

Absence of Association Between Asthma and High Serum Immunoglobulin E Associated *GPRA* Haplotypes and Adult Atopic Dermatitis

To the Editor:

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease that affects 10%–15% of children and 2%–10% of adults (Williams, 1997). The skin in AD is typically dry and excoriated due in part to reduced ability of the epidermis to hold water. Immunological mechanisms are thought to play a critical pathogenic role with AD being associated with high immunoglobulin E (IgE) levels and the accumulation of T lymphocytes of the Th2 subtype in involved skin (Galli *et al*, 2003). AD is also associated with atopic diseases in other organs such as asthma and allergic rhinitis (Mortz *et al*, 2001). Atopy reflects a genetic predisposition for excessive IgE antibody production with 80% of children with AD generating serum concentrations of IgE typically five fold increased (Stone, 1973; Johansson *et al*, 2001). Whilst the pathogenesis of AD is unclear, family and twin studies support a significant genetic component underlying predisposition to the phenotype (Kuster *et al*, 1990). Furthermore, genome-wide scans for susceptibility loci have reported a number of chromosomal loci linked to the disease (Lee *et al*, 2000; Cookson *et al*, 2001; Bradley *et al*, 2002).

Association studies provide a powerful method for isolating disease loci and a recent study of asthma patients and high serum IgE responders from isolated cohorts in Finland identified risk haplotypes from a 133 kb region on chromosome 7p (Laitinen *et al*, 2004). Located within this interval, a G protein-coupled receptor gene, *GPRA*, showed altered distribution patterns between bronchial biopsies from healthy and asthmatic individuals (Laitinen *et al*, 2004). This association has been further supported by two recent reports of asthma patients. These included a large German study (Kormann *et al*, 2005) and a cohort drawn from five Western European countries (Melén *et al*, 2005). Evidence of association was not observed in a Korean cohort (Shin *et al*, 2004), in which only a single-nucleotide polymorphism (SNP) was examined and as such may not have fully distinguished between the risk and non-risk haplotypes across the *GPRA* locus. The *GPRA* gene is also expressed in skin keratinocytes raising the possibility that variation in this gene may directly contribute to predisposition to AD (Laitinen *et al*, 2004).

To investigate inherited susceptibility to AD we examined seven SNP that characterize or “tag” the seven haplotypes spanning *GPRA* identified by Laitinen *et al* (2004). These were genotyped in 188 adult patients of Northern European descent and in 221 age- and sex-matched controls. Ethical approval was given by the medical ethics committee of St Thomas’s Hospital and informed written consent was obtained from all participating subjects. The study was conducted according to Declaration of Helsinki Principles. All patients were diagnosed using standard clinical criteria of Hanifin and Rajka by experienced dermatologists (Hanifin, 1992). Minimum age at study was 18 y indicating that this cohort had persistence or recurrence into adulthood. Further these patients in general had increased disease activity compared with childhood AD. Taken together this suggests that this patient group represents a more severe phenotype. The positions of the SNP markers are listed in Table I with respect to genomic contig NT_000380. Genotyping of samples was performed by size assessment of primer extension products using the MALDI-TOF mass spectrometry Sequenom system (Jurinke *et al*, 2002). Genotype frequencies were found to be in Hardy-Weinberg equilibrium. Allele frequencies for each SNP were compared between patients and controls using χ^2 contingency tables. Haplotypes of the seven SNP were constructed separately for patients and controls using PHASE (Stephens *et al*, 2000).

The allele frequencies for the seven markers in the patients and the controls are shown in Table I. No significant differences were observed in the allele frequencies between the patients and controls. Control allele frequencies were similar to those of the study by Laitinen *et al* (2004) and also to a German control population (Kormann *et al*, 2005). Haplotypes inferred using PHASE were used to assess whether specific haplotypes may harbor AD susceptibility alleles. These haplotypes were also compared with those observed in the asthma and high IgE cohorts (Laitinen *et al*, 2004). Similar haplotype frequencies were observed between the controls in this study and the controls reported in the asthma study and in those ascertained from Kormann *et al* (2005). An additional haplotype with a frequency greater than 2.5% was observed in this study by comparison with those reported in the asthma study (Hn, Table II). These haplotypes represented 96% of the haplotypes present in the cohort studied and no single haplotype accounted for more than 35% of the total. No overall statistical significant differences in haplotype frequencies were observed between AD patients and controls (Table II). These data imply

Abbreviations: AD, atopic dermatitis; IgE, immunoglobulin E; SNP, single-nucleotide polymorphism

Table I. Allele frequencies in atopic dermatitis (AD) patients and controls for single-nucleotide polymorphism (SNP) from the asthma susceptibility region *GPRA*

Marker ^a	Allele frequencies				p χ^2
	Patients		Controls		
	Frequency	Proportion	Frequency	Proportion	
515224*C	351	0.94	414	0.94	NS
515224*G	21	0.06	26	0.06	
522363*C	155	0.41	172	0.39	NS
522363*G	221	0.59	270	0.61	
529556*C	197	0.55	228	0.56	NS
529556*A	163	0.45	176	0.44	
546333*G	331	0.94	393	0.93	NS
546333*A	21	0.06	29	0.07	
555608*T	202	0.54	230	0.53	NS
555608*C	174	0.46	204	0.47	
563704*T	127	0.34	159	0.37	NS
563704*C	249	0.66	271	0.63	
585883*G	322	0.86	388	0.89	NS
585883*C	52	0.14	48	0.11	

^aPosition of SNP relative to genomic contig NT_000380 (Laitinen *et al*, 2004).

that variants in *GPRA* are unlikely to contribute to inherited predisposition to AD.

High IgE levels are a characteristic of AD and *GPRA* is associated with IgE levels. We, therefore, considered it prudent to determine if any association between *GPRA* SNP genotypes or haplotypes and IgE levels in the AD patients exists. The IgE data did not fit a normal distribution hence non-parametric rank-sum tests were used. No significant relationship was seen for any SNP genotype and IgE using the Kruskal–Wallis test or for any particular haplotype and IgE using the Mann–Whitney test. A subtype of AD exists in which the clinical criteria for AD are fulfilled in the absence of high serum IgE levels. This subtype may be caused through the activation of inflammation via different path-

ways not involving IgE. In this cohort only eight AD patients had normal serum IgE levels (<100 kU per liter), a sample set not large enough for statistical analysis. Hence at this time we simply note a decrease in the frequency of non-risk haplotypes (haplotypes h1, h3, and h6 in Table II; 56% in the controls, 38% in the AD patients with normal IgE levels) and a corresponding increase in risk haplotypes (haplotypes h2, h4, h5, h7) described for asthma and high serum IgE by Laitinen *et al* (2004).

In this report we have investigated the hypothesis that genetic variation in the *GPRA* gene is associated with AD, a disease in which high serum IgE levels are a characteristic feature. We found no significant differences in either individual allele frequencies or haplotype frequencies for seven

Table II. Haplotypes with a frequency greater than 2.5% within atopic dermatitis (AD) patient and control cohorts

Haplotype ^a	Markers							Haplotype frequencies	
	515224	522363	529556	546333	555608	563704	585883	Patients, n = 338	Controls, n = 364
H1	C	G	C	G	C	T	G	110 (33%)	121 (33%)
H2	C	C	A	G	T	C	G	65 (19%)	79 (22%)
H3	C	G	C	G	C	C	G	49 (14.5%)	42 (11.5%)
H4	C	C	A	A	T	C	C	20 (6.0%)	26 (7.0%)
H5	C	C	A	G	T	C	C	24 (7%)	13 (3.5%)
H6	C	G	C	G	T	C	G	31 (9%)	41 (11%)
H7	G	C	A	G	T	C	G	19 (5.5%)	19 (5.0%)
Hn	C	G	A	G	T	C	G	12 (3.5%)	11 (3.0%)

^aHaplotypes labelled according to Laitinen *et al* (2004), Hn indicates a haplotype not previously described.

SNP surrounding GPRA between patients and controls. In a small subset of AD patients with normal serum IgE levels potential association with *GPRA* was observed. But investigation of this will require a larger collection of adult AD patients with normal serum IgE levels. Although no significant association has been observed for the common form of high serum IgE level AD, the study by Laitinen *et al* (2004) provides evidence for the role of *GPRA* in atopy in general and identification of genes in the *GPRA* signalling pathway combined with data from genome-wide linkage studies should serve to identify further candidates for AD.

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