Neither adult nor childhood asthma with a marked atopy phenotype (ASE>3.5 kU/l and total IgE >mean +2 SD) was associated with this variant of *STAT6*. Again, no atopy phenotypes were associated with this variant.

Recent genetic and functional studies highlight the importance of variants of IL-4Rα in the development of atopy; Ile50 upregulates cellular IgE synthesis and is associated with atopic asthma in a Japanese population,[4 5] while Pro478 alters the binding affinity of IRS-1/2, and is associated with lower IgE levels.[7] No association was found between atopic asthma and these variants in a British population.[5] These findings emphasise the genetic heterogeneity of atopic disorders, even within one locus in different ethnic groups; they raise the question of interaction between variants of different components of the IL-4/IL-13 signalling pathway. *BCL6* might repress stat6 activated transcription[16 17] and a variant of *BCL6* has been associated with marked atopy.[18] Interactions among signal transduction molecules of IL-4Rα might explain the complex relationships between genetic variants and phenotypes in atopy and asthma.[1–3]

The gene encoding human *STAT6*, spanning 19 kb on 12q13-14, consists of 23 exons.[19] Genome wide searches for atopic asthma showed a strong linkage to 12q14-24.[20–22] Our present data, showing association between variants of *STAT6* and asthma with mild atopy, support the candidacy of *STAT6* as an "asthma" locus on 12q in a Japanese population. Also, the marked difference in genotype frequencies between the British and Japanese populations may explain the "genetic heterogeneity" among and within ethnic groups.[1–3] Since G2964A variant is located in 3'UTR of the gene, the functional role of this variant remains unknown; it may be in linkage disequilibrium with so far unidentified but functional variants in the regulating or coding parts of *STAT6* or variants of the immediately adjacent genes. Further studies are needed to clarify the role of the *STAT6* variants in predisposing to asthma among different ethnic groups.

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No linkage or association of the *IL-4Rα* gene Q576R mutation with atopic asthma in Italian families

EDITOR—Atopy, a familial clinical syndrome of asthma, rhinitis, and eczema, is characterised by IgE mediated allergy, which results from genetic and environmental events. The disease is immunologically defined by the presence of specific IgE antibodies to common allergens and raised total serum IgE concentrations. Also, because of its high prevalence, age dependent penetrance, and assumed heterogeneity, the mode of inheritance is unknown.[1] Several genes which affect IgE responsiveness have been reported.[2] Among the genes that have been reported to affect the atopic phenotype, that for IL4 regulates the production of IgE.[3 4] Through its receptor (IL4-R), IL4 signals target cells and tissues to mount a response. The IL4-R α chain binds IL4 and mediates its effect through kinases attached to the intracellular domain.[5] The *IL4-Rα* coding gene has been localised to the short arm of chromosome 16 (16p12.1).[6] Sharing of maternal markers flanking the *IL4-Rα* gene was recently found in atopy.[7] A gain of function mutation in the *IL4-Rα* gene was recently described, and it was reported to be associated with higher levels of expression of CD23 by IL-4, severe atopic dermatitis, raised serum IgE level, or a specific response to a common allergen in a case-control study with 20 affected and 30 unaffected adults in the North American population.[8] This association study needs confirmation in a different sample.

In the present study we have investigated the frequency of the *IL4-Rα* Q576R mutation in a large sample of Italian atopic asthmatic families to determine its involvement in genetic susceptibility to some atopic asthma related phenotypes.

A panel of 851 subjects was analysed, recruited from 192 families with one or more affected children, of whom 133 were attending the Allergy and Pulmonology Clinic of the Department of Paediatrics of the University of Verona, as described previously,[9] and 59 families attending the Institute of Paediatrics of the Hospital in Bolzano, both located in north east Italy. All the subjects were tested for clinical history, total serum IgE level, skin prick test (SPT), and bronchial hyper-responsiveness (BHR). Clinical asthma was defined according to the American Thoracic Society criteria,[10] including the response to a respiratory questionnaire. BHR to methacholine was defined as PC 20<25 mg/ml. Atopy was defined by the presence of one or both of the following criteria: (1) positive SPT to one or more common aeroallergens, such as house dust mites, cat, dog, *Alternaria*, grass pollen, *Parietaria*; (2) raised circulating total IgE (from 0 to 10 years of age: age adjusted standard curve, levels above the 90th centile; after 10 years of age: 200 kU/l). Among the families, 522 atopic subjects, 477 with a positive SPT, 288 with raised total serum IgE levels, 298 with clinical asthma, and 281 with BHR were analysed. The total is greater than the total number of people, as two or more phenotypes could occur together in the same person.

Genomic DNA was extracted from whole blood, according to standard protocols. To achieve an easy and rapid evaluation of the guanine for adenine substitution at nucleotide 1902 of the *IL4-Rα* gene, a new protocol based on RG-PCR and nuclease digestion[11] was developed. This method is faster than the single strand conformation polymorphism technique (SSCP) and DNA sequencing analysis previously described.[8] A mutant oligonucleotide which inserted in the product of DNA amplification a new restriction site for *Dde*I was synthesised. The primers used were a novel 21-mer forward oligonucleotide (5'-TCGGCCCCCACCAGTGGCT**C**T-3') with an A1899C substitution, and the same reverse oligonucleotide previously described.[8] The PCR product is a 298 bp fragment. Restriction analysis with *Dde*I results in two fragments of 277 and 21 bp for the Q576 allele, and a single fragment of 298 bp for the 576R allele, as shown in fig 1. All the subjects were genotyped. The data were analysed using the affected sib pairs method implemented in SIBPAL (SAGE package) and the transmission disequilibrium test.[14] The frequency of the 576R mutation calculated in the family founders was 18% (PIC=0.25). In the normal controls, the allele R frequency was 17.5% (18/104 alleles). This frequency is similar to that reported for US controls (10%).[8]

Linkage analysis indicated no significant increase in allele sharing for atopy, SPT, IgE, BHR, or asthma, respectively. No allele transmission disequilibrium was observed. Association studies were performed with 52 adult unrelated controls in whom positivity for each of the phenotypes was excluded. Cases were collected from family founder members according to the phenotype (asthmatic: 45, BHR: 60, IgE: 71, SPT: 153, atopy: 175). The case-control analysis did not show a variation in the distribution of alleles between cases and controls in the phenotypes investigated. The statistical tests have sufficient power (80%) to detect an association with an odds ratio of 3 for IgE, SPT, atopy, and of 5 for asthma and BHR.

As the informativity of Q576R was limited (PIC 0.25), further linkage analyses with additional *IL4-Rα* polymorphisms[13] may better define the putative involvement of the gene in hereditary susceptibility to atopic asthma. Wang *et al*[14] have recently noted that mutation

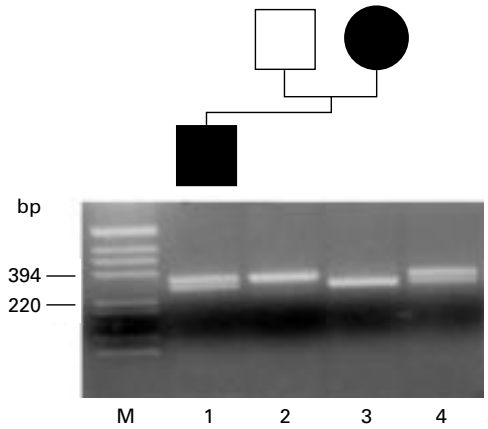

Figure 1 Detection of the IL4-Rα mutation with DdeI restriction analysis of PCR products after modification of the restriction site during PCR. (Top) Pedigree of family BZ78. Filled symbols denote affected family members. (Bottom) Electrophoretic analysis. Lanes 1-3: family members shown above, genotypes are QR, RR, QQ, respectively; lane 4: QR control. M: molecular weight marker (1 kb ladder, GIBCO-BRL).

Q576R does not have a direct effect on IL-4 signal transduction in murine cells and suggested that the hypersensitive induction of CD23 in cells derived from human allergy patients may be the result of different or additional alterations in the IL-4 signalling pathway. Another *IL4-Rα* gene mutation, Ile50Val, was reported to be associated with atopic asthma, increased total IgE, and increased mite specific IgE in a Japanese population, and to upregulate B lymphocyte growth and IgE production in response to IL4 challenge.[15 16] This mutation will now be investigated in the Italian group of patients.

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Importance of the autosomal recessive retinitis pigmentosa locus on 1q31-q32.1 (RP12) and mutation analysis of the candidate gene *RGS16* (*RGS-r*)

EDITOR—Retinitis pigmentosa (RP, MIM 268000) is the term applied to a clinically and genetically heterogeneous group of retinal degenerations primarily affecting the rod photoreceptors. RP is characterised by progressive loss of vision, initially manifesting as night blindness and reduction in the peripheral visual field, and later involving loss of central vision.[1] Ophthalmoscopic examination typically shows pigmentary disturbances of the mid-peripheral retina. RP may be inherited as an autosomal recessive, autosomal dominant, digenic, or X linked trait. Autosomal recessive RP (ARRP) accounts for around 20% of all cases of RP, while sporadic RP, which is presumed to be recessive in most cases, accounts for a further 50%.[2]

Mutations causing autosomal recessive RP (ARRP) have been found in the genes encoding rhodopsin,[3] the α and β subunits of rod phosphodiesterase,[4 5] the α subunit of the cyclic GMP gated channel protein,[6] and the genes *RPE65*,[7 8] *RLBP1*,[9] *ABCR*,[10] and *TULP1*.[11 12] In addition, genetic linkage studies have identified ARRP loci at 1q31-q32.1,[13 14] 2q31-q33,[15] 6cen-q15,[16] and 16p12.1-p12.3.[17] (MIM numbers for the loci identified by these studies are 180380, 180071, 180072, 123825, 180069, 180090, 601708, 600132, 600105, and 602594 respectively. No MIM number has yet been assigned to the 2q31-q33 and 6cen-q15 loci.) Of these four loci, only linkage to the 1q31-q32.1 locus (RP12) and the 6cen-q15 locus has been reported in more than one pedigree.

The first report of linkage of ARRP to 1q31-q32.1 was in a large inbred Dutch family with ARRP in which most patients exhibited para-arteriolar preservation of the retinal pigment epithelium (PPRPE).[13] This was followed by linkage of a second, consanguineous, pedigree from Pakistan.[14] In both the Dutch and the Pakistani families there was evidence that only the branches of the family with PPRPE were linked to 1q31-q32.1, while other branches of each family had classical RP and were unlinked to 1q. In both cases the authors attributed this finding to non-allelic heterogeneity. The occurrence of two very similar eye conditions in one family would presumably be because of the high prevalence of recessive disorders in inbred populations. Recently the critical interval for the RP12 locus was reduced to a 3 cM region between the markers D1S412 and AFM207wb12.[18]

The human gene for a retinally expressed regulator of G protein signalling (*RGS16*/*RGS-r*) has been mapped to 1q25-1q31.[19] Since it has been shown that RGS16/RGS-r can interact with the α subunit of transducin, a heterotrimeric G protein that is an integral part of the rod phototransduction cascade, the *RGS16*/*RGS-r* gene is an excellent functional candidate for ARRP.[20 21] We sought to determine: (1) how important the 1q31-q32.1 locus is as a cause of ARRP by performing linkage analysis and homozygosity mapping in a panel of ARRP families from Europe and Asia, and (2) whether mutations in *RGS16*/*RGS-r* are responsible for ARRP at this locus (RP12). In order to perform linkage analysis and homozygosity mapping, 14 large/consanguineous families with ARRP from the UK (two families), Pakistan (nine families), and Spain (three families) were ascertained. In addition, PMK214, the family originally studied by Leutelt *et al*,[14] was included for mutation screening of the candidate gene *RGS16*/*RGS-r*. Clinical examination of all affected subjects showed typical features of retinitis pigmentosa (pigmentary retinopathy, associated with symptoms of night blindness and loss of visual fields). Informed consent was obtained by local clinicians.

To identify the locus responsible for the disease in each family we performed a linkage or homozygosity analysis. The initial set of markers chosen to be analysed were those corresponding to the loci of genes responsible for ARRP, and those known to flank the ARRP loci for which no gene has as yet been identified (table 1). When evidence of linkage was obtained for the 1q31-q32.1 locus, further polymorphic markers from this region were analysed to determine whether recombinant subjects within the family would permit further refinement of the locus. Marker order was determined from the Généthon sex averaged genetic map.[22] Primers were obtained from the MapPairs set (Research Genetics, Huntsville, AL), or synthesised commercially according to data from GDB (Johns Hopkins University: http://gdbwww.gdb.org/).

Non-radioactive PCR was performed with 300 ng of genomic DNA, 10 pmol of each primer, 200 μmol/l dNTPs, 1.5 mmol/l $MgCl_2$, and 1 unit of *Taq* DNA polymerase. A three stage PCR consisting of 35 cycles of 94°C, 50-62°C, and 72°C, each for one minute, was used. The amplified products were then separated by electrophoresis on 6-8% non-denaturing polyacrylamide gels (Protogel, National Diagnostics) and stained with ethidium bromide. Two point lod scores were calculated using the LINKAGE package.[23]

Primers for heteroduplex analysis and direct sequencing of the five exons and the intron-exon boundaries of the *RGS16*/*RGS-r* gene were designed using the published

Table 1 Known loci for ARRP including some of the polymorphic markers used in this study

Locus	*Gene*	*Reference*	*Markers used*
1p31	*RPE65*	7	D1S1665, D1S1669
1p13-21	*ABCR*	10	D1S236, D1S2813
1q31-q32.1	*RP12*	13, 14	F13B, D1S2622
2q31-q33		15	D2S152, D2S157
3q21-q24	*Rhodopsin*	3	D3S1292, D3S1589
4p16.3	*PDEB*	6	*PDEB* intragenic, D4S412
4p14-q13	*CNGC*	5	D4S189, D4S405
5q31.2-q34	*PDEA*	4	D5S536, D5S412
6p21	*TULP1*	11, 12	D6S291, D6S276
15q26	*RLBP1*	9	D15S127, D15S124
16p12.1-p12.3		17	D16S287, D16S292