

Short reports

Department of Plastic Surgery, Maasland Hospital, Sittard, The Netherlands
G A M Malfeyt

Department of Pathology, Maasland Hospital, Sittard, The Netherlands
Th L Ceelen

Department of Clinical Genetics EE2422, Erasmus University Rotterdam and Academic Hospital Rotterdam Dijkzigt, PO Box 1738, 3000DR Rotterdam, The Netherlands

S Verhoef
A Tempelaars
D Lindhout
D J J Halley
A M W van den Ouweland

Department of Clinical Genetics, Maastricht University, Maastricht, The Netherlands
C T R M Schrandt-Stumpel

Department of Pathology, Erasmus University Rotterdam, Rotterdam, The Netherlands
V D Vuzevsky

Institut für Humangenetik, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany
L A J Janssen

Correspondence to: Dr Verhoef.

Received 5 January 1998
Revised version accepted for publication 17 March 1998

Familial cylindromatosis mimicking tuberous sclerosis complex and confirmation of the cylindromatosis locus, CYLD1, in a large family

S Verhoef, C T R M Schrandt-Stumpel, V D Vuzevsky, A Tempelaars, L A J Janssen, G A M Malfeyt, Th L Ceelen, D Lindhout, D J J Halley, A M W van den Ouweland

Abstract

A large Dutch family had been known for many years to be affected with skin tumours labelled as adenoma sebaceum, which were inherited in an autosomal dominant fashion. Since this skin sign is considered pathognomonic for tuberous sclerosis complex, the condition in the family was labelled accordingly, in the absence of further clinical features of tuberous sclerosis complex-like mental retardation or epilepsy. The skin changes started at early puberty with small eruptions around the nose and progressed to larger tumours, with considerable variation in severity. Some affected members had required plastic surgical reconstruction following excision.

Linkage analysis in this family was performed for the two chromosomal regions involved in tuberous sclerosis complex on chromosomes 9q34 and 16p13, but no positive linkage was found.

On critical re-evaluation of the clinical and pathological data and renewed assessment, the working diagnosis was changed to autosomal dominant cylindromatosis. The recently published candidate region for cylindromatosis on chromosome 16q12-13 was subsequently proven to be

positively linked with a lod score of 3.02 with marker D16S308. Review of pathological specimens confirmed the diagnosis of cylindromatosis. DNA analysis of tumour tissue showed loss of heterozygosity for the cylindromatosis CYLD1 locus. These results confirm the candidate locus for cylindromatosis on chromosome 16q12-13.

(J Med Genet 1998;35:841-845)

Keywords: tuberous sclerosis; cylindromatosis; CYLD1 gene; chromosome 16

Hereditary multiple epithelioma, or cylindromatosis (MIM No 132700), is a rare autosomal dominant disease, characterised by the appearance of multiple skin adnexal tumours with different histological descriptions and names. The primary localisations of these usually benign lesions are the head and neck. The genetic aspects of this disease were comprehensively reviewed by van Balkom and Hennekam,¹ who used the name dermal eccrine cylindromas for the condition. Recently, the gene for familial cylindromatosis, referred to as CYLD1, was mapped to the long arm of chromosome 16, band 16q12-13, in a genome search involving two large families.² So far, there is no evidence for locus heterogeneity.

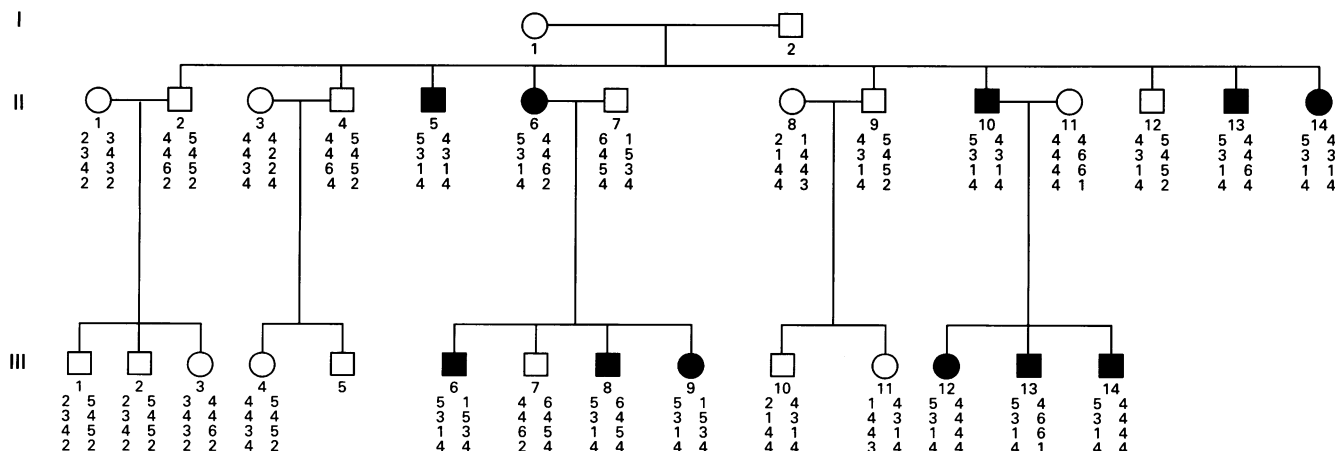


Figure 1 In the pedigree the haplotypes of the markers of the 16q12-13 region are depicted. The markers used from the CYLD candidate region from proximal to distal (top to bottom) were D16S411, D16S304, D16S308, and D16S419 (affected haplotype 5-3-1-4).

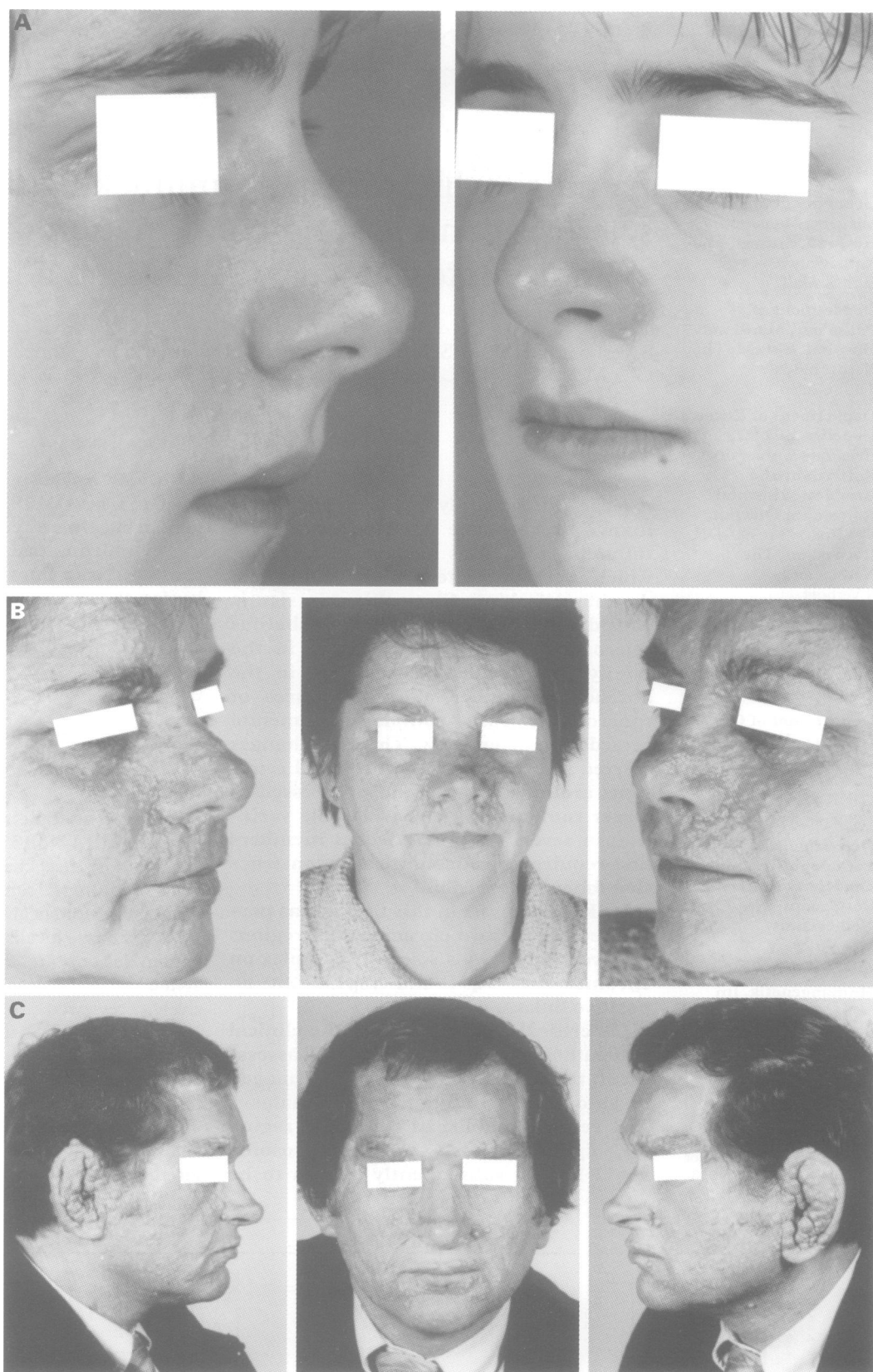


Figure 2 (A) Patient III.12 showing small lesions on the cheeks and in the nasolabial folds. (B) Patient II.6 showing facial tumours in the nasolabial fold, mimicking angiofibroma. The histological diagnosis was cylindroma. (C) Patient II.10 with large tumours in the nasolabial fold, on the cheeks, and on both ears.

The skin abnormalities of cylindromatosis may be confused with some of the manifestations of tuberous sclerosis complex (TSC), previously designated epiloia or Morbus Bourneville-Pringle. TSC is an autosomal dominant disease with a prevalence of between 1:6000 and 1:10 000 at birth, characterised by

the presence of hamartomas that can appear in any organ system. The organs most often involved are the skin, central nervous system, eyes, kidneys, and heart. The clinical expression of TSC is extremely variable and occasionally families can have a predominance of skin manifestations. Two genes are involved in

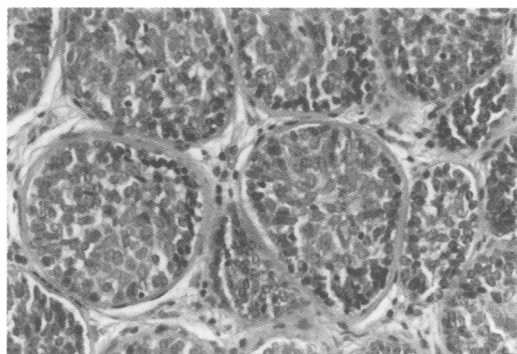


Figure 3 Pathological analysis of one of the tumours of patient II.14. Lobules of basophilic epithelial cells, surrounded by hyaline membranes, are present. Diagnosis: cylindroma (H&E).

tuberous sclerosis complex. Both genes have recently been isolated, the TSC1 gene on the long arm of chromosome 9, band 9q34,³ and the second gene, TSC2, on the short arm of chromosome 16, band 16p13.3.⁴

We describe a family in which a clinical diagnosis of tuberous sclerosis complex was made on the basis of the presence of so called "sebaceous adenoma", a skin sign pathognomonic for the disease, in several of its members. Linkage analysis of the two regions for the TSC1 and TSC2 genes, and subsequently the candidate region for the CYLD1 gene, as well as review of the skin pathology and loss of heterozygosity studies, were performed. These enabled us to resolve this differential diagnostic problem and confirm the candidate region of CYLD1 on chromosome 16q12-13.

Patients and methods

A total of 27 members of family T6065, of which 10 members are affected, were sampled (fig 1). The family was recruited through index patients II.6 and II.10. All participating family members were seen by a clinical geneticist (CSS) at the time of venepuncture and completed a questionnaire on their state of health, with special focus on skin signs and other possible signs of tuberous sclerosis complex.

EDTA blood samples were collected from affected and unaffected subjects and partners, initially for linkage analysis for both the TSC1 and TSC2 regions. DNA extraction from

peripheral leucocytes was performed according to standard procedures. Linkage analysis was undertaken mainly using microsatellite markers with the linkage computer program MLINK. Chromosome 9q markers used flanking the TSC1 region were D9S149, D9S66, and D9S114. For chromosome 16p, markers 3'HVR, KG8, 16AC2.5, and SM7, flanking the TSC2 gene, were tested. KG8 is a marker in the adjacent PKD1 gene showing 0% recombination with the TSC2 gene. Subsequently, the cylindromatosis candidate region on chromosome 16q was tested with markers D16S411, D16S304, D16S308, and D16S419. Calculations were performed using a gene frequency of 0.1%, a penetrance of 80%, and a 1% phenocopy rate. Allele frequencies were presumed equal.

Some of the previously removed and analysed tumours were re-evaluated histologically and investigated for loss of heterozygosity for both TSC loci and for the familial cylindromatosis CYLD1 gene candidate region.^{2,5} For the LOH studies, DNA isolated from blood and from tumour tissue from subject II.5 was run on an ALF automated sequencer (Pharmacia), using markers D9S66 (TSC1 locus), 16AC2.5 (D16S291, TSC2 locus), and D16S411 (CYLD1 candidate region).

Results

The multiple dermal tumours in family T6065 were mostly restricted to the face, neck, and scalp, and sometimes the ears. Clinical records and re-evaluation showed no evidence for the presence of hypomelanotic macules, shagreen patches, fibrous forehead plaques, or (peri)ungual fibromas in any of the affected family members. The age of onset of the skin signs was between 10 and 20 years of age in most subjects, with slow progression, sometimes more rapid during puberty, pregnancy, or in periods of increased psychological stress (fig 2A).

Patient II.5 had tumours removed recently, which were sent in for pathological review (VDV).

Patient II.6 (fig 2B) had a clinical diagnosis of facial adenoma sebaceum in 1986. CT scan of the brain and skull x ray were normal. In 1990, again the diagnosis of "typical Morbus Pringle-Bourneville" (TSC) was made by one of her physicians. Pathological analyses of her tumours, reviewed on more than one occasion, gave different diagnoses of basalioma, trichoepithelioma, or cylindroma.

Patient II.10 (fig 2C) had a clinical diagnosis of "Morbus (Bourneville)-Pringle" (TSC) in 1977. On several occasions skin tumours had been removed and invariably histology had been reported as trichoepithelioma. He underwent clinical examination in 1986 of his brain (CT scan), kidneys (ultrasound), eyes, skin, and teeth. Apart from his skin lesions, then referred to as "adenoma sebaceum", he had no other possible signs of tuberous sclerosis complex. In this patient, some lesions were so severe that skin grafts were necessary after removal.

Patient II.14 had a previous clinical diagnosis of "Morbus Pringle-Bourneville" (TSC).

Table 1 MLINK analysis for family T6065 for the regions containing TSC1 (chromosome 9q34), TSC2 (chromosome 16p13.3), and the candidate region for the CYLD1 gene (chromosome 16q12-13). Gene frequency 0.01%, penetrance 80%, phenocopy rate 1%, allele frequencies presumed equal. Markers are ordered top to bottom, proximal to distal. The maximum lod score of 3.02 is attained at D16S308

	Theta					
	0%	5%	10%	20%	30%	40%
Chromosome 9						
D9S149	-3.92	-2.00	-1.32	-0.66	-0.29	-0.08
D9S66	-5.09	-2.38	-1.46	-0.61	-0.24	-0.06
D9S114	-5.23	-2.53	-1.57	-0.66	-0.25	-0.06
Chromosome 16p						
3'HVR	-6.84	-2.25	-1.24	-0.39	-0.10	-0.02
KG8	-0.01	-0.01	-0.01	-0.01	-0.002	-0.001
16AC2.5	-6.77	-1.87	-1.01	-0.30	-0.07	-0.01
SM7	-2.85	-0.61	-0.13	0.18	0.17	0.06
Chromosome 16q						
D16S411	1.69	1.54	1.38	1.04	0.66	0.25
D16S304	2.21	2.00	1.78	1.30	0.77	0.24
D16S308	3.02	2.75	2.46	1.84	1.14	0.39
D16S419	2.28	2.06	1.84	1.34	0.80	0.26

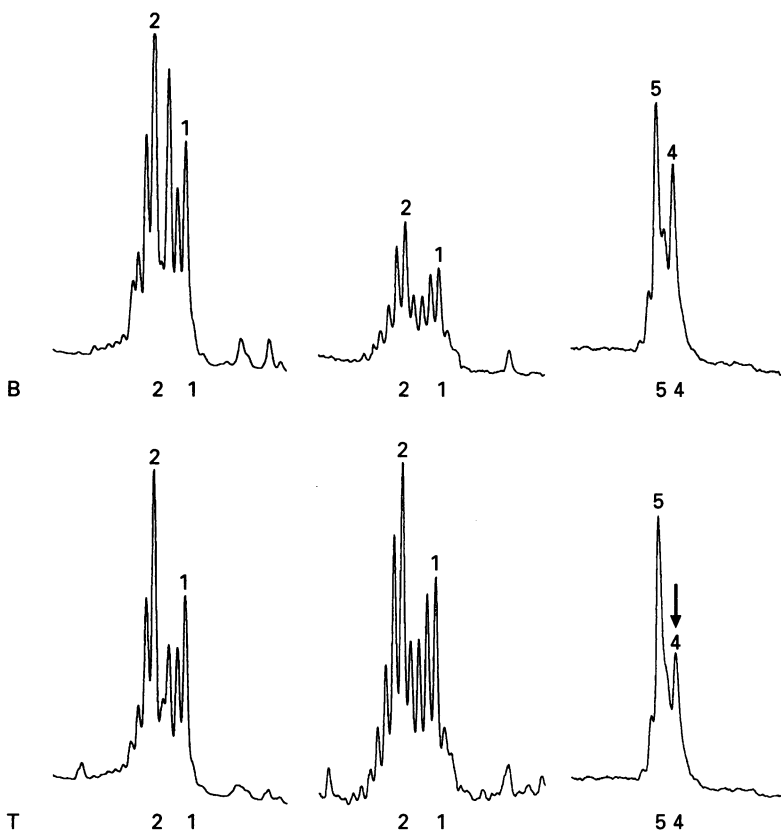


Figure 4 Loss of heterozygosity (LOH) studies of blood (B) versus tumour (T) DNA of patient II.5. Left and middle: chromosome 9q34 (TSC1) marker D9S66 and chromosome 16p13 (TSC2) marker 16AC2.5 (D16S291), respectively, showing no LOH. Right: chromosome 16q12-13 (CYLD1) marker D16S411, showing partial loss of allele 4 of the unaffected haplotype (arrow) in tumour.

Two tumours removed in 1988 were diagnosed as eccrine spiradenomas and three others removed in 1990, 1992, and 1994 as cylindromas. The tumour removed in 1990 was recently re-examined (VDV), confirming the diagnosis of cylindroma (fig 3).

None of the affected family members had suffered from medical complications characteristic of tuberous sclerosis complex, like epilepsy, mental retardation, renal cysts, or renal angiomyolipomas.

Lod scores proved negative for both the TSC1 and the TSC2 locus using flanking markers (table 1). Subsequent linkage for the candidate region for familial cylindromatosis on chromosome 16q12-13 gave a highest lod score of 3.02 with marker D16S308, with no recombinations in affected subjects. The affected haplotype was, proximal to distal, 5-3-1-4 (D16S411, D16S304, D16S308, D16S419).

Pathological review of three tumours from the scalp of patient II.5 resulted in a histopathological diagnosis of trichoepithelioma for all three tumours. In this tumour tissue, loss of heterozygosity for the chromosome 16q marker D16S411 could be shown, with loss of the normal number 4 allele (fig 4). The other markers on chromosome 16q were uninformative in this subject.

Discussion

The clinical picture in this family gave rise to confusion about the exact diagnosis, despite the fact that in some of the pathological reports

a diagnosis of cylindromatosis had been correctly made. The tumours had been associated with adenoma sebaceum and therefore with tuberous sclerosis complex.

Familial multiple cylindromatosis and multiple epitheliomas are considered one clinical entity. The two main manifestations, cylindroma and (tricho)epithelioma, have been reported within one family and in a single person.⁶ The most severe type of manifestation is the so called "turban tumour", as was reported by Biggs *et al.*² In the present family, T6065, an "unusual type of tuberous sclerosis complex, manifesting itself only in the skin, as sebaceous adenoma" was apparent. The localisation of these tumours was reported to be predominantly in the nasolabial folds, as is also considered characteristic of tuberous sclerosis complex. Some more severely affected subjects had tumours elsewhere (fig 2). Family members had not been further analysed clinically because the condition was known to be familial and considered to be mild, apart from the severe cosmetic problems it was causing. Patients II.6 and II.10 had been investigated fully for other signs of tuberous sclerosis, but no other manifestations of tuberous sclerosis complex had been found. Removal or biopsy, followed by histological analysis, had been performed by a plastic surgeon or dermatologist in several members of the family. On these occasions, the pathological findings had been interpreted as "in agreement with a diagnosis of tuberous sclerosis". The term sebaceous adenoma, suggesting a skin adnexal origin, is now considered a misnomer for angiofibroma as found in definite cases of tuberous sclerosis complex. The tumour is not derived from the eccrine tissue of the sebaceous gland, but it contains cells with fibroblastic, vascular, and neuronal properties,^{7,8} more in line with the hamartomatous changes that are seen elsewhere in the body in tuberous sclerosis complex. The term sebaceous adenoma could have contributed to the diagnostic confusion in this family. The name facial angiofibroma is proposed as a more descriptive and distinguishing term.

After the exclusion of linkage to either the TSC1 or TSC2 locus, analysis using polymorphic markers of the chromosome 16q12-13 region gave convincing evidence that the diagnosis in this family should be familial cylindromatosis. Our results independently confirm the localisation of the proposed CYLD1 locus on chromosome 16q12-13, with a maximum lod score of just over 3.0 with marker D16S308. The maximum lod score in the original article was attained with marker D16S304.²

Not until the linkage analysis had excluded both candidate regions for tuberous sclerosis complex did a renewed look at the family data permit a change in the suspected diagnosis. In addition to the linkage analysis results, the observation of loss of heterozygosity of the relevant chromosome 16q region in lesional tissue confirmed that the CYLD1 candidate region on chromosome 16q contained the locus for cylindromatosis in this family. This case may serve to illustrate the need for careful verifica-

tion of the diagnosis in the case of genetic counselling for diseases that are known for their clinical variability.

The authors thank the family for their encouragement to do this study, and Francis van der Lubbe for the clinical pictures. This work was supported by the Dutch Praevention Fund, grant No 28-7123-1.

- 1 van Balkom IDC, Hennekam RCM. Dermal eccrine cylindromatosis. *J Med Genet* 1994;31:321-4.
- 2 Biggs PJ, Wooster R, Ford D, *et al*. Familial cylindromatosis (turban tumour syndrome) gene localised to chromosome 16q12-q13: evidence for its role as a tumour suppressor gene. *Nat Genet* 1995;11:441-3.
- 3 van Sleightenhorst M, de Hoogt R, Hermans C, *et al*. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science* 1997;277:805-8.
- 4 The European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 1993;75:1305-15.
- 5 Biggs PJ, Chapman P, Lakhani SR, Burn J, Stratton MR. The cylindromatosis gene (CYLD1) on chromosome 16q may be the only tumour suppressor gene involved in the development of cylindromas. *Oncogene* 1996;12:1375-7.
- 6 Gerretsen AL, Beemer FA, Deenstra W, Hennekam FAM, van Vloten WA. Familial cutaneous cylindromas: investigations in five generations of a family. *J Am Acad Dermatol* 1995;33:199-206.
- 7 Nickel WR, Reed WB. Tuberous sclerosis. *Arch Dermatol* 1962;85:209-26.
- 8 Ishibashi Y, Watanabe R, Nogita T, Takahashi T, Onodera K, Kimura G. Abnormal gene expression of stroma cells in patients with tuberous sclerosis. *Ann NY Acad Sci* 1991;615:228-42.