Atopy in Australia

Sir — Shirakawa *et al.*[1] reported in *Nature Genetics* last year a strong association between atopy and mutations in the sixth exon of the FcεRI β-subunit gene, notably Ile181Leu. This finding has been replicated in a population sample from Busselton, Western Australia, where the frequency of Leu181 was 2.7% (28 carriers in 1020 individuals)[2]. We have typed 939 subjects for this mutation as part of an Australia wide twin-family study of asthma. The sample comprised 610 twins (one member of each monozygotic pair and both members of each dizygotic pair) ascertained for a history of wheeze or asthma, 198 of their parents, and 131 unrelated controls (of unknown asthma status) recruited from our laboratory staff, spouses and blood donors. The twins in the sample underwent a histamine inhalation challenge, epicutaneous skin testing for 11 common aeroallergens, and measurement of total serum Immunoglobulin E (IMx Total IgE assay, Abbott Laboratories, USA).

We used a modification of the ARMS assay[1] (details may be obtained from the authors). Each run was accompanied by a successful positive control run, and the wild type allele (Ile181) was detected in all cases. However, we were unable to detect any Leu181 mutations in our sample. As these DNAs have amplified at many other STR loci successfully, the only artefactual explanation for this finding considered was the presence of interfering factors introduced during processing that might specifically affect binding of the Leu181 primer. However, dilutions of up to 1:99 of the known positive control in pooled DNA from the twins did not interfere at all with detection of the (control) mutant band present, making any contaminant hypothesis untenable. In addition, we did not detect the Leu181 or Leu183 mutations using either the original ARMS assay[1], or direct sequencing (fluorescent cycle sequencing, Applied Biosystems), in the 19 subjects with the highest summed skin prick test wheal diameters (geometric mean sIgE of 321.5 IU ml^{-1}) or their available parents (n=20; 12 mothers, eight fathers). Four of this group had a maternal but no paternal history of symptomatic atopy. These individuals would have a high probability of carrying a mutant allele according to the earlier work[1,3].

Previous failures to replicate linkage of atopy to the 11q13 region have variously been ascribed[3,4] to (i) the presence of maternal inheritance of atopy (possibly mediated by imprinting); (ii) use of different disease definitions; and/or (iii) genetic heterogeneity[5]. Our finding of a high heritability of atopy in the twins tested despite the absence of any Leu181 mutations supports the hypothesis of genetic heterogeneity. It seems most likely from our results that the Leu181 mutation is far less common than 2.7% in the white Australian population[6], including atopic asthmatics. We can only speculate why the mutations found in the Busselton sample were as frequent as they were, but feel confident that we have excluded experimental artefact as a reason for the strong discordance between our results and those of Shirakawa *et al.*[1] and Hill *et al.*[2].

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IN REPLY — Duffy *et al.* suggest that the prevalence of the FcεRI-β polymorphisms described by Shirakawa *et al.* is 0% in Australian atopic twins. We too have tested for these polymorphisms in an Australian population. We studied approximately 1,000 individuals in 230 nuclear families[7]. We found Ile181Leu in the company of Val183Leu (Leu181/Leu183) in 4% of the subjects, and failed to detect Ile181Leu alone. We have directly sequenced (by radioactive methods) one positive individual from each of the 15 families, in the presence of negative controls, and confirmed the presence of the Leu181/Leu183 variants. The mutation was strongly associated with atopy.

We have discovered a high false-negative rate for PCR-based tests for these variants, as some sequence-positive individuals have been negative when re-typed by the ARMS assay. We have never found a false-positive individual. There appears to be differential PCR amplification of wild-type and Leu181. Leu183 alleles, which may depend on the quality and concentration of DNA in the assay.

The reasons for the false-negative tests have not yet been resolved. The polymorphisms are found in a structurally complex region. There is a microsatellite repeat near the start of exon 6, which may affected amplification in this area. Chromosome 11q13 contains at least three highly homologous genes in close proximity; FcεRI-β, CD20 and hTM4 (ref. 8). It is not known how many other members of this family exist, or if the region contains pseudogenes or multiple copis of active genes in variable numbers, as is the case of the nearby PGA locus[9]. The results of Duffy *et al.* should therefore be taken in the context of a difficult assay for rare polymorphisms. Nevertheless, it is clear that the Leu181/Leu183 allele cannot account for all of the linkage of chromosome 11q atopy.

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