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Genotype–phenotype analysis of the CXCL16 p.Ala181Val polymorphism in inflammatory bowel disease

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Abstract To identify if genetic determinants of CXCL16 modulate the susceptibility and phenotype of inflammatory bowel diseases (IBD), we analyzed genomic DNA from 574 individuals (365 IBD patients, 209 healthy controls) for the CXCL16 p.Ala181Val polymorphism. In this study, we demonstrate that in Crohn's disease (CD), the CXCL16 p.Ala181Val polymorphism is not a disease susceptibility gene but associated with younger age at disease onset (p=0.016) and higher frequency of ileal involvement (p=0.024; OR 2.17; 95% CI 1.12–4.21) in ValVal carriers compared to a higher frequency of colonic involvement in AlaAla carriers (p=0.009; OR 2.60; CI 1.29–5.25). Carriers of at least one Val allele and one CARD15/NOD2 variant had a higher incidence of a stricturing and penetrating phenotype (p=0.030, OR 4.04, CI 1.27–12.84) and of stenoses (p=0.014; OR 3.97; CI 1.38–11.40) than patients carrying NOD2 variants only, suggesting that this polymorphism contributes to a severe disease phenotype in CD.

Abbreviations: APC, antigen-presenting cell; CARD, caspase-activation recruitment domain; CD, Crohn's disease; DC, dendritic cell; fs, frameshift; GALT, gut-associated lymphoid tissue; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; mAb, monoclonal antibody; NK, natural killer cell; NOD, nucleotide-binding oligomerization domain; OR, odds ratio; TACE, tumour necrosis factor-alpha-converting enzyme; TLR, Toll-like receptor; TNF, tumor necrosis factor; UC, ulcerative colitis; vs., versus; wt, wild-type.

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Introduction

The pathogenesis of inflammatory bowel diseases (IBD) is still not completely understood, but it is widely accepted that the disease results from an exaggerated mucosal immune response to luminal antigens in a genetically susceptible host [1,2]. Since the first report of *CARD15* as a major susceptibility gene in Crohn's disease (CD) [3,4], polymorphisms in several proteins involved in inflammation and bacterial host defense, such as the Toll-like receptor (TLR) 4 [5], the fractalkine receptor CX3CR1 [6], C-reactive protein (CRP) [7] or macrophage migration inhibitory factor (MIF) [8], have been shown to contribute to certain CD phenotypes.

Recently, it has been demonstrated that chemokines and chemokine receptors are also important in the intestinal immune response [6,9–13]. Another chemokine contributing to the defense against bacteria is CXCL16, the most novel member of the CXC chemokine family. Interestingly, CXCL16 shares structural similarities with CC chemokines and the CX3C chemokine fractalkine. CXCL16 and fractalkine are the only chemokines with a transmembrane region and a chemokine domain suspended by a mucin-like stalk [14]. Therefore, CXCL16 may exist as membrane-bound or cleaved, soluble form. CXCL16 expression has been shown in antigen-presenting cells (APCs), including subsets of CD19+ B cells and CD14+ monocytes/macrophages [14]. A recent study demonstrated that membrane-bound CXCL16 mediates adhesion and phagocytosis of both Gram-negative and Gram-positive bacteria [15]. Moreover, suppression of chemotactic activity of CXCL16 with anti-CXCL16 antibodies significantly inhibited bacterial phagocytosis by human APCs [15]. These findings suggest that CXCL16 may play an important role in facilitating the uptake of bacterial pathogens.

CXCL16 binds a distinct receptor recently identified and designated as CXCR6 [14,16], which was first recognized as a receptor for simian and human immunodeficiency viruses [17]. CXCR6 was originally described under three different names: STRL33 (seven transmembrane receptor-like from clone 33), Bonzo, and TYMSTR (T lymphocyte-expressed seven-transmembrane domain receptor) [17–19]. CXCR6 is expressed by CD4+, CD8+ and natural killer T cells in mice, whereas in humans CXCR6 is expressed by small subsets of Th1 or T-cytotoxic 1 (Tc1) cells [20]. Most interestingly, CXCR6+ T cells are particularly found among T cells at sites of tissue inflammation, such as rheumatoid joints and inflamed livers [20]. CXCR6 mRNA has been detected in lymphoid tissue such as thymus and spleen [17] but also in the small and large intestines [17,21,22]. Interestingly, we also demonstrated functional CXCR6 expression on intestinal epithelial cells [23], suggesting a role for this chemokine system in modulating the intestinal barrier function.

Recently, we demonstrated increased CXCL16 expression in the colon and sera of patients with active CD [23], pointing to a role for this chemokine system in the pathogenesis of IBD. This is supported by a very recent study showing that anti-CXCL16 antibody treatment significantly suppressed IL-1β production in a murine model of dextran sulfate sodium (DSS)-induced colitis [24]. Most recently, a sequence alteration has been described in exon 4 of the *CXCL16* gene [25], resulting in a C→T missense mutation in codon 181 replacing alanine (GCT) by valine (GTT; CXCL16 p.Ala181Val) [25]. This SNP has been shown to be associated with the severity of coronary artery stenosis [25] but its effect in IBD patients has not been investigated thus far. Given our preliminary results suggesting a role for this chemokine in CD [23], we therefore analyzed the effect of the CXCL16 p.Ala181Val polymorphism on IBD susceptibility and disease phenotype in a large German cohort ($n=574$) including 365 IBD patients.

Patients and methods

Study population of the genotype–phenotype analysis

The study population ($n=574$) was composed of 201 patients with CD, 164 patients with ulcerative colitis (UC), and 209 healthy unrelated controls. All patients and controls were Germans of Caucasian ethnicity recruited by the University-Hospital Munich-Grosshadern. Controls were healthy volunteers including blood donors matched by gender to the IBD study population. Details on gender and age distribution of patients and controls are provided in Table 1. Diagnosis of CD or UC was assessed according to established criteria [26]. Demographic and routine clinical data were recorded by patients' chart analysis and an interview at the time of enrollment. CD phenotypes were determined according to the Vienna classification [27]. In UC patients, anatomic location was determined based on the criteria pancolitis, left-sided colitis or colitis limited to the rectum (proctitis). The study was approved by the local Ethics Committee. All participating patients gave written, informed consent prior to genetic analysis.

Table 1 Demographic characteristics of the study population

	CD ($n=201$)	UC ($n=164$)	Controls ($n=209$)
Gender			
Male (%)	93 (46.3%)	83 (50.6%)	99 (47.4%)
Female (%)	108 (53.7%)	81 (49.4%)	110 (52.6%)
Age (year)			
Mean ± SD	40.3 ± 11.9	42.8 ± 14.7	48.6 ± 15.2
Range	17–75	18–84	23–86
Body mass index			
Mean ± SD	23.2 ± 4.0	24.1 ± 4.3	
Range	16–40	16–41	
Age at diagnosis (year)			
Mean ± SD	28.3 ± 11.9	31.9 ± 13.7	
Range	7–71	9–81	
Disease duration (year)			
Mean ± SD	11.9 ± 8.2	10.7 ± 7.8	
Range	1–43	0.5–39	
Positive family history of IBD (%)	25 (12.4%)	21 (12.8%)	