

Association between a common *IL10* distal promoter haplotype and IgE production in individuals with atopic dermatitis

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Summary

Atopic dermatitis (AD) is a genetically determined inflammatory skin disease characterized by abnormal cytokine production, including increased production of interleukin 10 (*IL10*). Single nucleotide polymorphisms (SNP) and haplotypes in the *IL10* gene promoter region on chromosome 1q31-32 have been implicated in several inflammatory diseases, but generally, only SNPs proximal to the transcription start site (TSS) have been investigated. The aim of this study was to identify proximal, distal and combined haplotype sets in the *IL10* promoter region and to study their association with clinical phenotypes in atopic dermatitis. SNPs at positions -3575, -2849, -2779, -2763, -1082, -851, -819 and -592 in the *IL10* promoter region were genotyped in individuals with atopic dermatitis ($n = 47$) and nonatopic control subjects ($n = 40$) using polymerase chain reaction-based techniques and induced heteroduplex generator (IHG) analysis. Pan-promoter, TSS-proximal and TSS-distal haplotypes were reconstructed using PHASE analysis. Fifteen haplotypes representing all eight SNPs were identified. Subgrouping identified four 4-locus and three 3-locus TSS-proximal haplotypes; and nine 4-locus TSS-distal haplotypes. No difference was found in haplotype or SNP frequencies between the AD and control groups, or between patients with mild or severe disease. However, a common 4-locus TSS-distal haplotype (TGAC) was significantly increased in patients with IgE levels over 1000 kIU L⁻¹. This study is the first to analyse the association between haplotype groups in the *IL10* promoter region and clinical phenotypes in AD. We have demonstrated a significant association between the TSS-distal haplotype TGAC, and IgE levels in AD patients.

It remains to be shown if there is an association between the TGAC haplotype and *IL10* production, which might account for the stimulation of IgE production.

Introduction

Atopic dermatitis (AD) is a common, debilitating inflammatory skin disease. Its prevalence has increased markedly over the last two decades and it is now the commonest chronic disease of childhood in Europe, the USA and Japan (Leung *et al.*, 2004). AD is thought to represent an interaction between both genetic and environmental influences with trigger factors including irritants, environmental allergens and microorganisms. There is substantial evidence to suggest that genetic factors have an important role in predisposition to atopic dermatitis. The risk of a child having atopic disease (asthma, eczema, allergic rhinitis) is 50% if one parent is affected, rising to 75% if both parents are affected. In addition, the concordance in monozygotic twins is 72% and 23% in dizygotic twins (Larsen, 1986).

There is considerable evidence to indicate that immune dysregulation occurs within the skin in atopic dermatitis, there being a predominance of T-helper type 2 (Th2) lymphocytes, with a resulting increased production of various cytokines including interleukin (*IL*)4, *IL*5, *IL*13 in acute eczematous lesions (Yoshizawa *et al.*, 2002). *IL*10 is produced by Th1 and Th2 lymphocytes and increased levels have also been identified in the skin of patients with atopic dermatitis. *IL*10 has been shown to have pleiotropic effects on immunoregulation in the skin including the down-regulation of Th1 type cytokines (Lalani *et al.*, 1997).

A number of candidate genes within various gene clusters, including those that determine the production or activity of cytokines (Arkwright *et al.*, 2001), have been implicated in predisposition to atopic disease. However, the importance of these genetic factors remains unclear. Multiple single nucleotide polymorphisms (SNP) in the *IL10* gene promoter region on chromosome 1q31-32 have been associated with susceptibility, severity and clinical outcome in a number of inflammatory diseases and it has been postulated that some of these SNPs influence the expression of *IL10*. Evidence for the heritable genetic basis of *IL10* production comes from monozygotic twin studies that indicate that genetic factors could account for up to 75% of the level of *IL10* production.

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The majority of previous studies of polymorphism in the *IL10* promoter region in atopic dermatitis and other disorders, have only investigated a very limited number of SNPs (Reich *et al.*, 2003). However, the finding that certain SNPs that were previously thought to be regulatory are highly conserved within certain ethnic groups (Mok *et al.*, 1998) suggests that the degree of polymorphism in the *IL10* promoter region is likely to be much greater than previously believed. The study of only limited numbers of SNPs may therefore fail to identify significant associations between disease and *IL10* promoter polymorphisms. The aims of this study were to identify pan-promoter, TSS-proximal and TSS-distal haplotypes of *IL10* by reconstruction from SNP genotyping data, and to investigate associations with clinical phenotypes in individuals with atopic dermatitis.

Materials and methods

Study participants

A physician-administered questionnaire was completed for each participant. Caucasian patients over the age of 18 years with a history of atopic dermatitis ($n = 46$) were recruited from the dermatology departments at the Bristol Royal Infirmary, Bristol and Royal United Hospital, Bath. The diagnosis of atopic dermatitis was made according to the modified Hanifin and Rajka diagnostic criteria for AD as defined and validated by the UK Working Party (Williams *et al.*, 1994). The patients were examined and disease severity was assessed according to the eczema area and severity index (EASI) score (Hanifin *et al.*, 2001). Unrelated, healthy individuals with no personal history of atopic disease (asthma, eczema, allergic rhinitis) were recruited as control subjects ($n = 40$). Six millilitres of venous blood was collected from all study participants. Total serum IgE levels were determined by a bead capture system (Euro/Dpc Ltd, Llanberis, Wales, UK; Immunolyte 2000). The study was approved by the United Hospitals Bristol NHS Foundation Trust (UHBT) and Bath local research ethics committees.

Genotyping of *IL10* promoter region polymorphisms

Genomic DNA was extracted (QIAamp DNA minikit; QIAGEN, Hilden, Germany) from buffy coats derived from whole blood. Sequences within the *IL10* promoter region were then amplified using polymerase chain reaction (PCR) (*Taq* PCR Master Mix Kit, QIAGEN) in order to generate genomic amplicons. Induced heteroduplex generator (IHG) analysis was used to investigate SNP genotypes at eight positions (−3575A>T, −2849A>G, −2779A>G, −2763A>C, −1082A>G, −851G>A, −819C>T, −592C>A) relative to the transcription start site (TSS) in the *IL10* promoter region, using previously published techniques (Wood *et al.*, 2001).

Statistical analysis

Haplotypes were reconstructed from genotype data using a Bayesian statistical algorithm (PHASE 2.1 software;

Stephens *et al.*, 2001; Stephens & Donnelly, 2003). Haplotypes reconstructed were from all eight loci 'pan-promoter' SNPs (−3575, −2849, −2779, −2763, −1082, −851, −819 and −592); 4-locus TSS-proximal SNPs (−1082, −851, −819 and −592); 3-locus TSS-proximal SNPs (−1082, −819 and −592); and 4-locus TSS-distal SNPs (−3575, −2849, −2779, −2763). Haplotype frequencies were compared between: AD patients and controls; AD patients with mild and moderate/severe disease; and AD patients with IgE levels < 1000 kIU L^{−1} and > 1000 kIU L^{−1}. Haplotype odds ratios were determined by a standard haplotype-vs.-rest of cohort method and by a haplotype-vs.-reference haplotype method, or where appropriate using the chi-squared or Fisher's exact test. Haplotypes with a frequency of < 0.1 were combined for statistical purposes as 'others' (Table 2).

Results

Characteristics of the study population are shown in Table 1. There was a sex ratio of 1:1.5 F:M for both groups. A significantly elevated IgE level was found in the AD group vs. the controls. Twenty-five per cent of the AD group and 93% of the control group were found to have normal IgE levels (< 89 kIU L^{−1}). Fifty-seven per cent of the AD group had a history of asthma and 51% had a history of allergic rhinitis in addition to atopic dermatitis. Based on the EASI score, most of the AD patients were classified as having mild eczema at the time of examination (54%) with 20% and 26% having moderate and severe disease respectively.

In AD patients and controls, fifteen 8-locus ('pan-promoter') haplotypes were identified in the reconstruction. There were four 4-locus TSS-proximal and three 3-locus TSS-proximal haplotypes; and nine 4-locus TSS-distal haplotypes. No difference was found in any of the haplotype or SNP frequencies between the AD and control groups, or between patients with mild or moderate/severe disease (data not shown). However, comparison of AD patients with IgE levels below and over 1000 kIU L^{−1} (Table 2) revealed that the most frequent 4-locus TSS-distal haplotype

Table 1. Characteristics of the study population

	Atopic dermatitis ($n = 46$)	Healthy controls ($n = 40$)
Sex		
Male	18 (39%)	16 (40%)
Female	28 (61%)	24 (60%)
Age (years)		
Mean (SD)	39.43 (12.93)	46.72 (11.16)
Range	20–69	24–63
Serum IgE levels (kIU L ^{−1})		
Mean (SD)	5429 (10174)	46.92 (111)
Disease severity (EASI score)		
Mean (SD)	10.85 (9.34)	0
Duration of symptoms (years)		
Mean (SD)	32.58 (16.68)	0

Table 2. Haplotype odds ratio analysis of *IL10* promoter polymorphisms in AD patients. Odds ratios (OR) and 95% confidence intervals (CI) are shown for patients with high IgE levels (> 1000 kIU L⁻¹, ' > 1000 ') versus normal or low/moderate IgE levels (< 1000 kIU L⁻¹, ' < 1000 '), for each of the four haplotype reconstructions (A–D). Cohort OR represents the haplotype vs. rest of cohort (all other haplotypes) odds ratio; ref Haplo OR represents the haplotype vs. reference haplotype (most frequent haplotype) odds ratio. Where odds ratios were not applicable due to low haplotype numbers, Fisher's exact test was used. Haplotypes are shown in the left hand columns and denote the alleles at each of the loci shown in the order noted in the heading of each table: the reference haplotype is shown in bold typeface. N/A, not applicable

	> 1000	< 1000	Cohort OR (CI)	Ref Haplo OR (CI)
A: 8-locus pan-promoter (–3575, –2849, –2779, –2763, –1082, –851, –819, –592)				
AAAAGGCC	10	10	0.94 (0.31–2.85)	0.69 (0.17–2.78)
TGACAGCC	13	9	1.53 (0.52–4.54)	1.0
TGACAGTA	13	5	3.07 (0.90–12.05)	1.8 (0.40–8.74)
Others	10	20	N/A	N/A
Total	46	44		
B: 4-locus TSS-proximal (–1082, –851, –819, –592)				
AGCC	13	11	1.18 (0.42–3.34)	1.53 (0.49–4.83)
AGTA	14	8	1.97 (0.66–5.96)	2.26 (0.68–7.66)
GGCC	17	22	0.59 (0.23–1.48)	1.0
GACC	2	3	$P = 0.67$ (Fisher)	$P = 1.0$ (Fisher)
Total	46	44		
C: 3-locus TSS-proximal (–1082, –819, –592)				
ACC	13	11	1.18 (0.42–3.34)	1.56 (0.51–4.77)
ATA	14	8	1.97 (0.66–5.96)	2.30 (0.71–7.58)
GCC	19	25	0.53 (0.21–1.34)	1.0
Total	46	44		
D: 4-locus TSS-distal (–3575, –2849, –2779, –2763)				
AAAA	10	12	0.74 (0.25–2.15)	0.43 (0.13–1.41)
TGAC	27	14	3.05 (1.18–7.96) ^a	1.0
AGAC	1	5	$P = 0.1$ (Fisher)	$P = 0.033$ (Fisher)
Others	8	13	N/A	N/A
Total	46	44		

^a Chi-square = 5.51; $P = 0.01$.

(TGAC) was significantly increased in patients with IgE levels > 1000 kIU L⁻¹ (OR = 3.05, CI = 1.18–7.96). Interestingly, the AGAC haplotype was significantly less frequent in this group of patients ($P = 0.033$, Fisher's exact test). The AGAC haplotype was also significantly less frequent in patients with moderate/severe disease (haplotype frequency = 0/40) than in those with mild disease (haplotype frequency = 7/52; $P = 0.032$, Fisher's exact test).

Discussion

IL10 is produced by a variety of cells within the skin including monocytes, T cells and dendritic cells. Increased levels of *IL10* in lesional skin (Ohmen *et al.*, 1995) and serum (Shin *et al.*, 2005) in individuals with AD have been observed previously. Although the role of *IL10* in the pathogenesis of cutaneous inflammation is currently unclear, it has been shown to induce B cell proliferation and

synthesis of IgE (Kobayashi *et al.*, 2002). Thus, increased levels of *IL10* may in part explain the increased levels of IgE also observed in patients with AD. IgE is thought to mediate inflammation in the skin via enhanced antigen presentation of allergens, activation of macrophages and by inducing the release of inflammatory mediators from mast cells (Kang & Stevens, 2003). Mast cell growth within the skin has also been shown to be stimulated by *IL10* (Thompson-Snipes *et al.*, 1991).

In addition to its pro-inflammatory effects, *IL10* is also a potent immunosuppressive cytokine, known to inhibit T cell mediated responses, with reduced *IL12* induced production of interferon gamma (Lester *et al.*, 1995). This may account for the reports of reduced allergic contact sensitivity responses in subjects with AD. Another proposed mechanism is the down-regulation of antimicrobial peptides (Howell *et al.*, 2005), potentially contributing to the observed increased susceptibility to cutaneous bacterial infection seen in AD.

Various single nucleotide polymorphisms within the *IL10* promoter region including locations –1082 (A>G) (Suarez *et al.*, 2003), –819 (Zhang *et al.*, 2002) and –592 (A>C) (Rosenwasser & Borish, 1997) have been demonstrated previously to result in differences in *IL10* production and *IL10* nuclear binding capacity. The observation that certain *IL10* promoter polymorphisms are seen in association with various inflammatory (Gibson *et al.*, 2001) and infectious diseases (Persico *et al.*, 2006) indicates these polymorphisms are likely to play an important role in determining susceptibility to both infection and inflammation.

In patients with atopic dermatitis, one study of the –1082 SNP in the *IL10* promoter region failed to demonstrate any association with AD (Reich *et al.*, 2003). However, a more recent study has shown that the *IL10* promoter haplotype *IL10*-ht 2 (–1082A, –819C, –592C, +117T) is seen in association with reduced levels of serum IgE in a gene-dose dependent manner (Shin *et al.*, 2005). *IL10* promoter polymorphisms have also been attributed to increased IgE production in patients with asthma (Karjalainen *et al.*, 2003). *IL10* promoter polymorphisms have been implicated in other skin disorders including malignant melanoma (Howell *et al.*, 2001), squamous cell carcinoma (Alamartine *et al.*, 2003) and psoriasis (Asadullah *et al.*, 2001).

The present study has revealed considerable complexity in the haplotype structure of the *IL10* promoter using eight SNPs within a 3.6-kb region upstream of the transcription start site. Analysis of the 4-locus distal haplotype revealed that the most frequently observed haplotype in both patients and controls, TGAC (–3575T, –2849G, –2779A, –2763C) was nearly twice as frequent in patients with high IgE levels (> 1000 kIU L⁻¹) than in patients with lower IgE levels (< 1000 kIU L⁻¹). However, we were unable to reveal any significant association between *IL10* promoter haplotype and either susceptibility to AD, or disease severity. There was no association between 8-locus pan-promoter or 3- or 4-locus TSS-proximal haplotypes and IgE levels in patients.

It was also observed that the infrequent AGAC haplotype was significantly less frequent in patients with high levels of IgE, and in AD patients with moderate to severe disease. Although this was of borderline statistical significance in each case, it could reflect the relatively small numbers of individuals recruited and a larger study will be needed to verify this association.

This study has emphasized the importance in disease association studies of the analysis of promoter haplotypes, as opposed to isolated single nucleotide polymorphisms, and of the separate analysis of TSS-proximal and TSS-distal haplotype sets within the *IL10* promoter.

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