Allelic association of gene markers on chromosome 11q in Italian families with atopy

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In our study, the genetic linkage of the Fce RIβ gene with atopy in 77 affected sibling pairs recruited from an Italian panel of 201 subjects has been examined. Atopy was defined by the presence of a positive skin prick test to one or more common aeroallergens, a positive RAST test to one or more common aeroallergens and an elevated circulating total IgE. Genotype analysis was performed by PCR amplification of Fce RIβ CA and C111-319 CA microsatellites. All the family members were also tested for the Ile 181 mutation with the ARMS method and for Leu181/Leu183 polymorphism. Seventy-two point five percent (72.5%) of the affected sibling pairs shared their maternal allele and 27.5% did not. Therefore, an increased maternal allele sharing was observed: $\chi^2 = 8.10, p < 0.01$. Comparing paternal versus maternal allele sharing, a significant difference was observed for the C111-319 CA marker ($\chi^2 = 4.32, p < 0.05$). Atopy phenotype with positive skin prick test, RASTs, and high total serum IgE also showed greater sharing of maternal than paternal alleles in affected sibling pairs. Of the 201 subjects studied, 17 (8.4%) were positive for Leu181. Ten of these were children and seven (70%) had inherited the variant maternally. The seven children had maternally inherited Leu181/Leu183 and were atopic. Within this sample the maternal inheritance of Fce RIβ Leu181/Leu183 was associated with an increased risk of IgE responses to common allergens, raised eosinophil counts and increased skin prick test reactions. Therefore, the variant identified a genetic risk factor for atopy.

Conclusion: The central role of Fce RIβ in atopy and the linkage data presented here point to the possibility that genetic variation in Fce RIβ or its controlling element may cause differences in the extent of IgE responses between atopic and non-atopic subjects. A search for such mutations or polymorphisms will need to take into account some carriers of atopy among the normal population and genetic heterogeneity among atopic individuals.

Key words: Amplification refractory mutation system, atopy, chromosome 11q13, Fce RIβ gene

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of atopic children with the best available microsatellite DNA markers, to establish the prevalence of Fce RIβ Leu181 mutation and Leu181/Leu183 polymorphisms on chromosome 11q13 and to examine whether maternally inherited alleles confer a risk of atopy.

Subjects and methods

Patient recruitment
A panel of 201 subjects (100M, 101F) was studied. This panel consisted of 30 families from the region of Sicily (Southern Italy) recruited from among atopic children attending the Allergy and Immunology Clinic of the Department of Paediatrics of the University of Messina. The study was approved by the committee for ethics in medical research and was therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All the subjects gave their informed consent prior to their inclusion in the study.

In each family at least two atopic subjects were present, and the total number of children in each family ranged from two to four. Median age of the children was 13 y (range 6–35 y), and median age of parents was 40 y (range 33–57 y).

Phenotype analysis
A respiratory questionnaire based on the American Thoracic Society questionnaire but including questions on rhinitis and allergies was given (17).

Atopy was defined by the presence of one or more of the three following criteria (18, 19): (i) positive skin prick test (3 mm > negative control) (20) to one or more common aeroallergens; (ii) positive RAST test (>0.35 RU/ml) to one or more common aeroallergens; (iii) elevated circulating total IgE (>100 KUL in adults) >1 SD above an age-related geometric mean in children.

Skin prick tests were performed for Dermatophagoides pteronyssinus and farinae antigens, common types of grass, moulds and cat and dog epithelia with commercial extracts (Bayrofarm, Milano, Italy). Specific and total IgE were assayed by an enzyme-linked immuno sorbent test (ELISA-PRIST, RAST, Pharmacia, Uppsala, Sweden).

Genotype analysis
Genotype analysis was performed on genomic DNA extracted from whole blood. Two microsatellite DNA markers were used (9). PCR amplification parameters of Fce RIβ CA microsatellite were: 95°C for 5 min, 30 cycles at 95°C for 30 s, 55°C for 45 s and 72°C for 45 s, followed by a final step of 7 min at 72°C. The microsatellite C111-319 CA PCR conditions were 95°C for 5 min, 32 cycles at 95°C for 45 s, 55°C for 30 s and 72°C for 45 s, then 7 min at 72°C. The alleles were visualized on 8% non-denaturing polyacrilamide gels by ethidium fluorescence, and they were identified by the migration rate relative to co-electrophoresed standard samples.

Sib-pair analysis
Allele numbers from pedigrees were counted, treating each sibship in extended pedigrees as independent (21). The significance was calculated on the assumption that the expected proportion of sibling pairs sharing or not sharing an allele from the specified parent was 0.5. Sibling pairs were included in the analysis only if the two alleles from the specified parent could be individually recognized. χ² estimation was used to test the significance of differences between sharing of maternal and paternal alleles.

Non-parametric linkage and association was performed with the Non-parametric Analysis Of Linkage and Association (NOPAR) program, which calculated a z-score for each locus and allele. The nominal significance level takes z as a normal deviate, corresponding to large samples of independent observations. Reliability of this result was confirmed by 10000 simulations of the sample, altering genotypes of children and quantitative scores.

Mutation analysis
The Fce RIβ Leu181 mutation was detected by the amplification refractory mutation system PCR (ARMS) (22). The ARMS assay was carried out with the following oligonucleotide primers (a) 5A-TGT ATG TGT CAC TTT AAA AAG ACT GGT CAG; (b) 5B- TTT TCA TTT GTT GCT GTT CAA TAG GAA GTT; (c) 3W-AAT GGT GAG AAA CAG CAT CAT CAT TAC CAA; (d) 3K-TAA CAT ATC AGT CCT ATT ATC CCA ACC CTC. Genomic DNA samples (0.40 µg) were amplified in a total volume of 50 µl containing 0.5 µM oligonucleotide primers 5A, 3K and 5B; 0.1 µM 3W; 200 µM dNTPs; 5x reaction buffer (43 mM potassium chloride, 8.6 mM Tris-hydrochloric acid (pH 8.3), 2.5 mM magnesium chloride, 0.008% gelatin); and 1 unit DNA Taq polymerase Gold (Perkin Elmer, Milano, Italy).

The reaction mixture with enzyme was heated to 95°C for 14 min in a thermal cycler (Perkin Elmer 2400). The reaction conditions were then followed by 30 cycles at 95°C for 1 min, 55°C for 1 min and 72°C for 2 min; 6 cycles at 95°C for 45 s, 55°C for 1 min and 72°C for 45 s; and 1 cycle at 72°C for 7 min. Amplified products were electrophoresed in a 3% agarose gel (2.25 g Seakem agarose + 0.75 g NuSieve GTG-FMC per 100 ml TBE 1x) before ethidium fluorescence analysis. Three bands can result from the primer combinations: 5A/3K gave a 459 bp control band, 5B/3K gave a 353 bp band in the presence of the “wild type” Leu181 and 3W/5A gave a 163 bp band in the presence of Leu181 mutation.

A member of each family segregating Leu181 mutation was sequenced by the method of Sanger et
Table I. Distribution of atopic members in the families studied.

<table>
<thead>
<tr>
<th>Members affected</th>
<th>Families (n)</th>
<th>Subjects (n)</th>
<th>Atopics (total n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother, father, at least two children</td>
<td>6</td>
<td>44</td>
<td>37</td>
</tr>
<tr>
<td>Mother, father, one child</td>
<td>4</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>Mother, at least two children</td>
<td>8</td>
<td>44</td>
<td>32</td>
</tr>
<tr>
<td>Father, at least two children</td>
<td>6</td>
<td>42</td>
<td>28</td>
</tr>
<tr>
<td>Father, one child</td>
<td>3</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Two or more children, no parents affected</td>
<td>3</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>201</td>
<td>140</td>
</tr>
</tbody>
</table>

Results

We studied 201 subjects belonging to 30 families from the region of Sicily in Southern Italy, ascertained through atopic children: 140 of them were found to be atopic. The family composition is summarized in Table 1. In 10 families (33.4%), both parents were atopic, in 17 (56.6%) 1 parent was atopic and in 3 (10%) neither parent was atopic.

All subjects were typed with two microsatellites: Fce RIβ CA, located inside the gene and C111-319 CA, linked closely to it. At the Fce RIβ CA locus, six alleles were detected. At C111-319 CA locus, five alleles were detected.

With these markers, sib-pair analysis was performed in all the families. Affected sib-pair analysis was performed because it is independent of assumptions about penetrance, mode of inheritance and gene frequency. Seventy-seven genetically informative sibling pairs were examined. It was found that a total of 29 (72.5%) affected sibling pairs shared their maternal allele and 11 (27.5%) did not, while 26 (48.0%) shared their paternal allele and 38 (52.0%) did not. (These numbers are not the sum of the data reported for the two markers separately because some pairs were informative with both markers, and they were, therefore, counted once only.) These numbers are significantly different from the expected 50/50 distribution, because an increased maternal allele sharing was observed ($\chi^2 = 8.10$, $p < 0.01$) (Table 2). Moreover, comparing paternal versus maternal allele sharing, a significant difference was observed for the C111-319 CA marker (20 vs. 30 paternal sharing, 20 vs. 10 maternal not sharing, $\chi^2 = 4.32, p < 0.05$).

By contrast, there was no significant sharing of alleles in unaffected sibling pairs in the same families: 11 shares the maternal allele and 12 did not, while 10 shared the paternal allele and 13 did not.

No difference was found between the sibling pairs sharing and not sharing the paternal allele. By contrast, there was a significant difference ($p < 0.05$) between the subjects sharing the maternal allele and those not sharing (Table 3).

Non-parametric tests revealed no evidence for linkage for atopy to the two markers examined in this study.

All the family members were also tested for the Ile181Leu mutation with the ARMS method. An example of the results is given in Figure 1. Of the 201 subjects studied, 17 (8.4%) were positive for Leu181. Ten of these were children and seven of said figure (70%) had inherited the variant maternally, while three (30%) had inherited it paternally. A member of each family segregating Leu181 was sequenced by the method of Sanger et al. (23) to ensure accuracy of the PCR and to determine whether Leu183 was present.

Table 2. Sharing of alleles at the Fce RIβ locus in sib-pair with atopy.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Paternal allele, pairs (n)</th>
<th>Maternal allele, pairs (n)</th>
<th>$\chi^2$ Paternal vs. maternal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sharing (%)</td>
<td>Not sharing (%)</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>Fce RIβCA</td>
<td>17 (48.5)</td>
<td>13 (51.5)</td>
<td>0.187</td>
</tr>
<tr>
<td>C111-319CA</td>
<td>20 (50)</td>
<td>20 (50)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>26 (48)</td>
<td>38 (52)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* $\chi^2$ were calculated from expected frequencies of 50% for an allele shared and 50% for an allele not shared from the specified parents. The first two lines report informative pairs in which the parental allele could be identified at the two loci, respectively. The third line gives the total number of informative pairs. Please note that the total number of scored parental alleles is not the sum of the scored maternal and paternal alleles, as some pairs were informative with both markers, and they are therefore reported only once in the bottom line.

* $p = \text{ns}$; ** $p < 0.01$; *** $p < 0.05$. 

al. (23) to ensure accuracy of the PCR and to determine whether Leu183 was present.
results to house dust mite and grass pollen. The eosinophil counts in the seven children with the maternal variant were significantly raised. The three children who had inherited Leu181/Leu183 paternally were, by contrast, non-atopic, with negative skin prick and radioallergosorbent test results. Of the seven parents with Leu181/Leu183, two had negative skin prick and radioallergosorbent test results to house dust mite and grass pollen, whereas five were positive in both skin prick and radioallergosorbent tests to one or more allergens.

Discussion

The study of a complex disorder that is dependent for expression on both genetic and environmental factors involves many problems of definition and study design (7, 24). There are two different approaches to the study of the genetics of atopy. The first one is that of considering the disease phenotype in general; the second one is that of evaluating specific components correlated with the disorder and more likely to be controlled by a few genes. As previously published (18, 19), in our study atopy has been defined by the presence of a positive skin prick test to one or more common aeroallergens, a positive RAST test to one or more aeroallergens and an elevated circulating total IgE.

It is difficult to assign disease status because there are several factors that influence the variability of penetrance, such as age, sex and environment (25). The number of persons with atopy rises to a peak in the early teens, and then decreases after 30 y of age (26). Various studies indicate clearly that atopic disorder is due to the interaction of genetic and environmental factors, but they do not indicate how many factors there are for each phenotypic category of atopy and how interactive these may be. The outbred nature of man and the range of his culture and environment indicate that heterogeneity is most likely to exist, in which the factors underlying the phenotype in different individuals will differ significantly. All of these problems have to be considered, and they are certainly responsible for the discordant suggestions on the inheritance of atopy as a recessive, dominant, codominant or polygenic (25) disease. These controversial findings suggest that this phenotype has multifactorial causes and that it is probably genetically heterogeneous, as several genes located in different positions in the genome may determine a clinically similar phenotype.

Excellent candidate genes exist in regions that were identified in one or more linkage studies (18, 19, 27, 28). These candidates regulate either the immune response, such as IgE induction, or smooth muscle activity. Modification in the expression of these genes could significantly alter the IgE production pathway and lead to atopic phenotypes. Studies of the genetics of atopy and IgE production agree that a major locus contributes to the state, although both dominant and recessive patterns of inheritance have been proposed (29, 30). The gene is located on chromosome 11q13, in which the β subunit of the high-affinity receptor for IgE (Fce-RIβ) lies. The known roles of Fce-RI in antigen-induced mast-cell degranulation and in the release of cytokines that enhance IgE production make the gene for its β subunit a candidate for the chromosome 11 atopy locus. Studies by Cookson and co-workers (18, 19) have suggested evidence for linkage to markers located in this part of the genome. But there are also investigators who have been unable to replicate these findings (12–14).

In our study, a sib-pair analysis was performed by two microsatellites: Fce R1β CA, located inside the gene on chromosome 11q13, and CI11-319 CA, linked closely to it. The difference between the proportion

### Table 3. Sibling pairs sharing paternal or maternal chromosomes.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Paternal allele, pairs (n)</th>
<th>Maternal allele, pairs (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sharing</td>
<td>Not sharing</td>
</tr>
<tr>
<td>Atopy (any criterion)</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>Skin test (any antigen &gt;3mm)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Rast positive ≥ 2</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>High total serum IgE</td>
<td>35</td>
<td>33</td>
</tr>
</tbody>
</table>

* p = ns; ** p < 0.05.
sharing paternally derived alleles and that sharing maternally derived alleles was significant (p < 0.05). In particular, comparing paternal versus maternal allele sharing, a significant difference was observed for the C111-319 CA marker (p < 0.05). By contrast, there was no significant sharing of alleles in unaffected sibling pairs in the same families.

When we defined atopy by the presence of any one of the three criteria previously described, a significant difference of the affected sibling pairs sharing their maternal allele from the expected 50/50 distribution was found. Thus, detection of transmission of atopy was through the maternal line. There was no excess of females or males in our sample and the proportion of males and females affected with atopy were similar. These findings, especially since they include all subjects genotyped by us, confirmed that our finding of linkage in chromosome 11q was not an artefact of phenotype definition, and that neither linkage nor its significance depended on the specific parameters applied to the linkage test.

Sequencing of Fce RIβ has recently detected two variants in addition to the more usual wild type: these are known as Leu181/Leu183 and Leu181 and are characterized by three nucleotide substitutions in the sixth exon, resulting in the Ile181Leu and Val183Leu substitutions within the fourth transmembrane domain. Leu181 has been detected in 10 of 60 (17%) of English families ascertained through an asthmatic proband (19), and Leu181/Leu183, originally identified on the chromosome conferring atopy in a family, has been found in 4–5% of an Australian population sample. Maternal inheritance of both these variants was associated with severe atopy in double-blind studies. These findings are not confirmed by other studies (14) and by research performed in a population of Northern Italy (16).

This is the first study that shows the frequency of Leu181 mutation and Leu181/Leu183 polymorphism in a population of Southern Italy. In our study, all the family members were tested for the Ile181Leu mutation with the ARMS method. Of the 201 subjects studied, 17 (8.4%) were positive for Leu181. Ten of these were children; seven (70%) had inherited the variant maternally. Moreover, the seven children who had maternally inherited Leu181/Leu183 were atopic. Within this sample the maternal inheritance of Fce RIβ Leu181/Leu183 was associated with an increased risk of IgE responses to common allergens, raised eosinophil counts and increased skin prick test reactions. Therefore the variant identified a genetic risk factor for atopy.

It is likely that other common polymorphisms in and around Fce RIβ exist and that haplotypes of these may better define genetic risk due to Fce RIβ.

However, the Fce RIβ polymorphisms cannot alone explain the genetics of atopy. The TCR-α region on chromosome 14 (35) and the interleukin-4 cluster on chromosome 5 (27) also show linkage to IgE responses. Prospective testing for Fce RIβ polymorphisms and examination of the effects of other genes and the environment are required before genotyping at the Fce RIβ locus can be used clinically for genetic risk estimation.

References

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