

Exposing the human nude phenotype

The recent discovery of the human counterpart of the hairless mouse phenotype¹ has helped our understanding of the molecular genetics of hair growth. But there are no reports of a defect in the human homologue of the best known of the 'bald' mouse phenotypes, the nude mouse². This may be because affected individuals are so gravely ill from the accompanying immunodeficiency that their baldness goes unnoticed. We have carried out a genetic analysis that reveals a human homologue of the nude mouse. The nude mouse is characterized by a congenital absence of hair and a severe immunodeficiency², resulting from mutations in the *whn* (winged-helix–nude; *Hfh11nu*) gene, which encodes a member of the forkhead/winged-helix transcription factor family with restricted expression in thymus and skin³. The simultaneous occurrence of severe functional T-cell immunodeficiency, congenital alopecia and nail dystrophy (MIM database no. 601705) in two affected sisters led to the recognition that the clinical phenotype was reminiscent of the nude mouse⁴. We therefore investigated whether this syndrome represents the human counterpart of the nude mouse phenotype. We obtained DNA samples from members of the sisters' family in a small village in southern Italy. The affected sisters were born with a complete absence of scalp hair (Fig. 1a), eyebrows and eyelashes and had dystrophic nails, and no thymic shadow was evident upon X-ray examination. The first affected child revealed a striking impairment of T-cell function shortly after birth, and died at the age of 12 months. Her sister had similar immunological abnormalities, but bone-marrow transplantation at five months of age led to full immunological reconstitution, although the alopecia and nail dystrophy are still present⁴. We performed linkage analysis using microsatellite markers near the human *WHN* locus on chromosome 17, and found a lod score of 1.32, suggestive of linkage. We then sequenced the human *WHN* gene⁵ and found a homozygous C-to-T transition at nucleotide position 792 of the *WHN* cDNA (GenBank accession no. Y11739) (Fig. 1b). This leads to a nonsense mutation at residue 255 (R255X) in exon 5, and predicts the complete absence of functional protein as a result of nonsense-mediated decay of messenger RNA. Because the proband's bone-marrow transplant was from her brother, we examined her leukocyte DNA both before and after the graft for the presence of chimerism. Genotyping the proband before the transplant showed that her leukocyte DNA was homozygous only for the mutant allele (Fig. 1c). Four years after the transplant, we detected the haplotype specific for the wild-type paternal *WHN* allele received from the brother, as well as the mutant allele, indicative of chimerism. Gender determination revealed that the proband's leukocyte DNA was genotypically XX before the transplant, and the brother's DNA was XY. Afterwards, the proband's leukocyte DNA was found to be XY (Fig. 1c), providing evidence of long-term engraftment and expansion of the bone-marrow graft. The *WHN* gene encodes a transcription factor, which is developmentally regulated and directs cell-fate decisions⁶. In mammals, *whn* is expressed specifically in the epithelial cells of the skin and thymus, where it helps to maintain the balance between growth and differentiation^{7,8}. Recent evidence⁹ has highlighted the importance of the thymic microenvironment in determining the T-cell repertoire, as both positive and negative selection of developing T cells depends on cell–cell interactions with the thymic epithelium. In *whn* knockout mice, the defect has been localized to the differentiating thymic microenvironment rather than to a defect in the developing T cells⁷. The proband was free of infections for four years after the bone-marrow transplant, indicating that T-cell function was at least partly restored. This is probably due to mature T cells of donor origin, although we cannot exclude the possibility that

positive selection of T lymphocytes occurs in the periphery despite the mutated *whn* gene. Our findings provide evidence of a human immunodeficiency caused by a gene expressed not in haematopoietic cells¹⁰, but in specific epithelial cells. In the human hair follicle, expression of *WHN* is sharply demarcated in defined cell populations (Fig. 1d). Although nude mice appear to be completely naked, the dermis contains a normal number of hair follicles, but they are incompletely developed. The fact that only short, bent hairs occasionally emerge from the epidermis is thought to result from impaired keratinization¹¹. Together with the *hairless* gene¹, our finding extends the evidence implicating cell-type-specific transcription factors in hair-follicle cycling and morphogenesis, and indicates that baldness is an extremely complex phenotype.

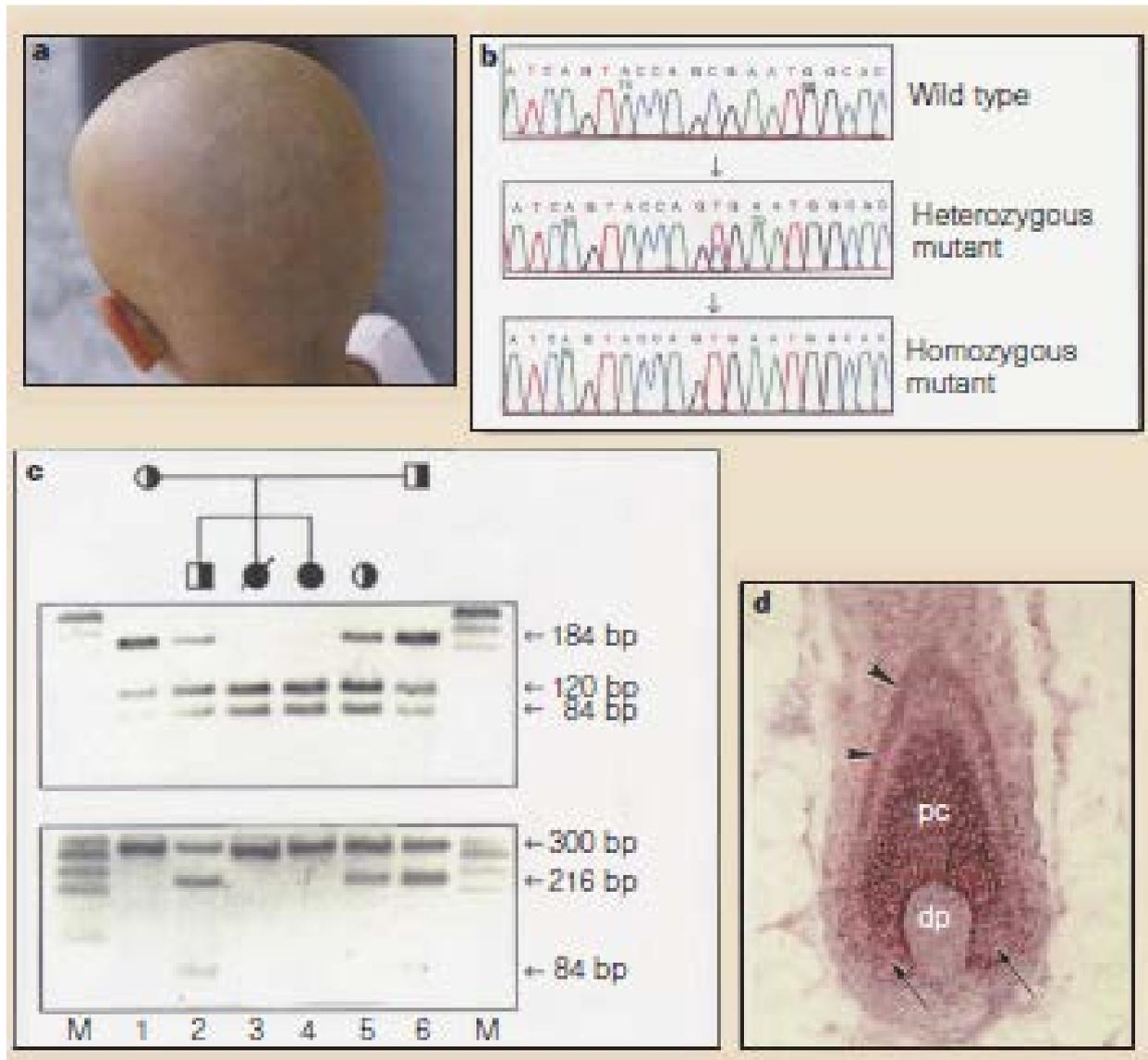


Figure 1. Molecular analysis of the human nude phenotype. a, A five-year-old child with congenital alopecia and T-cell immunodeficiency. b, Sequence analysis of a nonsense mutation in exon 5 of the *WHN* gene. Top, homozygous wild-type sequence from an unrelated, unaffected control individual. Middle, sequence from a heterozygous carrier of the mutation R255X; arrow indicates a double T&C peak. Bottom, homozygous mutant R255X sequence from the affected individual; arrow indicates mutant T only, leading to a C-to-T transition (CGA to TGA) and a substitution of an arginine residue

by a nonsense mutation. c, Restriction-enzyme digestion confirms the mutation. The mutation introduced a restriction site for BsrI and, after digestion of the 184-base-pair (bp) polymerase chain reaction (PCR) product containing exon 5, the product generated from the mutant allele should cleave into two bands of 120 and 64 bp. Top, the unaffected parents and brother had three bands of 184, 120 and 64 bp (lanes 1, 2 and 6), indicating that they were heterozygous carriers of the mutation R255X. Both patients had only the two digested bands of 120 and 64 bp (lanes 3 and 4), consistent with the presence of the mutation in the homozygous state. Bottom, evidence for long-term engraftment of the bone-marrow transplant. Gender determination of family members revealed a genotypically XX pattern of an undigested 300-bp band in the mother (lane 1) and affected patients (lanes 3 and 4), and a genotypically XY pattern consisting of the 300-bp band and two additional bands of 216 and 84 bp, indicative of the Y chromosome, in the brother (lane 2) and father (lane 6). Lane 5, peripheral blood leukocytes from the patient after the transplant, demonstrating an XY genotype and the presence of the normal WHN allele, providing evidence for fraternal chimerism and persistence of the graft. M, size markers. d, WHN mRNA expression in normal human scalp skin. In the hair bulb, WHN mRNA is localized to the differentiating cells of the hair follicle precortex (pc) and the innermost cell layer of the outer root sheath (arrowheads); the dermal papilla (dp) fibroblasts and hair matrix below the level of Auber (small arrows) remain negative for WHN mRNA.

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