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SHORT COMMUNICATION

Association of a chromosome 1q21 locus in close proximity to a late cornified envelope-like proline-rich 1 (*LELP1*) gene with total serum IgE levels

Mamta Sharma · Kamiya Mehla · Jyotsna Batra · Balaram Ghosh

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Abstract The chromosomal region 1q21 has been linked to atopic dermatitis in previous studies. Seven polymorphic repeats were identified in a 0.5 Mb region of chromosome 1q21 encompassing a small proline-rich protein (*SPRR*) gene cluster, a few *S100* gene family members, loricin, and several uncharacterized genes. These repeats were genotyped by fragment length polymorphism analysis in 133 atopic case–parent trios, of which 111 probands had atopic asthma. Our trio-based analysis for association with atopy and atopic asthma revealed no significant allelic or genotypic association for any of the seven loci tested. However, a significant association was observed with locus 5 (3.65 Mb of contig NT_032962) and $\log_{10}$ serum IgE levels ($F = 3.93$, $P = 0.0008$). Results were also replicated in an independent case-control cohort ($N_p = 165$, $N_c = 166$), where a significant association with $\log_{10}$ serum IgE levels was observed in the patient group ($P = 0.0005$). Interestingly, locus 5 is 6.2 kb upstream of a late cornified envelope-like proline-rich 1 (*LELP1*) gene which encodes a novel small proline rich protein. Further, we have also found a significant association of rs7534334 (tagged SNP from *LELP1*) SNP with $\log_{10}$ serum IgE levels in the patient group ($P = 0.0029$). Thus, our results identify a chromosomal region in close proximity to a novel gene and highlight the need for intense research on *LELP1* and other genes close by with respect to atopic disorders.

Keywords Asthma · Atopy · 1q21 · IgE · QTDT · LELP1

Introduction

Atopic asthma is a complex polygenic disorder characterized by allergen-induced inflammation of the airways and high Th2-mediated IgE humoral response (Ghosh et al. 2003). The chromosome 1q21 region has been shown to be linked to atopic dermatitis and psoriasis, an inflammatory skin condition, with a peak linkage overlying the epidermal differentiation complex (EDC) spanning a region of 2.05 Mb (Cookson et al. 2001; Capon et al. 1999; Bhalerao et al. 1998). The EDC contains various important genes, such as involucrin (*IVL*), loricin (*LOR*), the small proline-rich protein (*SPRR*) gene family, profilaggrin (*FLG*) and trichohyalin (*THH*) encoding structural components of the epidermis, and the S100 gene family encoding calcium-binding proteins involved in signal transduction and cell cycle progression (Marenholz et al. 1996). Interestingly, in recent studies, two null mutations in the *FLG* gene were found to be associated with atopic dermatitis and asthma occurring in the context of atopic dermatitis (Palmer et al. 2006, Marenholz et al. 2006).

Towards the telomeric end of the EDC, within a span of 0.5 Mb, several genes such as a *SPRR* gene cluster, loricin, *S100A8*, *A9*, *A12*, *A15*, and several uncharacterized genes are present (Fig. 1). The serum

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M. Sharma · K. Mehla · J. Batra · B. Ghosh (✉)
Molecular Immunogenetics Laboratory,
Institute of Genomics and Integrative Biology,
Mall Road, Delhi 110007, India
e-mail: bghosh@igib.res.in

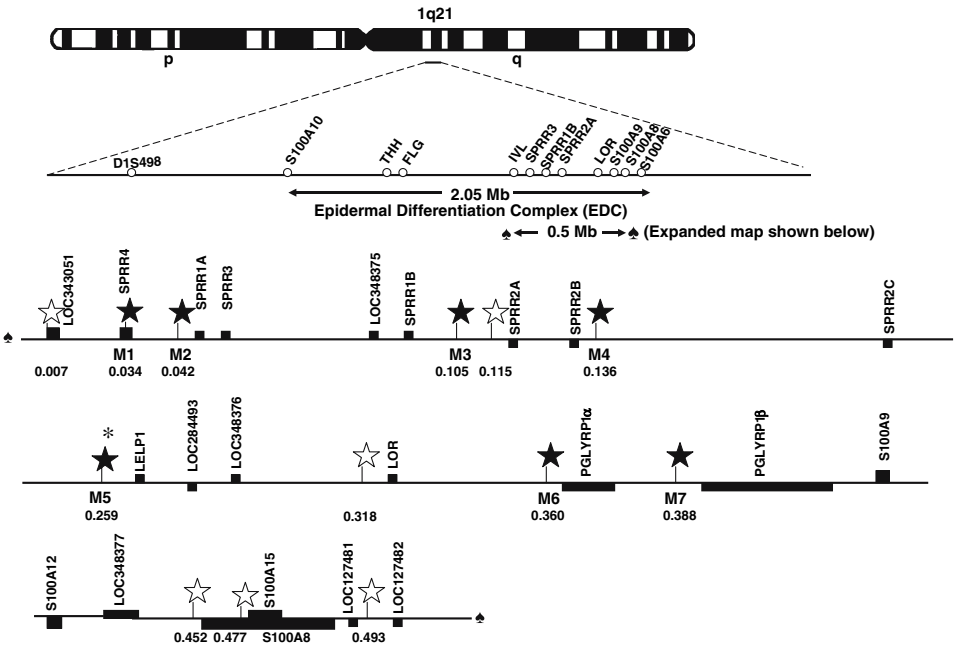

Fig. 1 Earlier linkage studies with atopic disorders identified a peak of linkage at the D1S498 marker overlying the EDC spanning a region of ~2 Mb on chromosome 1q21. This region has an S100 gene cluster and the epidermal differentiation genes. Simple repeats were identified in this 0.5 Mb region (3.4–3.9 Mb of contig NT_032962), spanning the important inflammatory candidate genes on chromosome 1q21. The position of each repeat is given in Mb within the 0.5 Mb region. Polymorphic and nonpolymorphic repeats are marked with *solid* and *open stars*, respectively. Significant association with serum IgE levels was observed at the 0.259 Mb position, as shown by an *asterisk*

levels of the S100A8 and A9 protein complex (calprotectin) are found to be raised above normal levels under many inflammatory conditions (Sorg et al. 1992). Moreover, the S100A8 homodimer is a potent chemotactic factor for leukocytes (Passey et al. 1999) and S100A12 exhibits proinflammatory activity on endothelial cells and inflammatory cells (Hofmann et al. 1999). Microarray studies on skin biopsy samples of patients suffering from atopic dermatitis revealed an increased expression of *S100A7*, *S100A8* and downregulation of filaggrin and loricrin (Sugiura et al. 2005). Recent studies using allergen-induced asthma models revealed overexpression of the *SPRR2A* and *SPRR2B* genes in subsets of bronchial epithelial cells and mononuclear cells (Zimmermann et al. 2005).

Thus, a number of inflammatory candidate genes and several uncharacterized genes are present within a distance of 0.5 Mb on chromosome 1q21. However, an association study to identify the genes associated with atopic disorders from this region has not yet been performed. In this study we have investigated the genetic association of simple repeat markers in this region with atopy, atopic asthma and serum IgE level phenotypes in the Indian population. The association was further validated using tagged single nucleotide polymorphisms (SNPs) within the *LELP1* gene.

Materials and methods

The study population consisted of 133 atopic case–parent trios, of which 111 probands had atopic asthma. Patients and the family members were recruited from hospitals in North India. An independent case (atopic asthma)-control (normal nonasthmatic individuals) cohort (N_p = 165, N_c = 166) was also collected. Ethical approval was obtained from the Institutional Review Board. Written informed consent was obtained from all participating individuals. Patients were diagnosed by physicians for asthma according to the guidelines of the National Asthma Education and Prevention Program (Expert Panel Report 2, 2002) and were examined for a self-reported history of breathlessness, wheezing and for family history (Nagpal et al. 2005). Atopic individuals gave a positive skin prick test (SPT) for at least one of the fifteen common environmental allergens and suffered from asthma, allergic rhinitis or atopic dermatitis. The demographic and clinical details of the study population are given in Table 1. Genetic homogeneity between the patients and the control group was confirmed by genotyping various loci that have yet to be linked to asthma-related disorders (Batra et al. 2005). Total serum IgE levels were estimated using enzyme-linked immunosorbent assay

Table 1 Demographic profile of the study population

	Trio-based study	Case-control study	
	Atopic asthmatic probands	Atopic asthmatics	Controls
Number of individuals:	111	165	166
Age	16.59 (±10.9)	25.87 (±16.7)	23.74 (±8.6)
Mean $\log_{10}$ IgE	3.01 (±0.69)	3.07 (±0.74)	2.21 (±0.76)
Sex ratio (M:F)	0.60:0.40	0.59:0.41	0.53:0.47
Asthma	All	All	None
SPT positivity/atopy*	All	All	None
Smoking history	None	None	None
Frequent episodes of wheezing	All	All	None
FEV1/FVC at the time of attack	<70%	<70%	NA
Percent reversibility using bronchodilator	>12%	>12%	ND
Asthma therapy	All	All	NA

Patients and their family members were recruited from states in North India, viz: Delhi, Haryana and Punjab. Controls were also collected from the same area

Parentheses contain standard deviation values. *NA* indicates not applicable and *ND* indicates not done

*Patients tested positive for at least one of the 15 most common environmental allergens

(ELISA) (Bethyl Labs, Montgomery, TX, USA). The NCBI nucleotide sequence NT_032962 was taken and the region 3.41–3.9 Mb was scanned for simple repeats using the RepeatMasker program. A total of 13 such repeats (Fig. 1) were identified. Primers were designed using DNASTAR PrimerSelect software in order to amplify the identified repeat regions (Supplementary Table 1). The forward primers were fluorescently labeled with FAM/HEX. PCR amplification was performed from the DNA from 20 normal individuals, and samples were electrophoresed using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) as per the manufacturer's instructions. Fragment lengths were determined using the GeneScan/Genotyper v3.7 (Applied Biosystems). The seven polymorphic loci (Fig. 1, marked with solid stars) were genotyped in all of the individuals. Two tagged SNPs (rs11576296 in intron 1 and rs7534334, 255 bp downstream of the *LELP1*) in/around the *LELP1* gene were identified using the HAPMAP database and were also genotyped using the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems) using the primers detailed in Supplementary Table 1. These samples were subsequently electrophoresed using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems) as per the manufacturer's instructions.

Mendelian inconsistencies during the transmission of alleles at the seven loci were checked using the Pedcheck program. An extended transmission disequilibrium test (E-TDT) for multi-allelic marker (http://www.mds.qmw.ac.uk/statgen/dcurtis/software.html) was carried out for the seven multi-allelic markers. In TDT, a parent heterozygous for an associated allele A1 and nonassociated allele A2 should transmit A1 more often to an affected child than A2. A quantitative transmission/disequilibrium test (QTDT) (Abecasis et al. 2000) was carried out for the serum $\log_{10}$ IgE levels in families. The QTDT test statistics were obtained using likelihood-ratio tests, assuming normal distributions for the traits. An empirical *P* value that is based on the permutation of genotypes is obtained to protect against possible deviations from either normality or selection on the trait (http://www.sph.umich.edu/csg/abecasis/QTDT/download/index.html). A global *P* value was calculated for multi-allelic marker alleles using the multi-allelic option with the orthogonal test. When this option is used, rare alleles (i.e., those with a frequency of ≤5% in the total genotyped sample) are clumped together and individual effects for all other alleles are estimated, producing a single *P* value per marker/phenotype pair. Allelic and genotypic associations with atopic asthma were analyzed in the case-control study using JMP Version 4.0.2 (SAS Institute Inc., Cary, NC, USA) or the Armitage trend test. Allelic or genotypic association with serum $\log_{10}$ IgE levels was assessed for the case and control groups using one-way ANOVA.

Results and discussion

In our study, we identified thirteen simple repeats in the 0.5 Mb region of chromosome 1q21 in close proximity to several important candidate genes in this region: SPRR genes, S100A8, S100A9, and several other uncharacterized genes (Fig. 1). Seven loci—at positions 3.43 (M1), 3.44 (M2), 3.5 (M3), 3.53 (M4), 3.65

(M5), 3.76 (M6) and 3.78 Mb (M7)—were found to be polymorphic whereas six loci—at positions 3.4, 3.5, 3.7, 3.85, 3.87 and 3.89 Mb—of contig NT_032962 were found to be nonpolymorphic in the Indian population (Fig. 1). The heterozygosity index of each polymorphic locus was greater than 0.75. Since the markers were in close proximity to several candidate genes in this region, they could be used in an association study, which involves directly analyzing the polymorphism within the gene of interest or the markers in strong linkage disequilibrium with this gene (Risch and Merikangas 1996). E-TDT analysis of 133 atopic trios and 111 atopic asthmatic trios revealed no significant association with either atopy or atopic asthma for any of the simple repeat markers tested. The association of the seven loci with $\log_{10}$ serum IgE levels was tested in 119 out of the 133 atopic trios, because serum IgE levels could not be estimated for 14 probands. Out of these 119 atopic probands, 98 had asthma. A significant association ($F = 3.926$, $P = 0.0008$) was observed with serum $\log_{10}$ IgE levels in the 119 families at locus 5 (Table 2) using the orthogonal association model. In addition, when dominance was taken to be a covariate in the orthogonal model, a significant association was observed at locus 5 ($F = 2.66$, $P = 0.0035$) and locus 6 ($F = 1.86$, $P = 0.0417$). The association at locus 5 was significant in both models after Bonforreni correction for seven tests (the number of markers tested) [$P = 0.0056$ (orthogonal model), $P = 0.0245$ (orthogonal dominance)].

Further, no allelic or genotypic association of the seven loci with atopic asthma was observed in an independent case-control study. However, ANOVA analysis for association with $\log_{10}$ IgE levels showed a significant association of locus 5 alleles in the patient group (Table 3). Using a Tukey–Kramer HSD test, we found a significant difference between the mean of $\log_{10}$ IgE for allele 6 and the mean of $\log_{10}$ IgE for allele 10 ($P < 0.01$). It should be noted that a similar association was not obtained in the control group, so this locus could be associated with IgE levels specifically in asthmatic individuals, or it could be associated with severity of asthma but not with IgE in general. Locus 5 is present 6.2 kb upstream of the *LELP1* gene that encodes a novel small proline-rich protein (SPRR). This gene is uncharacterized as yet, and its exact function is unknown. Still, in order to strengthen our results further, we selected tagged SNPs from the HAPMAP database present in and around the *LELP1* gene and analyzed their associations. None of the two SNPs (i.e., rs11576296 and rs7534334) showed a significant association with asthma in our case-control studies ($P > 0.5$). Nevertheless, rs7534334 (255 bp downstream of the *LELP1*) was found to be significantly associated with $\log_{10}$ IgE levels in the patient cohort (Table 3).

Recently, Palmer et al. (2006) identified an association of two null mutations in the *FLG* gene, present nearly 0.8 Mb upstream of *LELP1*, with atopic dermatitis as well as asthma. The filaggrin protein is directly implicated in atopic dermatitis because of its involvement in skin barrier formation. These mutations were found to be a strong predisposing factor for atopic dermatitis as well as asthma occurring in the context of atopic dermatitis (nearly 50% of the asthmatics had atopic dermatitis). However, no association was observed with asthma in the absence of atopic dermatitis (OR = 0.8). Atopic dermatitis constituted less than 5% of our total study population, so the association that we

Table 2 E-TDT and QTDT analyses at seven loci with atopic asthma ($N = 111$), atopy ($N = 133$) and total serum IgE ($N = 119$) phenotypes

Locus	E-TDT				QTDT			
	Repeat	Alleles	Atopic asthma (P = Allelic, Genotypic)	Atopy (P = Allelic, Genotypic)	Orthogonal		Orthogonal dominance	
					F	P value	F	P value
3.43 Mb (M1)	(CA)n	7	$P = 0.8, 0.7$	$P = 0.7, 0.6$	0.99	0.3990	1.87	0.0818
3.44 Mb (M2)	(CT)m (CA)n	8	$P = 0.47, 0.16$	$P = 0.77, 0.26$	1.52	0.2238	0.43	0.8243
3.5 Mb (M3)	(CA)n	8	$P = 0.59, 0.09$	$P = 0.63, 0.08$	1.39	0.2351	1.57	0.1448
3.53 Mb (M4)	(CA)n	11	$P = 0.56, 0.08$	$P = 0.49, 0.09$	0.34	0.7954	1.06	0.3930
3.65 Mb (M5)	(GT)n	15	$P = 0.16, 0.17$	$P = 0.37, 0.18$	3.93	0.0008*	2.66	0.0035*
3.76 Mb (M6)	(GA)m (GGAA)n	15	$P = 0.74, 0.37$	$P = 0.67, 0.18$	1.14	0.3450	1.86	0.0417*
3.78 Mb (M7)	(GT)n	8	$P = 0.13, 0.19$	$P = 0.28, 0.39$	0.59	0.6239	1.96	0.0778

QTDT analysis was performed using an orthogonal model. Dominance was used as a covariate in the orthogonal dominance model
*Significant P value

Table 3 Allelic/genotypic associations with $\log_{10}$ serum IgE levels assessed using one-way ANOVA

Locus 5 (allele no.) *	Atopic asthmatics (N = 165)					Controls (N = 166)				
	Number of individuals	Mean $\log_{10}$ serum IgE ± SD	DF	F Ratio	P value	Number of individuals	Mean $\log_{10}$ serum IgE ± SD	DF	F Ratio	P value
1	27	2.94 ± 0.54	9	3.4	0.0005	16	2.46 ± 0.64	11	1.27	0.24
3	52	3.03 ± 0.43				64	2.17 ± 0.77			
6	85	2.92 ± 0.48				82	2.32 ± 0.76			
7	8	3.49 ± 0.12				5	2.01 ± 0.49			
8	24	2.82 ± 0.62				18	2.42 ± 0.82			
9	6	2.80 ± 0.79				6	2.15 ± 0.75			
10	57	3.32 ± 0.88				77	2.24 ± 0.63			
11	20	2.81 ± 0.54				22	1.85 ± 0.65			
12	16	2.93 ± 0.48				10	2.21 ± 0.59			
13	9	2.70 ± 0.46				7	1.90 ± 0.86			
LELP1-rs11576296 (G/A)										
GG	38	3.02 ± 0.67	2	0.62	0.53	20	2.53 ± 0.89	2	1.74	0.17
GA	72	3.04 ± 0.78				103	2.18 ± 0.74			
AA	47	3.18 ± 0.74				36	2.21 ± 0.72			
LELP1-rs7534334 (C/T)										
CC	78	2.92 ± 0.59	2	6.08	0.0029	87	2.23 ± 0.83	2	8.2	0.44
CT	53	3.12 ± 0.72				64	2.15 ± 0.73			
TT	22	3.49 ± 0.91				14	2.43 ± 0.52			

*Alleles with counts of less than 5 were excluded from the analysis

obtained is unlikely to be due to the involvement of the *FLG* gene mutations. On the other hand, our findings of the association of a repeat and a SNP in the proximity of the *LELP1* gene with IgE levels could also be related to atopic dermatitis, as IgE levels are elevated in atopic asthma as well as atopic dermatitis (Jones et al. 1975). It would, therefore, be interesting to study this locus, specifically *LELP1* with atopic dermatitis, in the future. In any event, it is very likely that the EDC region on the 1q21 chromosome contains multiple loci such as *FLG* and *LELP1*, which are involved in atopic disorders.

Interestingly, the *SPRR2A* and *SPRR2B* genes from the same SPRR family to which the *LELP1* belongs were observed to be induced in the lungs of allergen-challenged mice via in situ hybridization (Zimmermann et al. 2005). This induction was found to be dependent on IL-13 and STAT-6. Studies in *STAT-6* knock-out mice suggest that it is an essential transcription factor for class switching to IgE (Shimoda et al. 1996). Thus, the expression of the novel small proline-rich protein LELP1 might be regulated by the same STAT-6 protein that regulates the expression of IgE. This could explain the association of *LELP1* with serum IgE levels. However, this could also be due to linkage disequilibrium of the locus 5 marker and/or rs7534334 SNP with genetic variations in some other genes in this vicinity. Nevertheless, our study narrows down the chromosome 1q21 region associated with serum IgE levels, and it should prove helpful for identifying gene(s) associated with atopic disorders.

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