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SHORT REPORT

Molecular evidence for absence of Y-linkage of the Hairy Ears trait

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The human Hairy Ears phenotype has traditionally been regarded as the only Y-linked heritable trait. Here, we use Y-chromosomal DNA binary-marker haplotyping to show that a cohort of southern Indian Hairy-Eared males carries Y chromosomes from many haplogroups of the Y-phylogeny, which, under a hypothesis of Y linkage, would require multiple independent mutations within a single population. We further show that there is no significant difference between the Y-haplogroup spectrum in Hairy-Eared males and that in a geographically matched control sample of unaffected males. The trait cannot, therefore, be Y-linked in southern Indians, and by extension, is unlikely to be so in any population.
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Introduction

For over 40 years there has been debate over the mode of inheritance of the human Hairy Ears trait (Hypertrichosis Pinnae Auris (MIM 139500 or 425500)), attracting the early interest of such luminaries as Curt Stern[1,2] and JBS Haldane,[3] and characterized by the unusual feature of several geneticists studying themselves and their own families.[4–6]

The trait involves the development of hairs on the outside of the pinna of the ear, and observation of simple holandric (father-to-son) inheritance in a five-generation Italian pedigree reported[7] in 1907 suggested that this phenotype was Y-linked. As has recently been discussed,[8] Hairy Ears was one of a set of 17 examples of suggested Y-linkage later re-examined by Stern[1] and generally debunked. However, while Stern was categorical in his rejection of most of the traits, his opinion on Hairy Ears was that 'judgement may well be postponed'. Subsequently, southern Indian pedigrees[4] showed strong evidence of Y-linkage, but there were later complications including claimed illegitimacies,[3] apparently reduced penetrance, variability in diagnostic criteria, age-dependence of onset, cosmetic hair removal[9] and secondary ear-baldness.[2] Crucially, some pedigrees appeared to show that daughters (unaffected) of affected males were unable to pass on the trait,[10] consistent with Y-linkage rather than the alternative hypothesis of sex-limited expression of an autosomal dominant gene. However, more recently, three female subjects have been described who themselves manifest a form of the trait.[11] Despite this confusion, Hairy Ears is still widely discussed in textbooks under the heading of Y-linkage (eg Griffiths *et al*[12]).

Here, we use a novel means to address this long-standing question by asking whether there is any association between this putatively Y-linked phenotype and Y-chromosomal DNA haplotype.

Materials and methods

Buccal swab samples were obtained with informed consent from 50 Hairy-Eared males and 50 unaffected males, both groups being ascertained by visual inspection. DNA was extracted using the QiaAmp DNA mini-kit (Qiagen).

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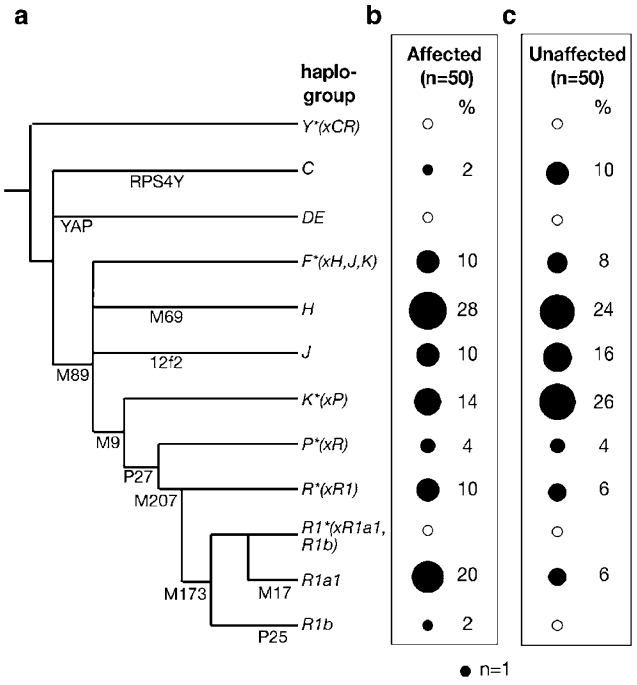

Figure 1 Y-chromosomal haplogroups in Hairy-Eared and unaffected control males. (**a**) Binary marker phylogeny of the Y chromosome, showing mutations on the branches of the tree, and haplogroup names[16] to the right. (**b**) Haplogroup profile of a cohort of 50 unrelated Hairy-Eared males. Areas of filled circles are proportional to haplogroup frequency, and unfilled circles indicate unobserved haplogroups. Percentages are given to the right of circles. (**c**) Haplogroup profile of a control cohort of 50 unrelated unaffected males.

Y-chromosomal binary markers (see Figure 1a) were typed either by methods listed previously,[13] or by the SNaPshot (Applied Biosystems) minisequencing method using modified versions of primers already described,[14] and analysis on an ABI3100 platform (Applied Biosystems).

Haplogroup frequencies in case and control groups were compared using the Arlequin package[15] by a population differentiation test, analogous to Fisher's exact test.

Results

We hypothesized that if the generally rare trait of Hairy Ears were indeed Y-linked, then manifesting males within a given population would be likely to share the same causative mutation on the Y chromosome. Since the male-specific portion of the Y chromosome is nonrecombining, this would imply that all affected males should belong to a particular Y-chromosomal lineage (haplogroup), as defined by binary markers representing unique events in human evolution. Although a number of cases have been reported from other parts of the world, the trait is particularly prevalent in southern India, where in one study it was found in almost 35% of males.[11] We therefore haplotyped a cohort of 50 unrelated Hairy-Eared males from the region around Chennai, India, using 11 Y-chromosomal binary markers[16] (Figure 1a).

The markers define a possible 12 haplogroups. Figure 1b shows that the affected males belong not to a single haplogroup, but to nine distinct haplogroups spread widely in the Y chromosome phylogeny and with a time to most recent common ancestor estimated at $68\,500 \pm 6000$ years ago.[17] If the trait is indeed Y-linked, then we must assume that there have been multiple causative mutations within this single population, and this therefore strongly suggests that the trait is not linked to the Y chromosome.

We then compared the haplogroup frequencies of the affected males with those of 50 unaffected unrelated males from the same geographical region (Figure 1c). Judged by a population differentiation test,[15] there is no statistically significant difference between the two groups ($P = 0.20$) further refuting any possible association between the Y chromosome and the Hairy Ears trait.

Discussion

Given this evidence against Y-linkage, and the obvious influence of hormones upon sexual dimorphism in body-hair patterns, sex-limited expression of an autosomal (or pseudoautosomal) dominant trait seems the most parsimonious explanation of the mode of inheritance of Hairy Ears in southern Indians. If this is so, rigorous pedigree analysis is expected to reveal the passage of the trait via nonmanifesting females. An alternative explanation, which would account for the occasional incidences of affected females,[11] would be partial sex linkage: a dominant allele at a pseudoautosomal locus close to the boundary with sex-specific DNA could occasionally exchange between the sex chromosomes and therefore occur on Y chromosomes of different haplogroups and also on X chromosomes. However, given the high frequency of the trait and the variety of Y haplogroups with which it is associated, we would expect to see more female subjects than the very few that have so far been observed; also, the potential candidate genes in the proximal parts of the pseudoautosomal regions are far from promising.

This study also suggests that, in the absence of strong evidence to the contrary, cases of Hairy Ears in other populations should be regarded by default as *not* Y-linked. It is worth noting that a recent comprehensive cataloging of genes on the Y chromosome[18] reveals no persuasive candidates for hypertrichotic phenotypes. Our findings contribute another nail to Stern's[1] coffin of Y-linked traits, but two heritable traits still remain: one is the interesting

and novel example of nonsyndromic hearing impairment recently reported in a large Chinese pedigree;[19] the other, paradoxically, and thanks to modern reproductive therapy, is one certainly not envisaged by Stern – natural male infertility.[20]

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