Association of the FcεRIβ gene with bronchial hyper-responsiveness in an Italian population

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Abstract

A study of two DNA polymorphisms (i2 *RsaI*, E237G) in the gene for the beta subunit of the IgE high affinity receptor (FcεRIβ) was performed in 168 Italian families with atopic asthmatic children. The prevalence of the E237G allele in the Italian population was 4%, so this polymorphism was unsuitable for this study. The i2 *RsaI* polymorphism minor allele frequency was 44%, and it had a PIC value of 0.37. Linkage analysis indicated a significant allele sharing in affected sib pairs for bronchial hyper-responsiveness (BHR, $p=0.048$), but not for allergic asthma. These data indicate an association of bronchial hyper-responsiveness with the FcεRIβ gene.

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Bronchial asthma is an important medical problem in the paediatric population. In many cases, childhood asthma is associated with atopy, a familial IgE mediated syndrome, which underlies asthma, allergic rhinitis, and eczema. Moreover, asthma is characterised by increased bronchial responsiveness to methacholine (BHR), which can be quantified by challenge testing.¹

Previous studies have found linkage of atopy and BHR to markers on chromosome 11q13.^{2,3} The beta subunit of the high affinity receptor for IgE (FcεRIβ) has been identified as a candidate gene for this linkage,⁴ suggesting the presence of a major gene influencing IgE levels and atopy. Recent studies were performed with the use of two new polymorphisms of the FcεRIβ gene. The *RsaI* polymorphism in intron 2 was associated with atopic clinical disorders, including eczema, allergic asthma, and allergic rhinitis in a Japanese population⁵; no association was found for intrinsic asthma, which manifests as typical bronchial hyper-responsiveness, but is not atopic in origin. The statistical association with atopic diseases was not confirmed in another study in another Japanese population sample, in which intrinsic asthma or bronchial hyper-responsiveness were not assessed.⁶ Another FcεRIβ gene polymorphism, Gly237Glu, located in exon 7, was associated with bronchial hyper-reactivity and atopy in an Australian population.⁷ The E237G polymorphism was also associated with atopic asthma in a Japanese population.⁸

We wished to reproduce these studies in an Italian population, in which our previous stud-

ies had not shown a significant association between two microsatellite markers of the gene and atopic asthma in 45 families; in that study, BHR was not considered. The BHR data from those families are included in the present analysis.

A panel of 168 families (659 subjects) from the Veneto region was recruited from atopic asthmatic children attending the Allergy and Pulmonology Clinic of the Department of Paediatrics of the University of Verona.⁹ All the subjects were tested for clinical history, total serum IgE level, skin prick test (SPT), and BHR. Asthma was defined according to the American Thoracic Society standard. Affected subjects consisted of atopic patients, defined as total serum IgE > 100 kU/l or a positive skin prick test to one or more common airborne allergens or both (391 subjects), and patients with bronchial hyper-reactivity to methacholine, defined as PC 20 < 25 mg/ml (248 subjects). These two groups were partly overlapping, so some subjects (220) were included in both groups. Healthy controls (50 subjects) were also screened for total serum IgE, SPT, and BHR.

We investigated the i2 *RsaI* polymorphism of the FcεRIβ gene in all the subjects; the E237G

Figure 1 Analysis of the i2 *RsaI* FcεRIβ gene polymorphism in families 29 and 69. Top: pedigrees. Bottom: silver stained polyacrylamide gel showing PCR products after restriction and electrophoresis. Filled symbols denote affected family members. One homozygote ++ is shown in lane 4 and two homozygotes -- in lanes 5 and 6; the other subjects are heterozygotes.

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Table 1 Sib pair analysis for *FcεRIβ* gene and BHR, atopy, skin prick test, and proportion of intron 2 polymorphism alleles shared

Trait	Pairs	Mean	SD	Z value	p value	SimIBD p value
BHR	57	0.5457	0.2038	1.6926	0.048	0.021
Atopy	137	0.5263	0.2159	1.4236	0.078	0.044
SPT	117	0.5186	0.2173	0.9264	0.178	0.014

Data obtained with SAGE SIBPAL 2.7.4 (columns 3 to 6) or SimIBD on 10 000 replicates (column 7).

polymorphism was tested in 286 subjects from 81 out of 168 families. We examined the data using the affected sib pair method SIBPAL to detect genetic linkage using the SAGE package. A non-parametric simulation based statistic (SimIBD) was also used.¹⁰

DNA was extracted from blood samples using standard methods.

The E237G polymorphism was tested as described previously⁷ with an ARMS-PCR (amplification refractory mutation system) technique. The E237G polymorphism was not sufficiently informative (G 4%, PIC=0.07) to be of use in the analyses. This is a lower frequency for the Gly237 allele compared to that described in the Australian population⁷ and in the Japanese population,⁸ 5.3% and 6%, respectively.

PCR for the i2 *RsaI* polymorphism was performed as described previously⁹ with the following primers, which we have designed on the published gene sequence¹¹: *FcεRIβ*/3 5'-CGAGAATGTTGCAGGGAGTTA-3' and *FcεRIβ*/4 5'-TAGCAGTCAGAATTTGTGTTACC-3'. The products were incubated with restriction enzyme *RsaI*. Two alleles were detected, - and +, corresponding to fragments of 730 bp, and two smaller fragments, as shown in fig 1 for two families. The frequency of the + allele was 44% (PIC=0.371). This is very different from the frequency of 82% obtained in the Japanese population^{7,8} and it gives better informativeness for the marker in linkage analyses in the Italian population.

The results of linkage analysis between the i2 *RsaI* polymorphism and BHR, atopy, or SPT is shown in table 1. The data indicate a significant parental allele sharing in sib pairs with BHR (p=0.048). The simulation based identity by descent (SimIBD) statistic with 10 000 replicates confirms this significant sib pair result for BHR (p=0.021) and also extends it to atopy (p=0.044) and skin prick test (p=0.014). No positive TDT was found between the polymorphism and any of the categorical phenotypes. These results are in partial agreement with data obtained in the Japanese population, as reported above.^{5,6}

This study is an extension of our recent report on the Italian population, in which two

microsatellite markers, 319 [CA]_n and *FcεRIβ* i5 [CA]_n, located in front of the gene and in intron 5 of the gene, respectively, were analysed.⁹ The order of the four markers is: 319[CA]_n - i2 *RsaI* - i5[CA]_n - E237G. Twenty-six four marker haplotypes were observed out of 140 possible haplotypes and 10 out of 28 three marker haplotypes in all the families described previously⁹ and here. Significant i2 *RsaI* - i5[CA]_n - E237G marker haplotype associations were observed with BHR, atopy, and SPT in 46, 126, and 101 informative affected sib pairs, respectively (SAGE, p=0.0289, 0.0127, and 0.0432, respectively). No particular haplotype was significantly associated with any phenotype (extended TDT¹²). For marker 319[CA]_n, three recombinants with the above cited intragenic three marker haplotype out of 250 informative meioses were observed (θ=1.2%). These data indicate an association of BHR, and perhaps also of atopic asthma and skin prick test, with the *FcεRIβ* gene.

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