ronine 1.6 ng/dl; normal range 0.8–2.67ng/dl; thyroid-stimulating hormone, 0.5 IU/L; normal range, 0.35–6.5 IU/L). Body temperature was in the normal range (36.6 °C). As in *ob/ob* mice[1], a sympathetic system dysfunction (low sympathetic tone) was observed in the patient. This was assessed by a cold pressor response test in which the patient showed a systolic blood pressure response of 7.8 mmHg (normal range, 10–13 mmHg) and a diastolic blood pressure response of 7.2 mmHg (normal range, 9–15 mmHg) after forearm immersion in ice-cold water for 1 min[7], an orthostatic hypotension test (fall of bood pressure from 140/100 mmHg to 105/70 mmHg, 1 min after standing up[8]) and the absence of a response to median nerve and auditory stimulation in sympathetic skin response test[9].

In contrast with *ob/ob* mice, plasma cortisol levels of patient 24 measured at 08.00 am after an overnight fast were not elevated (18.8 µg/dl; normal range, 5–25 µg/dl). ACTH levels were in the upper part of the normal range (91.1 pg/ml; normal, less than 90 pg/ml). The administration of 1 mg dexamethasone at 11.00 pm reduced plasma cortisol levels to less than 5 µg/dl at 8.00 am.

Wild-type and mutant *LEP* cDNAs were transiently transfected into COS-1 cells. 48 h later, leptin concentration measured by RIA was 26.5 ng/ml in the culture medium of cells transfected with the wild-type construct, whereas no leptin could be detected in the medium of cells transfected with the empty vector or with the mutated construct. Immuno-blot experiments confirmed that only cells transfected with wild-type *LEP* secreted leptin into the medium (Fig. 3, lane 2), whereas the mutant leptin was recovered in the cell lysate (Fig. 3, lane 6) indicating that the mutant protein was indeed synthesized in transfected cells but was not secreted into the medium. This suggests that the mutation does not induce protein breakdown but rather impairs the normal processing of the leptin through the secretory pathway[10–12].

The mutation described in the family studied here (a C→T transition in the first base of codon 105) is identical to the mutation which leads to the appearance of a premature stop codon in the *ob/ob* mouse[13]. The phenotype of the adult patients homozygous for this mutation suggests that leptin not only controls body mass but may also be a necessary signal for the initiation of human puberty.

Acknowledgements

We thank M. Hattab, K. Siquier and S. Simon for expert technical assistance. We are grateful to the staff of Nuclear Medicine and Biochemistry Departments of Gulhane School of Medicine for their help with hormone and biochemichal measurements. We are also grateful to A.M. Proenza for allowing us to use some of the DNA from Turkish patients in this study. This work was mainly supported by the CNRS, the Université de Paris VII, the Fondation de France and the INSERM. A.S. is a Marie Curie fellow of the European Union, which also supported this work through the MIEC grant.

Andreas Strobel[1,2], Tarik Issad[1], Luc Camoin[1], Metin Ozata[3] & A. Donny Strosberg[1]

[1]Institut Cochin de Génétique Moléculaire, Laboratoire d'Immuno-Pharmacologie Moléculaire, CNRS UPR 0415 et Université de Paris VII, 22, rue Méchain, 75014 Paris, France. [2]Institute of Physiological Chemistry and Pathobiochemistry, Johannes Gutenberg University Mainz, Duesbergweg 6, D-55099 Mainz, Germany. [3]Department of Endocrinology and Metabolism, Gulhane School of Medicine, Etlik-Ankara 06018, Turkey. A.S. and T.I. contributed equally to this work. Correspondence should be sent to A.D.S. e-mail: strosberg@icgm.cochin.inserm.fr

1. Bray, G.A. & York, D.A. *Physiol. Rev.* **59**, 719–809 (1979).
2. Considine, R. V. *et al. J. Clin. Invest.* **95**, 2986–2988 (1995).
3. Maffei, M. *et al. Diabetes* **45**, 679–682 (1996).
4. Carlson, B. *et al. Obes. Res.* **5**, 30–35 (1997).
5. Chehab, F.F., Lim, M.E. & Ronghua, L. *Nature Genet.* **12**, 318–320 (1996).
6. Montague, C.T. *et al. Nature* **387**, 903–908 (1997).
7. Sayinalp, S., Sozen, T., & Ozdagan, M. *Diabetes Res. Clin. Pract.* **26**, 21–28 (1997)
8. Ewing, D.J., Martyn, C.N., Young, R.J. & Clarke, B.F. *Diabetes Care* **8**, 491–498 (1985).
9. Shahani, B.T., Halperin, J.J., Boulu, P. & Cohen, J. *J. Neurol. Neurosurg. Psychiatry* **37**, 536–542 (1984).
10. Karaplis, A.C., Lim, S.K., Baba, H., Arnold, A., & Kronenberg, H.M. *J. Biol. Chem.* **270**, 1629–1635 (1984).
11. Smit, L.S. *et al. Hum. Mol. Genet.* **4**, 269–273 (1995).
12. Olias, G., Richter, D. & Schmale, H. *DNA Cell. Biol.* **15**, 929–935 (1995).
13. Zhang, Y. *et al. Nature* **372**, 425–432 (1994).

A mutation in *PDS* causes non-syndromic recessive deafness

As much as 10% of all hereditary deafness may be Pendred syndrome, an autosomal recessive disorder characterized by congenital sensorineural deafness and thyroid goitre (OMIM entry 274600; ref. 1). Variable intrafamilial expression has been noted, particularly with respect to goitre which may appear congenitally or in mid-childhood, but is often postpubertal[2]. The delay in the onset of goitre can make accurate diagnosis difficult until thyroid enlargement becomes apparent. This situation occurred in an Israeli-Druze family in which deafness was found to be linked to 7q31, defining the *DFNB4* non-syndromic deafness locus[3]. Affected members of this family were later found to have goitres and, thus, Pendred syndrome, rather than non-syndromic deafness (C. Baldwin, pers. comm.).

In a large consanguineous family from southwest India, 10 individuals (between 5 and 38 years old) are affected with congenital, profound, non syndromic autosomal recessive deafness (Fig. 1). Medical histories were obtained from all affected individuals in the family and their first degree relatives to exclude pre-, peri- and postnatal and environmental causes of deafness. Pure-tone audiometry tests showed that all affected individuals in the family demonstrated profound, prelingual, sensorineural deafness. Physical examinations were performed to exclude stigmata of syndromic deafness. Particular attention was paid to the thyroid; one of the authors (A.K.L.) examined all deaf individuals in this family. No palpable goitre was present in any of the affected individuals. Serum T3, T4 and thyroid-stimulating hormone (TSH) levels were normal in all tested individuals: three affected (II-5, II-12 and II-14, 18 to 28 years old) and one unaffected (II-11). The perchlorate discharge test[2] was not available in the area where the family resides. Thyroid scans, performed with radioactive $^{99m}TcO_4$ on individuals II-7, II-12 and II-11, showed normal thyroid uptake with a homogeneous distribution of the radiotracer[4].

Cochlear malformations of the Mondini-type are typically found in individuals with Pendred syndrome[5]. Axial and coronal computerized tomography of

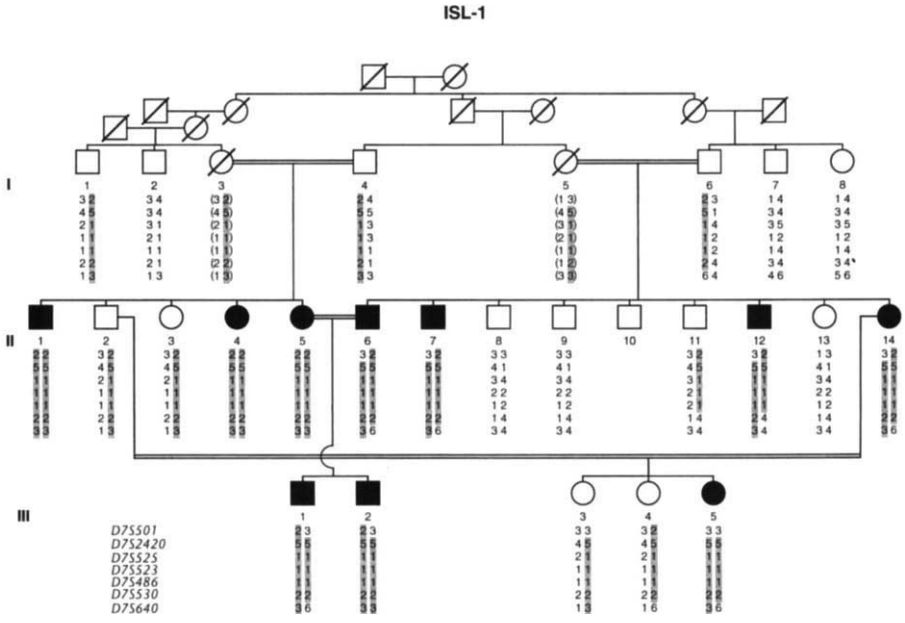

Fig. 1 Pedigree and haplotype data for an Indian family ISL-1 segregating non-syndromic deafness. Genotypes of markers *D7S501*–1cM –*D7S2420*–1cM –*D7S525*–2cM –*D7S523*–1cM –*D7S486*–9cM –*D7S530*–5cM –*D7S640* are shown. The deafness gene maps to approximately a 14-cM interval between markers *D7S501* and *D7S530*. The disease chromosomes are shaded. Deduced haplotypes are shown within parentheses.

the temporal bone was also conducted on individuals II-5, II-12, II-14 and II-11, revealing no Mondini-type cochlear malformation, though all three affected have bilateral large vestibular aqueducts. The cochlea, internal auditory canal, vestibule, semicircular canals, middle ear, mastoid and ossicular chain were normal on radiographic examinations conducted in these same individuals.

Linkage analysis of this family locates the gene within a 14-cM interval on chromosome 7q31 between markers *D7S501* and *D7S530*. Using FASTLINK version 5.1, the maximum LOD score is 7.0 for the marker *D7S525* at $\theta=0$, using the allele frequency calculated by typing 92 unrelated unaffected individuals from the same geographic area. This interval contains the *PDS* gene known to be mutated in Pendred syndrome[6].

Pendred syndrome has been linked to a single locus in more than 19 families from different geographic areas and ethnic backgrounds, suggesting locus homogeneity for the disorder[7]. The finding of several independent *PDS* point mutations and no large deletions indicates that a single gene is responsible for Pendred syndrome[6]. In light of its physical location, *PDS* represents an excellent candidate gene for the 7q31-linked deafness in this Indian family.

PDS contains 21 exons with a predicted open reading frame of 2,343 bp. The predicted protein, pendrin, consists of 780 amino acids (86 kD) and is thought to be a sulphate transporter[6]. Mutation analysis of *PDS* in the Indian family revealed two single-base changes in exon 13 in the coding region of this gene (Fig. 2*a*). The first is a G→A transition at nucleotide position 1713, resulting in a predicted Gly→Ser substitution at position 497 (G497S). The second is an A→C transversion at nucleotide position 1692, which results in a predicted Ile→Leu substitution at position 490 (I490L). All affected individuals were homozygous while their parents were heterozygous for both mutations. These two nucleotide changes were not detected in any of the non-carriers in the family or in 92 unaffected, unrelated individuals from the same geographic area. Both of

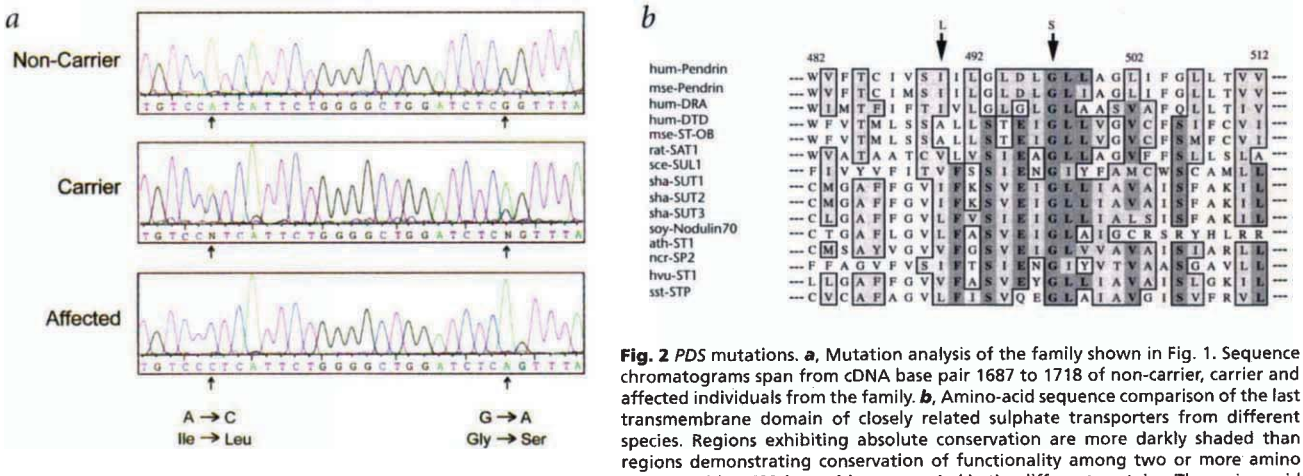

Fig. 2 *PDS* mutations. ***a***, Mutation analysis of the family shown in Fig. 1. Sequence chromatograms span from cDNA base pair 1687 to 1718 of non-carrier, carrier and affected individuals from the family. ***b***, Amino-acid sequence comparison of the last transmembrane domain of closely related sulphate transporters from different species. Regions exhibiting absolute conservation are more darkly shaded than regions demonstrating conservation of functionality among two or more amino acids. The Gly residue at position 497 (arrow) is conserved while the amino acid at position 490 (arrow) is more varied in the different proteins. The amino-acid sequences were derived from the following GenBank records: human pendrin (AF030880), mouse pendrin (L.A.E. and E.D.G., unpublished), human DRA (L02785), human DTD (U14528), mouse sulphate transporter protein (D42049), rat sulphate anion transporter (L23413), *Saccharomyces cerevisiae* sulphate permease (Z35134), *Stylosanthes hamata* (tropical forage legume) root sulphate transporters 1 (X82255), 2 (X82256) and 3 (X82454), *Glycine max* (soybean) sulphate transport protein homologue (S34800), *Arabidopsis thaliana* sulphate transporter (D89631), *Neurospora crassa* sulphate permease II (M59167), *Hordeum vulgare* (barley) sulphate transporter (X96431) and *Sporobolus stapfianus* (resurrection grass) sulfate transporter protein (X96761).

these changes occur within the last predicted transmembrane domain.

The Gly residue at position 497 is conserved in all 15 of the closest-related sulphate transporter proteins, including those from human, mouse, rat, soybean, barley, resurrection grass and yeast (Fig. 2*b*), whereas there are only 16 amino acids strictly conserved in the entire sequence among these proteins (data not shown). Substitution of the Gly→Ser in this conserved position introduces a polar constraint inside the transmembrane domain and would likely alter the conformation of the protein. The functional significance of the I490L substitution, if any, is less obvious. It appears to be only found in conjunction with the G497S substitution, as neither change was detected in 184 random normal chromosomes from the same geographic area. It is plausible that I490L could also create a subtle alteration in the protein. The previously reported three mutations in *PDS* included two frameshifts (predicted to result in truncated proteins) and one missense mutation causing a change from Phe→Cys in a highly conserved residue 667. These mutations associated with Pendred syndrome may result in a more severely altered protein than the missense mutations found in the Indian family with non-syndromic deafness. Alternatively, some of the features of Pendred syndrome may be non-penetrant due to the genetic background of the individual.

The family reported here is striking in that goitre is absent in all affected individuals, indicating that certain *PDS* mutations may reliably produce deafness in the absence of other syndromic features. It is also noteworthy that about 50% of children with sensorineural hearing loss have enlarged vestibular aqueducts[8]. These results suggest that *PDS* mutations may account for a larger proportion of congenital deafness than the anticipated 10%, as a diagnosis of Pendred syndrome would not be made in the absence of goitre.

Mutations in a single gene giving rise to different clinical phenotypes has been observed for other genes. Mutations in *CFTR*, for example, can be associated with different clinical phenotypes in cystic fibrosis[9]. Mutations in *MYO7A* are responsible for autosomal recessive non-syndromic deafness (*DFNB2*), autosomal dominant non-syndromic deafness (*DFNA11*) and Usher 1B, a deaf-blind syndrome[10–13]. This variable manifestation in phenotype may be explained by allelic heterogeneity or by the influence of genetic background. *PDS* represents another example where allelic variants or genetic background are associated with both syndromic and non-syndromic forms of deafness, but the molecular mechanisms whereby mutations in the same gene can cause either syndromic or non-syndromic deafness are not yet understood.

Acknowledgements

We are grateful to the family members for their participation in this study. We thank J. Battey, R. Morell, D. Drayna and S. Sullivan for critical comments and reviewing of the manuscript. This work was supported by the National Institute on Deafness and Other Communication Disorders (NIDCD) intramural research project Z01 DC00026. We also thank everyone in the Laboratory of Molecular Genetics, NIDCD, for their support and helpful discussion.

Xiaoyan C. Li[1], Lorraine A. Everett[2], Anil K. Lalwani[3], Dilip Desmukh[4], Thomas B. Friedman[1], Eric D. Green[2] & Edward R. Wilcox[1]

[1]Laboratory of Molecular Genetics, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, Maryland 20850, USA. [2]Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892, USA. [3]Division of Otology, Neurotology, and Skullbase Surgery, Department of Otolaryngology, Head and Neck Surgery, School of Medicine, University of California San Francisco, A730, 400 Parnassus Ave., San Francisco, California 94143, USA. [4]Rotary Deaf School, Ichalkaranji-Tilawani, 416115, Dist. Kolhapur, Maharashtra, India. Correspondence should be addressed to E.R.W. (e-mail: edwilcox@pop.nidcd.nih. gov) or E.D.G. (e-mail: egreen@nhgri.nih.gov).

1. Batsakis, J.G. & Nishiyama, R.H. *Arch. Otolaryngol.* **76**, 401–406 (1962).
2. Reardon, W. & Trembath, R.C. *J. Med. Genet.* **33**, 1037–1040 (1996).
3. Baldwin, C.T. *et al. Hum. Mol. Genet.* **4**, 1637–1642 (1995).
4. Hjelstuen, O.K. *Analyst* **120**, 863–866 (1995).
5. Johnsen, T. *et al. J. Laryngol. Otol.* **101**, 1187–1192 (1987).
6. Everett, L.A. *et al. Nature Genet.* **17**, 411–422 (1997).
7. Gausden, E. *et al. J. Med. Genet.* **34**, 126–129 (1997).
8. Arcand, P. *et al. J. Otolaryngol.* **20**, 247–250 (1991).
9. Welsh, M.J. & Smith, A.E. *Cell* **73**, 1251–1254 (1993).
10. Gibson, F. *et al. Nature* **374**, 62–64 (1995).
11. Liu, X.Z. *et al. Nature Genet.* **16**, 188–190 (1997).
12. Liu, X.Z. *et al. Nature Genet.* **17**, 268–269 (1997).
13. Weil, D. *et al. Nature Genet.* **16**, 191–193 (1997).