

FAST-TRACK PAPER

Association of atopic dermatitis to the beta subunit of the high affinity immunoglobulin E receptor

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Summary

IgE dysregulation is a major pathogenic feature of atopic dermatitis and other IgE-mediated allergic diseases such as asthma and rhinitis. Allergen complexed to IgE binds to the high affinity receptor for IgE (FcεRI) on the surface of epidermal Langerhans cells, mast cells and basophils, triggering the release of inflammatory mediators. The β subunit of FcεRI has been localized to human chromosome 11q12–13, and variants within this gene have been shown to associate with asthma and measures of atopy. We have tested several polymorphisms within FcεRI-β for association to atopic dermatitis in a panel of 60 families (panel A), recruited through a proband with atopic dermatitis. The findings were tested in a second panel of families (panel B). Significant sharing of maternal alleles was seen for atopic dermatitis and allele 2 of *RsaI* intron 2 (*RsaI_in2*2*) ($P=0.0022$) and allele 1 of *RsaI* exon 7 (*RsaI_ex7*1*) ($P=0.0036$) FcεRI-β gene polymorphisms. These findings were replicated in Panel B, confirming the association of FcεRI-β *RsaI* polymorphisms with atopic dermatitis. The combined significance of the association of atopic dermatitis to *RsaI* polymorphisms was $P=0.0002$ (*RsaI_in2*2*) and $P=0.00034$ (*RsaI_ex7*1*). The polymorphisms also showed association with asthma: $P=0.0068$ (*RsaI_in2*2*) and $P=0.018$ (*RsaI_ex7*1*). Polymorphisms within the FcεRI-β gene are strongly associated with atopic dermatitis.

Atopic dermatitis is a chronic inflammatory skin disease affecting approximately 15% of children in the U.K.¹ It is characterized by intense pruritus, a predilection for the flexural regions, a chronic relapsing course and a personal or family history of atopy. Total serum IgE levels are elevated in 80% of cases and most patients demonstrate positive immediate skin tests or specific IgE titres to common inhalant allergens.² Atopic dermatitis is a complex multifactorial disease sharing many pathogenic features with allergic asthma and rhinitis. Twin studies have identified a substantial genetic contribution to the development of atopic dermatitis, but as with asthma, the mode of transmission does not conform to a simple Mendelian pattern of inheritance.³

In predisposed individuals, contact of the skin with environmental antigen leads to the formation of

allergen–IgE complexes. These complexes bind to high affinity IgE receptors (FcεRI) on the surface of epidermal Langerhans cells, triggering the release of preformed inflammatory mediators.⁴ Upregulation of FcεRI expression has been shown to occur in lesional skin of atopic dermatitis patients, facilitating the uptake and processing of antigen by Langerhans cells and the dermal inflammatory response.⁵

The gene for the β subunit of FcεRI has been localized to chromosome 11q12–13 and is in close genetic linkage to atopy.^{6–9} This locus shows strongest linkage through maternally-derived alleles, consistent with the recognized pattern of maternal inheritance of atopy.^{10–13} Several polymorphisms within the FcεRI-β gene have been shown to associate with skin prick test responses to allergens, bronchial hyper-reactivity and asthma. These include a coding variant E237G within exon 7¹⁴ and three noncoding variants, a *RsaI* polymorphism in intron 2 (*RsaI_in2*),¹⁵ a dinucleotide

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microsatellite repeat in intron 5¹³ and an *RsaI* polymorphism in the untranslated region of exon 7 (*RsaI_ex7*).¹⁶ Other coding polymorphisms, *I181L* and *I181L/V183L* have proved difficult to detect consistently and are of uncertain status.^{17,18}

In order to test the importance of FcεRI-β in the genetic determination of atopic dermatitis, we analysed the association of FcεRI-β gene polymorphisms to atopic dermatitis and asthma in two independent panels of subjects, recruited through probands with active atopic dermatitis. Atopic dermatitis was rigorously defined according to the U.K. working party's set of validated diagnostic criteria.^{19,20} The transmission disequilibrium test (TDT) was used to explore the association of these polymorphisms to asthma and atopic dermatitis and to search for evidence of a maternal effect.

Materials and methods

Sixty nuclear families (Panel A) comprising 277 individuals, age range 1–50 years, were recruited from the dermatology clinics at Great Ormond Street Hospital for Children, NHS Trust (GOSH) through a single proband with active atopic dermatitis. Only those probands over the age of 1 year with at least one sibling were included in the study. Most individuals were Caucasian (90.5%), the remaining individuals being either Asian (1%), African (4%) or of mixed racial origin (4.5%). A questionnaire, which included the diagnostic criteria for atopic dermatitis defined by the U.K. working party,^{19,20} and a set of questions based on the American Thoracic Society's questionnaire for asthma and allergic rhinitis, was completed for each individual. Each family was examined for evidence of atopic dermatitis by the same doctor. A positive diagnosis of atopic dermatitis was made in individuals with a history of pruritus plus three or more of the following criteria: onset of rash under 2 years, history of flexural involvement, a personal history of asthma or hay fever (or respiratory atopy in a first degree relative in children under 4 years), a history of generalized dry skin or visible flexural dermatitis. Severity of atopic dermatitis was assessed using the scoring system of Rajka and Langeland²¹ which categorizes patients into having mild (3.0–4.0), moderate (4.5–7.5) or severe (8.0–9.0) skin disease on the basis of surface area involvement, continuity of disease and nocturnal pruritus.

Atopy was defined as the presence of a positive skin prick test response 3 mm greater than the negative

control, or a positive specific IgE, raised total serum IgE, or any combination of these features.²² Skin prick testing (Dome-Hollister-Stier, Spokane, WA, U.S.A.) to house dust mite (*Dermatophagoides pteronyssinus*), timothy grass (*Phleum pratense*), alternaria (*Alternaria alternata*), cat dander (*Felis domesticus*), egg white and cow's milk was carried out on all individuals. Total and specific IgE to the same panel of allergens were measured by a fluorescent enzyme immunoassay (Pharmacia CAP system, Pharmacia, Uppsala, Sweden). A specific ELISA class I result (>0.35 kU/L) was considered positive. A raised total serum IgE level was taken to be greater than 100 kU/L in adults (mean + 2 SD) or greater than 1 SD above the geometric mean of the published normal values for children according to their age. Automated assays of eosinophil and white cell counts were conducted. DNA was isolated from peripheral blood leucocytes by standard phenol–chloroform extraction followed by ethanol precipitation.

All positive results were tested in a second independent panel of families (Panel B) of similar racial origin (Caucasian 87%, Asian 6%, African 5%, Oriental 2%). These families were recruited in a previous study, on the basis of at least two first degree relatives with active atopic dermatitis, from the outpatient clinics at GOSH.²³ The first affected child from each of these pedigrees was chosen for TDT analysis. DNA was available for analysis from 88 of these families (402 individuals).

Genotype analysis

For detection of the *RsaI* polymorphisms within intron 2 and the untranslated region of exon 7 of FcεRI-β, a specific polymerase chain reaction (PCR) was carried out followed by restriction digestion of the PCR products. The amplification refractory mutation system was used to test for the presence of the E237G variant within exon 7 of the FcεRI-β gene.

For *RsaI_in2*, amplification of genomic DNA (100 ng) was performed in a thermal cycler (Perkin Elmer Cetus, Warrington, U.K.) using 35 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C for 1 min. The reaction mix contained 0.3 μmol/L of each primer, 200 μmol/L dNTPs, 0.1% (v/v) NP40, 0.1% (w/v) gelatin, 0.1 mol/L KCl, 0.1 mol/L Tris-HCl pH 8.8, 2 mmol/L MgCl₂ and 0.75 units of *Taq* DNA polymerase in a final volume of 15 μL. Primers were 5' CAG AAT GTT CTC ATG ACT GAA TTG 3' and 5' CAA GTA CAG AGC AGA CAA CTG 3'.

For *RsaI_ex7*, amplification of genomic DNA (200 ng) was performed in a Hybaid Omnigene (Teddington,

Middlesex, U.K.) thermal cycler (block control) using 32 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min. The PCR reaction mix contained 0.2 µmol/L of each primer, 200 µmol/L dNTPs, 67 mmol/L Tris-HCl pH 8.8, 16 mmol/L (NH₄)₂SO₄, 0.01% Tween-20, 1.5 mmol/L MgCl₂ and 0.75 units *Taq* DNA polymerase in a final volume of 15 µL. Primers were 5' TCA CTG TGT ATC ATG CTA AGC 3' and 5' TGA TAC AAT ACT GCA TCG TGG 3'.

Five microlitres of amplification products were digested with 4 units of *RsaI* enzyme (New England Biolabs, Hitchin, Herts, U.K.), together with the recommended buffer, in a final volume of 10 µL, for 1 h at 37 °C. Digested products were resolved on a 2% (w/v) agarose gel containing 1 µg/mL ethidium bromide, run for 60 min at 70 V in a BioRad (Hemel Hempstead, Herts, U.K.) wide subcell. Restriction digestion of the intron 2 PCR product yielded three genotypes with an additional constant 148 bp band being present as a result of the primers used. The 1,1 genotype was denoted by the presence of an 800 bp and 148 bp band pattern, the 2,2 genotype by the presence of a 500 bp, 280 bp and 148 bp band pattern and the 1,2 genotype by an 800 bp, 500 bp, 280 bp and 148 bp band pattern. Digestion of the exon 7 polymorphism potentially gave rise to three genotypes: a single 481 bp band denoted the 1,1 genotype, a 481 bp, 295 bp and 187 bp band pattern denoted the 1,2 genotype and a 295 bp and 187 bp band pattern denoted the 2,2 genotype.

For the E237G variant in exon 7, the following primers were used to test for the presence of the E237G polymorphism at nucleotide residue 7297 of the published sequence of the *FcεRI-β* gene:¹⁴ B7FA1, TGG CCA GCT AGT CTG GTT TGG TTT TCT GGA (30mer); B7FA2, GGA GCA TAT TAA GGT GGA CAG AAG CAG CAG (30mer); B7M1, ATT CAG CTA CTT ACA GTG AGT TGG AAG ACC CAG GCG G (37mer) and B7W2, AC GTG ATT CTT ATA AAT CAA TGG GAG GAG ACA ATT (36mer). A total reaction volume of 50 µL containing 1 µmol/L of each of the primers B7FA1, B7FA2 and B7W2 and 0.5 µmol/L of the primer B7M1, × 10 KCl buffer (1% (v/v) NP40, 1% (w/v) gelatin, 1 mol/L KCl, 1 mol/L Tris-HCl pH 8.3), 2 mmol/L MgCl₂, 200 µmol/L dNTPs, and 50 ng of genomic DNA was used. A hot start PCR was done with the addition of 2 units of *Taq* DNA polymerase (Boehringer Mannheim, Lewes, U.K.) after an initial 5 min denaturation at 95 °C. PCR conditions then followed 35 cycles of 94 °C for 1 min, 62 °C for 2 min and 72 °C for 2 min followed by a single cycle of 72 °C for 10 min. Amplified products were

then visualized in an ethidium-bromide stained 4% (w/v) agarose gel (3: 1 NuSieve: LMP agarose) and run for 120 min at 60 V in a BioRad wide subcell. The primers B7FA1-B7FA2 gave a 446 bp control band, B7FA1-B7W2 a 280 bp band in the presence of adenine at nucleotide position 7297 of the published sequence and B7M1-B7FA2 a 238 bp band in the presence of guanine at position 7297.

Statistical analysis

The TDT implemented in the GAS program (version 2.1) (A.Young, unpublished), was used to test for transmission of alleles at a particular locus from heterozygous parents to their affected offspring. The proportion of alleles transmitted from both mother and father was tested against a null hypothesis of 50% transmission by χ^2 analysis.

Results

The mean age of probands in Panels A and B was 6.4 and 8.1 years, respectively. Ninety-three per cent of probands in panel A were atopic and 63% asthmatic. The majority had moderate to severe skin disease with a mean severity score of 7.6 and mean geometric serum IgE of 1224.1 kU/L. Atopy and a history of asthma were present in 96 and 57%, respectively, of probands in Panel B. The mean severity score was 6.1 and mean geometric serum IgE 566.8 kU/L.

Genetic analysis

In Panel A subjects, transmission of allele 2 of the *RsaI* intron 2 polymorphism (*RsaI_in2*2*) ($P=0.0022$) and allele 1 of the *RsaI* exon 7 polymorphism (*RsaI_ex7*1*) ($P=0.0036$) to offspring with atopic dermatitis occurred significantly more frequently than expected by chance (Table 1). The difference between the proportion sharing maternal vs. paternal alleles was highly significant: $P=0.0019$, $\chi^2=9.66$ (*RsaI_in2*2*) and $P=0.0031$, $\chi^2=8.78$ (*RsaI_ex7*1*). No significant association of these polymorphisms was found with asthma.

The association of the two *RsaI* polymorphisms with atopic dermatitis was confirmed in Panel B families, in which an excess sharing of maternally-derived alleles for both *RsaI_in2*2* ($P=0.018$) and *RsaI_ex7*1* ($P=0.02$) was found. Furthermore, an association with allergic asthma was present in maternally derived alleles: *RsaI_in2*2* (x transmitted:y not transmitted, 15:6, $P=0.039$) and *RsaI_ex7*1* (14:6, $P=0.058$).

Table 1. Transmission disequilibrium test: sharing of *RsaI* alleles with atopic dermatitis

Panel	<i>RsaI</i> polymorphism	Allele	Paternal			Maternal		
			Transmitted	Not transmitted	<i>P</i>	Transmitted	Not transmitted	<i>P</i>
A	<i>in2</i>	1	17	10	0.12	3	16	NS
		2	10	17	0.88	16	3	0.0022
A	<i>ex7</i>	1	9	16	0.89	17	4	0.0036
		2	16	9	0.11	4	17	NS
B	<i>in2</i>	1	24	17	0.17	10	23	NS
		2	17	24	0.83	23	10	0.018
B	<i>ex7</i>	1	18	21	0.63	24	11	0.02
		2	21	18	0.37	11	24	NS

NS, not significant.

Combining the data from panels A and B significantly strengthened the associations with atopic dermatitis and asthma. Significance was strongest when examining the TDT sharing of maternal *RsaI* alleles with atopic dermatitis (Table 2). The significance of transmission of *RsaI_in2*2* was $P=0.0002$ and, for *RsaI_ex7*1*, $P=0.00034$. Transmission of these alleles was also significantly associated with asthma when maternally-inherited: *RsaI_in2*2* ($P=0.0068$) and *RsaI_ex7*1* ($P=0.018$) (Table 3).

The E237G coding variant was present in 6.5% of subjects from Panel A, but failed to associate significantly with atopic dermatitis ($P=0.38$) or asthma ($P=0.5$). For this reason, the polymorphism was not examined in Panel B subjects.

Discussion

This study has shown significant association of atopic dermatitis to two non-coding *RsaI* polymorphisms within the FcεRI-β gene. The association was demonstrated only in maternally-derived alleles. We were able

to replicate these findings in a second independent panel of families and to show significant association of the polymorphisms to allergic asthma.

Strict adherence to a set of six diagnostic criteria, as defined by the U.K. working party,^{19,20} ensured an accurate diagnosis of atopic dermatitis. Children were selected as probands in view of the high rate of spontaneous remission of atopic dermatitis with age. The exclusion of infants under 1 year of age reduced the problems in differentiating infantile seborrhoeic dermatitis from atopic dermatitis.

The positive results of this genetic association study are in contrast to the results of a previous linkage study in Panel B subjects, which failed either to detect or exclude linkage of atopic dermatitis to markers within the 11q12–13 region.²³ This is probably because the markers tested were situated telomeric to the FcεRI-β gene. Furthermore, tests of association are more powerful than linkage studies in detecting genetic effects in complex disorders.²⁴

The transmission of FcεRI-β alleles to offspring with atopic dermatitis was almost exclusively through the

Table 2. Transmission disequilibrium test: sharing of *RsaI* alleles with atopic dermatitis in combined panels A and B

<i>RsaI</i> polymorphism	Allele	Paternal			Maternal		
		Transmitted	Not transmitted	<i>P</i>	Transmitted	Not transmitted	<i>P</i>
<i>in2</i>	1	41	27	0.057	13	39	NS
	2	27	41	0.94	39	13	0.0002
<i>ex7</i>	1	27	37	0.87	41	15	0.00034
	2	37	27	0.13	15	41	NS

NS, not significant.

Table 3. Transmission disequilibrium test: sharing of *RsaI* alleles with asthma in combined panels A and B

<i>RsaI</i> poly-morphism	Allele	Paternal			Maternal		
		Transmitted	Not transmitted	<i>P</i>	Transmitted	Not transmitted	<i>P</i>
<i>in2</i>	1	25	18	0.18	9	24	NS
	2	18	25	0.82	24	9	0.0068
<i>ex7</i>	1	16	22	0.79	23	10	0.018
	2	22	16	0.21	10	23	NS

NS, not significant.

maternal line. This is in agreement with a number of studies showing strong maternal linkage of FcεRI-β and other loci to measures of atopy and asthma.^{10–13} Foetal–maternal interactions are favoured as a cause for this phenomenon, with maternal modification of the infant's developing IgE response occurring through the placenta or breast milk; however, genetic mechanisms such as genomic imprinting remain a possibility.¹²

FcεRI-β polymorphisms have been associated previously with asthma and severe atopy.^{6–9} In a study of atopic Japanese adults, the *RsaI*_{*in2*} polymorphism was shown to associate significantly with atopic asthma, rhinitis and eczema.¹⁵ The findings of our study suggest that the combination of severe atopic dermatitis and asthma may form a distinct clinical subtype of atopic disease, attributable to FcεRI-β. However, the FcεRI-β E237G coding variant was found not to be associated with atopic dermatitis. The functional polymorphism in FcεRI-β responsible for atopic dermatitis is therefore still to be identified.

FcεRI is a multimeric receptor expressed either as a αγ₂ trimer or αγ₂β tetramer.²⁵ Functionally, the β subunit has been shown to act as a signal amplifier, providing a gain of five to seven-fold.²⁶ Increases in β amplifier gain could be influenced by variants within the FcεRI-β gene, thus potentiating allergic responsiveness. Alternatively, FcεRI-β variants may increase the proportion of αγ₂β receptors expressed, thereby increasing the strength of the FcεRI-mediated signal.

Further studies of the FcεRI-β locus and the identification of other relevant genes will hopefully lead to an improved understanding of the genetic basis of atopic dermatitis and its relationship to asthma.

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