]. The development of these diseases is known to be influenced by both environmental factors and complex genetic predisposition[6]. IBD patients have an increased risk for the development of colorectal cancer[7]. The relative risk of colorectal cancer in ulcerative colitis is increased, compared to Crohn's disease[8]. Smoking is an important environmental factor with different effects on IBD[4]. Many other environmental factors have been investigated, including infectious agents, diet, drugs, stress, and social status[9]. With genome-wide linkage analyses several loci of possible IBD susceptibility genes have been identified[10-18]. The CARD15 gene on chromosome 16 *via* the NOD2 protein, and the SLC22A4 and SLC22A5 genes on chromosome 5q *via* the OCTN1 and OCTN2 transporters has been found to confer increased risk for developing inflammatory bowel disease[19-22].

In the current study our aim was to examine whether CTLA4 gene +49A/G polymorphism confers susceptibility to Crohn's disease and ulcerative colitis in Hungarian population samples.

MATERIALS AND METHODS

Patients

We examined 130 patients with CD (55 males, 75 females, mean age 43.0 ± 1.42 years) and 150 patients with UC (63 males, 87 females, mean age 46.1 ± 1.30 years). Diagnosis of inflammatory bowel disease was established according to endoscopic, radiological, histological and clinical criteria following the Council for International Organizations of Medical Sciences in WHO and the International Organization for the Study of Inflammatory Bowel Disease[23-25]. A total of 170 selected controls (49 males, 121 females, mean age 57.7 ± 1.29 years) were used. The control subjects were age- and sex-matched clinically healthy subjects and did not receive any drug administration. The IBD patients were treated with various drugs, such as sulfosalazin, 5-aminosalicylic acid, budesonide, metilprednisolon or azathioprin. All of our patients and controls were unrelated Caucasians. During the entire study period the guidelines and regulations approved by the local Ethics Committee and the Helsinki Declaration of 1975 were followed. The study design was approved by the local Ethics Committee.

Methods

Genomic DNA was extracted from peripheral blood leukocytes with a routine salting out method. The CTLA4 gene (MIM: 123890) +49A/G (GenBank rs231775) SNP in exon 1 was examined. For the amplification of the target sequence the following primers were designed and used: 5'-CTTGAGGTTGTCTTTTCGAG-3' as the sense and 5'-TACTAAATACCTGGCGCTCT-3' as the antisense primer. The PCR amplifications were performed on MJ Research PTC 200 thermal cyclers using the following conditions: initial denaturation at 96℃ for 3 min followed by 35 cycles of denaturation at 96℃ for 45 s, annealing at 56℃ for 45 s, extension at 72℃ for 45 s and final extension at 72℃ for 10 min. The amplicons were digested by allele-specific restriction endonuclease, *Bse* XI. The amplicon contained an obligate cleavage site of the restriction enzyme for the suitable visual control of the efficacy of the digestion. In normal cases (AA) *Bse* XI cleaves the 573 bp PCR product into 51 and 522 bp long fragments. In heterozygotes (AG) 51, 235, 287 and 522 bp long products were detected. In GG genotype the digestion resulted in 51, 235 and 287 bp long fragments. The restriction fragments were separated by electrophoresis on agarose gels containing ethidium bromide and visualized by UV transillumination.

Statistical analysis

Statistical analysis was carried out using Excel and SPSS 11.5. for Windows. For statistics the χ^2 method (cross-table analyses) was used to analyze the possible associations between the diseases and the examined polymorphism.

RESULTS

Results for the genotyping of the CTLA4 gene +49A/G SNP in 130 patients with CD, 150 subjects with UC, and 170 healthy controls are summarized in Table 1. The allele frequencies were in Hardy-Weinberg equilibrium both in the patients and in the controls. We found no accumulation of either the G allele alone; either expressed as AG heterozygous genotype, or as the G allele frequency, nor increased prevalence rate of the homozygous GG genotype in any IBD type compared with the healthy, IBD free controls. Comparing the genotype frequencies separately in males and females, there were no gender differences in the distribution of the genotypes.

Table 1 Prevalence of the alleles of CTLA4 gene in patients with Crohn's disease and ulcerative colitis

		Crohn's disease $n = 130$	Ulcerative colitis $n = 150$	Controls $n = 170$
Exon 1 +49A/G	AA	47 (36.2%)	56 (37.3%)	70 (41.2%)
	AG	67 (51.5%)	66 (44.0%)	73 (42.9%)
	GG	16 (12.3%)	28 (18.6%)	27 (15.9%)
G allele frequency		38.10%	40.60%	37.40%

DISCUSSION

The CTLA4 gene on chromosome 2q33 region has been investigated in several diseases with chronic inflammatory nature[3]. Nistico *et al* identified a novel polymorphism, +49A/G in exon 1, which associates with a Thr to Ala substitution at position 17 of the amino acid sequence[26]. Several studies have reported controversial results on association of the +49A/G SNP in the CTLA4 gene with type I diabetes[27,28], Grave's disease[29,30], rheumatoid arthritis[31-34], multiple sclerosis[35] and celiac disease[3,36]. CTLA4 is a susceptibility gene also for two main types of inflammatory bowel disease; Crohn's disease and ulcerative colitis[2]. Machida *et al* found that in the Japanese population CTLA4 is one of the determinants of UC, and confers risk for development of CD associated with fistula formation[2], and the GG genotype was also more frequently observed in CD patients with fistula[2]. Xia *et al* found no association of CTLA4 +49G SNP with IBD in Dutch Caucasian patients and with UC in Chinese patients[37]. More confusing that in some studies the A variant was found as a susceptibility factor for rheumatoid arthritis and celiac disease[33,38].

In the present study we compared the heterozygous AG genotype, the homozygous GG variant, and the G allele frequency of the CTLA4 gene +49A/G polymorphisms, and could not detect accumulation of any of them in any type of the IBD. This shows that the G variant of the +49A/G allele of the CTLA4 gene does not represent an obligatory susceptibility factor for Crohn's disease or for ulcerative colitis. Separate comparison of the G and GG genotypes in each diseases also excluded the possibility of a gene dose effect, evidenced by the similar distribution of each.

Several explanations have been presented for the discrepancy between negative findings[37,39,40], like ours, and the positive findings of others[2]. The most plausible is the known genetic diversity of the different populations at the haplotype level. For the present study, it is of special

interest that the Hungarians are historically different from the surrounding nations in the Carpathian basin due to the Asian origin of the founder tribes[41]. Recent studies support a language dominance, therefore the majority of the Hungarian population does not differ from the Europeans with respect to their genetic grouping[42-44]. However, spread of a non-European, non-susceptibility haplotype variant containing the +49G allele of the CTLA4 gene at a high incidence rate in Hungarians cannot be ruled out. Further association studies with more detailed haplotype analysis should be performed to clarify this assumption, with special care to the differentiation of the +49G allele containing variants.

REFERENCES

1 **Fiocchi C**. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; **115**: 182-205
2 **Machida H**, Tsukamoto K, Wen CY, Narumi Y, Shikuwa S, Isomoto H, Takeshima F, Mizuta Y, Niikawa N, Murata I, Kohno S. Association of polymorphic alleles of CTLA4 with inflammatory bowel disease in the Japanese. *World J Gastroenterol* 2005; **11**: 4188-4193
3 **Naluai AT**, Nilsson S, Samuelsson L, Gudjonsdottir AH, Ascher H, Ek J, Hallberg B, Kristiansson B, Martinsson T, Nerman O, Sollid LM, Wahlstrom J. The CTLA4/CD28 gene region on chromosome 2q33 confers susceptibility to celiac disease in a way possibly distinct from that of type 1 diabetes and other chronic inflammatory disorders. *Tissue Antigens* 2000; **56**: 350-355
4 **Mahid SS**, Minor KS, Soto RE, Hornung CA, Galandiuk S. Smoking and inflammatory bowel disease: a meta-analysis. *Mayo Clin Proc* 2006; **81**: 1462-1471
5 **Danese S**, Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. *World J Gastroenterol* 2006; **12**: 4807-4812
6 **Hugot JP**, Cho JH. Update on genetics of inflammatory bowel disease. *Curr Opin Gastroenterol* 2002; **18**: 410-415
7 **Geier MS**, Butler RN, Howarth GS. Inflammatory bowel disease: Current insights into pathogenesis and new therapeutic options; probiotics, prebiotics and synbiotics. *Int J Food Microbiol* 2007; **115**: 1-11
8 **Hagymasi K**, Tulassay Z. Inflammatory bowel disease and colorectal cancer. *Orv Hetil* 2006; **147**: 1977-1982
9 **Jantchou P**, Monnet E, Carbonnel F. Environmental risk factors in Crohn's disease and ulcerative colitis (excluding tobacco and appendicectomy). *Gastroenterol Clin Biol* 2006; **30**: 859-867
10 **Hugot JP**, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Van Gossum A, Orholm M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996; **379**: 821-823
11 **Satsangi J**, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. 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
12 **Cho JH**, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann JS, Kirschner BS, Jabs EW, Weber J, Hanauer SB, Bayless TM, Brant SR. Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci USA* 1998; **95**: 7502-7507
13 **Cho JH**, Nicolae DL, Ramos R, Fields CT, Rabenau K, Corradino S, Brant SR, Espinosa R, LeBeau M, Hanauer SB, Bodzin J, Bonen DK. Linkage and linkage disequilibrium in chromosome band 1p36 in American Chaldeans with inflammatory bowel disease. *Hum Mol Genet* 2000; **9**: 1425-1432
14 **Duerr RH**, Barmada MM, Zhang L, Pfutzer R, Weeks DE. High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. *Am J Hum Genet* 2000; **66**: 1857-1862
15 **Hampe J**, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJ, Cardon LR, Sakul H, Harris TJ, Buckler A, Hall J, Stokkers P, van Deventer SJ, Nurnberg P, Mirza MM, Lee JC, Lennard-Jones JE, Mathew CG, Curran ME. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am J Hum Genet* 1999; **64**: 808-816
16 **Hampe J**, Frenzel H, Mirza MM, Croucher PJ, Cuthbert A, Mascheretti S, Huse K, Platzer M, Bridger S, Meyer B, Nurnberg P, Stokkers P, Krawczak M, Mathew CG, Curran M, Schreiber S. Evidence for a NOD2-independent susceptibility locus for inflammatory bowel disease on chromosome 16p. *Proc Natl Acad Sci USA* 2002; **99**: 321-326
17 **Rioux JD**, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001; **29**: 223-228
18 **Taylor KD**, Yang H, Rotter JI. Inflammatory bowel disease. II. Gene mapping. *Mol Genet Metab* 2001; **74**: 22-44
19 **Cho JH**. Advances in the genetics of inflammatory bowel disease. *Curr Gastroenterol Rep* 2004; **6**: 467-473
20 **Silverberg MS**. OCTNs: will the real IBD5 gene please stand up? *World J Gastroenterol* 2006; **12**: 3678-3681
21 **Peltekova VD**, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004; **36**: 471-475
22 **Bene J**, Magyari L, Talian G, Komlosi K, Gasztonyi B, Tari B, Varkonyi A, Mozsik G, Melegh B. Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease. *World J Gastroenterol* 2006; **12**: 5550-5553
23 **Podolsky DK**. Inflammatory bowel disease (1). *N Engl J Med* 1991; **325**: 928-937
24 **Podolsky DK**. Inflammatory bowel disease (2). *N Engl J Med* 1991; **325**: 1008-1016
25 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-19
26 **Nistico L**, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, Larrad MT, Rios MS, Chow CC, Cockram CS, Jacobs K, Mijovic C, Bain SC, Barnett AH, Vandewalle CL, Schuit F, Gorus FK, Tosi R, Pozzilli P, Todd JA. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum Mol Genet* 1996; **5**: 1075-1080
27 **Zalloua PA**, Abchee A, Shbaklo H, Zreik TG, Terwedow H, Halaby G, Azar ST. Patients with early onset of type 1 diabetes have significantly higher GG genotype at position 49 of the CTLA4 gene. *Hum Immunol* 2004; **65**: 719-724
28 **Ahmedov G**, Ahmedova L, Sedlakova P, Cinek O. Genetic association of type 1 diabetes in an Azerbaijanian population: the HLA-DQ, -DRB1*04, the insulin gene, and CTLA4. *Pediatr Diabetes* 2006; **7**: 88-93
29 **Vaidya B**, Oakes EJ, Imrie H, Dickinson AJ, Perros P, Kendall-Taylor P, Pearce SH. CTLA4 gene and Graves' disease: association of Graves' disease with the CTLA4 exon 1 and intron 1 polymorphisms, but not with the promoter polymorphism. *Clin Endocrinol* (Oxf) 2003; **58**: 732-735
30 **Han S**, Zhang S, Zhang W, Li R, Li Y, Wang Z, Xie Y, Mao Y. CTLA4 polymorphisms and ophthalmopathy in Graves' disease patients: association study and meta-analysis. *Hum Immunol* 2006; **67**: 618-626
31 **Lei C**, Dongqing Z, Yeqing S, Oaks MK, Lishan C, Jianzhong J, Jie Q, Fang D, Ningli L, Xinghai H, Daming R. Association of the CTLA-4 gene with rheumatoid arthritis in Chinese Han

population. *Eur J Hum Genet* 2005; **13**: 823-828

32 **Lee CS**, Lee YJ, Liu HF, Su CH, Chang SC, Wang BR, Chen TL, Liu TL. Association of CTLA4 gene A-G polymorphism with rheumatoid arthritis in Chinese. *Clin Rheumatol* 2003; **22**: 221-224

33 **Suppiah V**, O'Doherty C, Heggarty S, Patterson CC, Rooney M, Vandenbroeck K. The CTLA4+49A/G and CT60 polymorphisms and chronic inflammatory arthropathies in Northern Ireland. *Exp Mol Pathol* 2006; **80**: 141-146

34 **Lee YH**, Choi SJ, Ji JD, Song GG. No association of polymorphisms of the CTLA-4 exon 1(+49) and promoter(-318) genes with rheumatoid arthritis in the Korean population. *Scand J Rheumatol* 2002; **31**: 266-270

35 **Suppiah V**, Alloza I, Heggarty S, Goris A, Dubois B, Carton H, Vandenbroeck K. The CTLA4 +49 A/G*G-CT60*G haplotype is associated with susceptibility to multiple sclerosis in Flanders. *J Neuroimmunol* 2005; **164**: 148-153

36 **Martin-Pagola A**, Perez de Nanclares G, Vitoria JC, Bilbao JR, Ortiz L, Zubillaga P, Castano L. No association of CTLA4 gene with celiac disease in the Basque population. *J Pediatr Gastroenterol Nutr* 2003; **37**: 142-145

37 **Xia B**, Crusius JB, Wu J, Zwiers A, van Bodegraven AA, Pena AS. CTLA4 gene polymorphisms in Dutch and Chinese patients with inflammatory bowel disease. *Scand J Gastroenterol* 2002; **37**: 1296-1300

38 **Djilali-Saiah I**, Schmitz J, Harfouch-Hammoud E, Mougenot JF, Bach JF, Caillat-Zucman S. CTLA-4 gene polymorphism is associated with predisposition to coeliac disease. *Gut* 1998; **43**: 187-189

39 **Hou W**, Xia B, Yuan A, Li J, Yang Z, Mao L. CTLA-4 gene polymorphisms in Chinese patients with ulcerative colitis. *Inflamm Bowel Dis* 2005; **11**: 653-656

40 **Lankarani KB**, Karbasi A, Kalantari T, Yarmohammadi H, Saberi-Firoozi M, Alizadeh-Naeeni M, Taghavi AR, Fattahi MR, Ghaderi A. Analysis of cytotoxic T lymphocyte associated antigen 4 gene polymorphisms in patients with ulcerative colitis. *J Gastroenterol Hepatol* 2006; **21**: 449-453

41 **Semino O**, Passarino G, Quintana-Murci L, Liu A, Beres J, Czeizel A, Santachiara-Benerecetti AS. MtDNA and Y chromosome polymorphisms in Hungary: inferences from the palaeolithic, neolithic and Uralic influences on the modern Hungarian gene pool. *Eur J Hum Genet* 2000; **8**: 339-346

42 **Guglielmino CR**, Beres J. Genetic structure in relation to the history of Hungarian ethnic groups. *Hum Biol* 1996; **68**: 335-355

43 **Hooz I**. Alteration of nationality structure in the Carpathian Basin. *Stat Szle* 1996; **74**: 930-939

44 **Comas D**, Calafell F, Mateu E, Perez-Lezaun A, Bosch E, Martinez-Arias R, Clarimon J, Facchini F, Fiori G, Luiselli D, Pettener D, Bertranpetit J. Trading genes along the silk road: mtDNA sequences and the origin of central Asian populations. *Am J Hum Genet* 1998; **63**: 1824-1838

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