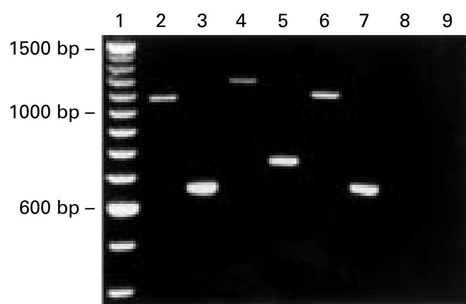

Figure 3 Allele specific amplification of cDNAs with and without the 134 bp insertion. The 7-DHCR cDNA without the 134 bp insertion was specifically amplified in two overlapping fragments from a patient heterozygous for the insertion (lanes 2 and 3) and a patient with two missense mutations (lanes 6 and 7) using primer sets $DHCR_{-58-38}$ and $DHCR_{979-967}$ (lanes 2 and 6), and $DHCR_{949-967}$ and $DHCR_{1563-1544}$ (lanes 3 and 7), respectively. The cDNA containing the 134 bp insertion was specifically amplified in two overlapping fragments from the heterozygous patient using primer sets $DHCR_{-58-38}$ and $DHCR\text{-}ins_{113-95}$ (lane 4), and $DHCR\text{-}ins_{21-39}$ and $DHCR_{1563-1544}$ (lane 5). Using the latter two primer sets, no fragments were amplified from the patient who had two missense mutations and lacked the splice site mutation causing the insertion (lanes 8 and 9).

transversion which results in the partial retention of intron 8 owing to alternative splicing.[7] After excluding the three sibs, this amounts to an incidence of ~35% (12/34 alleles) for the mutant allele in our group of patients. When patients previously reported by others are also included,[7 8] however, the incidence of this allele approximates ~25% (15/59 alleles). This makes this allele the most frequently occurring among SLO patients, since all other causative mutations identified to date have been recurrent among a limited number of patients[6–8] (unpublished results). The RT-PCR based methods presented in this paper provide an easy and rapid screening at the cDNA level for this frequently occurring mutant allele. The allele specific amplification of cDNAs in case of heterozygosity for the 134 bp insertion allows the establishment of compound heterozygosity without subcloning of the cDNAs, which is valuable in cases where no parental material is available.

The relatively high incidence of the splice acceptor site mutation among patients with SLO syndrome remains unexplained at present, but could be the result of a founder effect. This is supported by the complete absence of any of the quite common polymorphisms,[7] 189A>G, 207C>T, 231C>T, 438C>T, 1158C>T, and 1272T>C, in all 15 mutant alleles identified in our patients. On the other hand, this seems less likely as the allele was identified in unrelated patients of Dutch, German, Belgian, Spanish, and North American origin[6–8] (this paper).

H R WATERHAM*
W OOSTHEIM*
G J ROMEIJN*
R J A WANDERS*
R C M HENNEKAM†

**Laboratory for Genetic Metabolic Diseases (F0-226), Departments of Clinical Chemistry and Paediatrics, Emma Children's Hospital, Academic Medical Centre, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands*

†Department of Paediatrics, Emma Children's Hospital, Academic Medical Centre, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands

Correspondence to: Dr Waterham, h.r.waterham@amc.uva.nl

1 Smith DW, Lemli L, Opitz JM. A newly recognised syndrome of multiple congenital anomalies. *J Pediatr* 1964;**64**:210-17.
2 Kelley RI. A new face for an old syndrome. *Am J Med Genet* 1997;**65**:251-6.
3 Ryan AK, Bartlett K, Clayton P, *et al.* Smith-Lemli-Opitz syndrome: a variable clinical and biochemical phenotype. *J Med Genet* 1998;**35**:558-65.
4 Tint GS, Irons M, Elias ER, *et al.* Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. *N Engl J Med* 1994;**330**:107-13.
5 Schefer S, Salen G, Batta AK, *et al.* Markedly inhibited 7-dehydrocholesterol-Δ7-reductase activity in liver microsomes from Smith-Lemli-Opitz homozygotes. *J Clin Invest* 1995;**96**:1779-85.
6 Waterham HR, Wijburg FA, Hennekam RCM, *et al.* Smith-Lemli-Opitz syndrome is caused by mutations in the 7-dehydrocholesterol reductase gene. *Am J Hum Genet* 1998;**63**:329-38.
7 Fitzky BU, Witsch-Baumgartner M, Erdel M, *et al.* Mutations in the Δ7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome. *Proc Natl Acad Sci USA* 1998;**95**:8181-6.
8 Wassif CA, Maslen C, Kachilele-Linjewile S, *et al.* Mutations in the human sterol Δ7-reductase gene at 11q12-13 cause Smith-Lemli-Opitz syndrome. *Am J Hum Genet* 1998;**63**:55-62.
9 Ijlst L, Wanders RJA, Ushikubo S, *et al.* Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of the major disease-causing mutation in the α-subunit of the mitochondrial trifunctional protein. *Biochim Biophys Acta* 1994;**1215**:347-50.
10 Honda M, Tint GS, Honda A, *et al.* Measurement of 3β-hydroxysteroid Δ7-reductase activity in cultured skin fibroblasts utilising ergosterol as a substrate: a new method for the diagnosis of the Smith-Lemli-Opitz syndrome. *J Lipid Res* 1996;**37**:2433-8.
11 Van Rooij A, Nijenhuis AA, Wijburg FA, *et al.* Highly increased CSF concentrations of cholesterol precursors in Smith-Lemli-Opitz syndrome. *J Inherit Metab Dis* 1997;**20**:578-80.

J Med Genet 2000;**37**:389–392

Clinical and molecular cytogenetic studies of a large de novo interstitial deletion 16q11.2-16q21 including the putative transcription factor gene *SALL1*

EDITOR—Interstitial deletions of the long arm of chromosome 16 share common clinical features including growth retardation, failure to thrive, microcephaly, high and prominent forehead, prominent metopic suture, large anterior fontanelle, hypertelorism, broad nasal bridge, low set and dysplastic ears, cleft palate, micrognathia, short neck, narrow thorax, broad first toes, mental retardation, muscular hypotonia, congenital heart defects, and gastrointestinal as well as renal anomalies.[1] More than 26 patients with different interstitial long arm deletions of chromosome 16 have been reported.[2–29] Recently, mutations in the transcription factor gene *SALL1* on chromosome 16q12.1[30] were shown to result in Townes-Brocks syndrome, an autosomal dominantly inherited malformation syndrome characterised by malformations of the anus, hands, and ears as well as deafness.[31] We describe a fetus with the 16q deletion syndrome and additional features, including unilateral radial aplasia, ulnar hypoplasia, preaxial hexadactyly, and segmentation defects of the vertebral column. Some of these features overlap with the malformations seen in Townes-Brocks syndrome. We therefore investigated the hypothesis that the *SALL1* gene was included within the deletion.

The 31 year old, gravida 2, para 1 (her first child is a healthy boy) was referred at 24 weeks gestation because her fetus had cleft lip and palate detected by ultrasound screening. Biometry showed asymmetrical growth retardation with a thoraco-abdominal diameter of 43 mm (<5th centile) corresponding to 19 weeks' gestation, while the BPD (60 mm) and femur length (41 mm) were within normal limits. Careful examination by ultrasound showed multiple fetal abnormalities. Dilatation of the lateral ventricles, a dilated third ventricle, and a cavum septum pellucidum were noted. Aplasia of the right radius was

Figure 1 Postmortem picture of the fetus presented. Note the left sided cleft lip/cleft palate, right sided radial aplasia, and a narrow chest.

suspected. The left kidney was absent. The right kidney was supplied by two arteries. The heart showed tetralogy of Fallot with absent pulmonary valve, agenesis of the ductus arteriosus, and a right sided aortic arch. The lungs were hypoplastic. Amniotic fluid, placenta, and umbilical vessels were normal. A fetal blood sample was taken for chromosome analysis. Necropsy examination after the parents opted for termination of the pregnancy in the 25th week of gestation showed a male fetus 31 cm in length and 690 g in weight (corresponding to the 24th/25th week of gestation). The fetus had a large neurocranium. Facial features included a prominent nasal bridge, a broad nose, hypertelorism, and upward slanting palpebral fissures. The ears were dysplastic, small, and deeply set with a single, left sided preauricular tag. The fetus had left sided cleft lip and palate. The chest was narrow. There was anal stenosis. In the upper extremities, the right radius was absent, while the ulna and thumb were hypoplastic. The left hand displayed preaxial hexadactyly with a finger-like thumb. Crowding of the toes was seen on both feet (figs 1 and 2). *X* rays confirmed the right radial aplasia and a shortened and thickened ulna. There was a single ossification centre (1 mm in diameter) within the hypoplastic right thumb, and a single ossification centre (1 mm in diameter) within the additional left thumb. The distal ossification centre of the additional left thumb was missing. The spine showed multiple segmentation defects (fusion of the third and fourth thoracic vertebral bodies, malformation of the eighth thoracic vertebral body, and aplasia of the third to fifth sacral vertebral bodies). Examination of the heart disclosed a perimembraneous ventricular septal defect (4 mm in diameter), aplasia of the semilunar pulmonary valve, agenesis of the ductus arteriosus, and overriding of the right descending aorta. The left kidney was absent.

Chromosome analysis of fetal lymphocytes showed a large interstitial deletion of the long arm of chromosome 16 in all metaphases analysed. The result was confirmed by chromosome analysis of fetal fibroblasts. The karyotypes of the parents were normal. To define the exact site of the deletion, comparative genomic hybridisation (CGH) and fluorescence in situ hybridisation (FISH) analysis were performed. CGH was performed with fetal DNA as described elsewhere.[32] CGH analysis defined the deletion to encompass bands 16q11.2 to 16q21 (fig 3). FISH analysis was performed using YACs from within and outside the deletion. YACs 922f01 (D16S415), 965g04 (D16S514/ D16S739), and 957h03 (D16S400/D16S3129) residing in 16q12-16q21 were shown to give only one signal on the normal chromosome 16, but none from the deleted chromosome 16. The STS numbers mapped to the corresponding YAC clones are given in parentheses. YAC 821g09 (D13S3021) located in 16q22 was not deleted. In addition, FISH using a PAC clone (reference: LLNLP704M031126Q4) including the *SALL1* gene[33] showed heterozygous deletion of *SALL1* (fig 4). DAPI banding confirmed the loss of the whole heterochromatin of chromosome 16 harbouring the deletion. The resulting fetal karyotype was defined as 46,XY,del(16)(q11.2;q21) according to ISCN.[34]

The phenotype of the 16q deletion syndrome was attributed to the deletion of critical bands at 16q11.2-q13,[9 12 25] 16q21,[11 16 19 21 24] and 16q22.1.[14] Specific features, such as congenital cataract[20] and iris coloboma,[28] result from more distal deletions of 16q22.3 and 16q23.1-24.2. Some of the features documented in patients with proximal deletions of chromosome 16q are also seen in Townes-Brocks syndrome.[31 35 36] The clinical presentation of TBS is highly

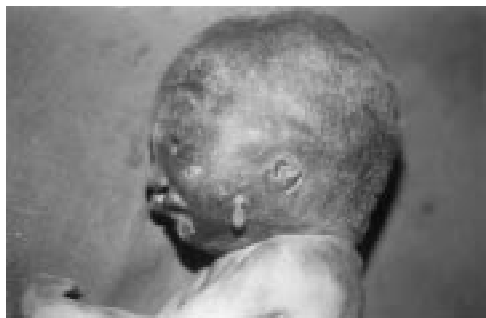

Figure 2 Lateral view of the fetal head. Note the dysplastic, small, and deeply set ears with a single left sided preauricular appendage, and retrognathia.

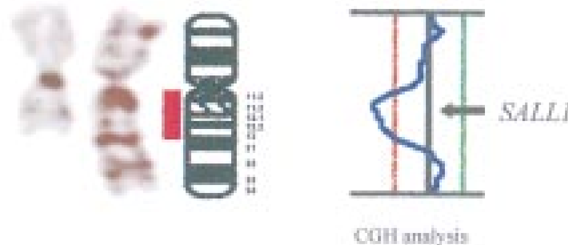

Figure 3 G banded chromosomes of fetal fibroblasts and CGH (comparative genomic hybridisation) analysis using fetal DNA showing an interstitial deletion 16q11.2-q21 according to ICSN[34] as illustrated by the ideogram. Fifteen metaphases were used for CGH analysis. The bar located on the left of the ideogram indicates the deleted region.

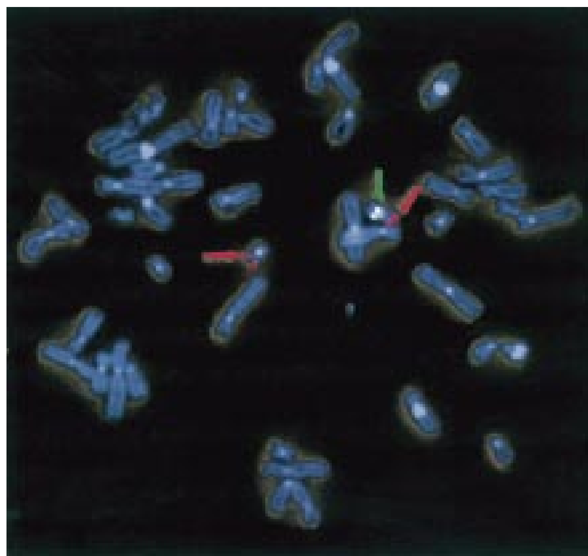

Figure 4 Two colour FISH analysis of fetal metaphase spreads (fibroblasts) using a PAC clone including SALL1.[33] The signal from the PAC clone, mapping to 16q12.1, gives only one signal on one chromosome 16, while the YAC clone (CEPH 765b06) located on the short arm of chromosome 16 (16p13) shows signals on both chromosomes.

Table 1 Clinical characteristics of patients with a deletion of the long arm of chromosome 16 including 16q12.1 or 16q13

		Reference						
		12	*17*	*18*	*25*	*11*	*16*	*19*
	Present case q11.2q21	*q12.1q13 (twins)*	*q12.1 (sibs)*	*q11.1q13*	*q12.1q13*	*q13*	*q13*	*q13q21*
Auricular malformation	+	+ +	+ +	+	+	+	+	+
Deafness	?	+ −	− −	?	SN		SN	−
Hands	Polydact	Small	T T	Small	SC	SC	Small	Polydact
Heart defects	+	− +	− −	+	−	+	−	+
Anal malformations	+	+ −	− −	−		−	Ectopic	+
Kidney hypo/agenesis	+	− −	+ +	?	−	+	+	−
Malposition of toes	+	+ +	+ +	+	+	+	−	−

SN = sensorineural deafness; polydact = preaxial polydactyly; T = proximally placed thumbs; SC = simian creases.

variable[37] within and between affected families. Characteristic features of TBS are anorectal abnormalities (imperforate anus, ectopic anus), abnormalities of the hands (preaxial polydactyly, triphalangeal thumbs, hypoplastic thumbs), abnormalities of the feet (syndactyly, club foot), deformities of the outer ear ("lop ears") and preauricular tags, and deafness.[38] Renal malformations include agenesis as well as hypoplastic or polycystic kidneys and may lead to renal failure.[39] The frequency of cardiac defects in TBS is high in those patients evaluated for cardiac defects by echocardiography.[40] Mental retardation has rarely been reported.[41 42]

TBS is caused by mutations of the *SALL1* putative transcription factor gene located on chromosome 16q12.1.[30] All mutations so far identified in TBS patients[33] are predicted to result in a prematurely terminated *SALL1* protein lacking all double zinc finger domains thought to be essential for *SALL1* gene function. Therefore, TBS is strongly suspected to result from haploinsufficiency for *SALL1*. This hypothesis is strengthened by the observation of a chromosomal translocation and a pericentric inversion in TBS patients, both involving a common breakpoint at 16q12.1.[43 44] The features of the fetus reported here show overlap with TBS. Our FISH studies prove that one allele of *SALL1* is indeed deleted. Therefore, we suggest that the ear anomalies, hand anomalies (preaxial polydactyly, hypoplastic thumb), anal stenosis, and the renal agenesis observed in the fetus result from heterozygous deletion of *SALL1*. The cardiac defects might also result from the *SALL1* deletion, since two TBS patients with tetralogy of Fallot have been described previously.[45 46] The anomalies of the radius and ulna seen in the fetus most likely result from the deletion of a contiguous gene in the deleted region. Vertebral segmentation defects have only recently been reported in a TBS patient.[47] However, a *SALL1* mutation has not been found in these patients.

Hoo *et al*[17] presented two mentally retarded sisters with a deletion of 16q12.1. Features described in these sisters included dysplastic ears with abnormal folding of the helices, proximally placed thumbs, and bilateral, symmetrically small kidneys. Twins with a deletion of 16q12.2-13 showed ectopic anus, congenital heart defects, and dysplastic ears.[12] A 10 year old boy with a de novo interstitial deletion 16q12.1q13 presented with micrognathia, a median cleft, low set dysplastic ears, a preauricular appendage, fusion of the third and fourth and the fifth and sixth vertebrae of the neck, hypospadias, hydrocephalus, sensorineural hearing deficits, and additional dysmorphic features.[25] Further clinical features described in patients with deletions of the proximal long arm of chromosome 16 are summarised in table 1. Whether or not *SALL1* is deleted in those patients reported with features similar to TBS and with deletions including 16q13 but not 16q12.1 remains to be elucidated.[11 16 19] Some families have been reported in which affected subjects show features typical of both TBS and Goldenhar syndrome/ oculoauriculovertebral spectrum.[48 49] One family was described with hemifacial microsomia and radial ray defects.[50] The affected members of this family displayed abnormal ears, multiple preauricular tags and pits, and unilateral micrognathia. In addition, triphalangeal thumbs, unilateral preaxial polydactyly, and a slightly anteriorly placed anus were present. Autosomal dominant inheritance was suggested in this family. Unilateral microphthalmia and facial asymmetry were observed in a family with anteriorly placed anus, irregular toes, and a digitalised thumb.[51] Gabrielli *et al*[48] described an infant with facial asymmetry, abnormal ears, preauricular tags, and anteriorly placed anus without triphalangeal thumbs or vertebral anomalies. Recently, a *SALL1* mutation was detected in the patient described by Gabrielli *et al*,[48] showing that *SALL1* mutations can also result in a Goldenhar-like phenotype.[33] It remains to be seen if the affected subjects in the families reported by Johnson *et al*[49] and Moeschler *et al*[50] also carry mutations in *SALL1*.

In summary, our results suggest that haploinsufficiency for *SALL1* might contribute to TBS-like features seen in the fetus presented here. In addition, certain clinical features in patients with proximal deletions of 16q including q12.1 are likely to result from haploid *SALL1* deletions. Nevertheless, the interstitial deletion in this fetus probably includes several hundred genes, arguing against a simplistic additive genetic model to explain the observed phenotype. Moreover, additional features of the 16q deletion syndrome, which are not part of the phenotypic spectrum of TBS, are likely to be caused by the deletion of additional genes located within the deletion. Some clinical features might be non-specific and cannot be attributed to the deletion of a single gene. FISH studies should indicate whether *SALL1* is deleted in those patients reported with features similar to TBS but with deletions including 16q13 but not 16q12.1. If a *SALL1* deletion cannot be confirmed in these patients, a TBS-like phenotype in these patients might still result from a position effect influencing *SALL1* gene expression.

Reference address for YACs: http://www.mpimg-berlin-dahlem.mpg.de; http://www.mpimg-berlin-dahlem.mpg.de/~ctyogen/CHRM16.HTM. We would like to thank Karin Lehmann and Gundula Leschick for expert technical assistance. Dr Friedrich C Luft helped us with the text. This work was funded in part by a grant from the Wilhelm-Sander-Stiftung to JK.

HANS KNOBLAUCH*
GUNDULA THIEL*
SIGRID TINSCHERT*
HANNELORE KÖRNER*
CORNELIA TENNSTEDT†
RABIH CHAOUI‡
JÜRGEN KOHLHASE§
CHRISTA DIXKENS§
CRISTOPHER BLANCK§

**Institut für Medizinische Genetik, Universitätsklinikum Charité, 10098 Berlin, Germany*

†Institut für Pathologie, Universitätsklinikum Charité, 10098 Berlin, Germany

‡Abteilung für Pränatale Diagnostik und Therapie, Universitätsklinikum Charité, 10098 Berlin, Germany

§Institut für Humangenetik, Universität Göttingen, Heinrich-Düker-Weg, 37073 Göttingen, Germany

1 Fryns JP, Bande-Knops J, Van den Berghe H. Partial monosomy of the long arm of chromosome 16: a distinct clinical entity? *Hum Genet* 1979;**46**:115-20.
2 Brenholz P, Wilmot P, Shapiro LR. Fragile sites on chromosome 16 and the 16q- syndrome. *Am J Hum Genet* 1982;**34**:119A.
3 Callen DF, Eyre H, Lane S, *et al*. High resolution mapping of interstitial long arm deletions of chromosome 16: relationship to phenotype. *J Med Genet* 1993;**30**:828-32.
4 Carter NP, Ferguson-Smith MA, Perryman MT, *et al*. Reverse chromosome painting: a method for the rapid analysis of aberrant chromosomes in clinical cytogenetics. *J Med Genet* 1992;**29**:299-307.
5 Casamassima AC, Klein RM Wilmot PL, Brenholz P, Shapiro LR. Deletion of 16q with prolonged survival and unusual radiographic manifestation. *Am J Med Genet* 1990;**37**:504-9.
6 Chen CP, Chern SR, Lee CC, Chen LF, Chuang CY. Prenatal diagnosis of *de novo* interstitial 16q deletion in a fetus associated with sonographic findings of prominent coronal sutures, a prominent frontal bone, and shortening of the long bones. *Prenat Diagn* 1998;**18**:490-5.
7 Cooke A, Tolmie J, Darlington W, Boyd E, Thomson R, Ferguson-Smith MA. Confirmation of a suspected 16q deletion in a dysmorphic child by flow karyotype analysis. *J Med Genet* 1987;**24**:88-92.
8 Crawford MN, Punnett HN, Carpenter GG. Deletion of the long arm of chromosome 16 and an unexpected Duffy blood group phenotype reveal a possible autosomal linkage. *Nature* 1967;**215**:1075-6.
9 Doco-Fenzy M, Elchardus JF, Brami G, Digeon B, Gruson N, Adnet JJ. Multiple critical smallest region of overlap in monosomy 16q syndrome. *Genet Couns* 1994;**5**:39-44.
10 Duca D, Meilã P, Anca I, *et al*. Sindroamele cromozomiale 16. *Pediatria* 1981;**30**:365-71.
11 Edelhoff S, Maier B, Trautmann U, Pfeiffer RA. Interstitial deletion of 16(q13q22) in a newborn resulting from a paternal insertional translocation. *Ann Genet* 1991;**34**:85-9.
12 Elder FFB, Ferguson JW, Lockart LH. Identical twins with deletion 16q syndrome: evidence that 16q12.2-q13 is the critical band region. *Hum Genet* 1984;**67**:233-6.
13 Ferguson-Smith MA, Aitken DA. Heterozygosity at the alpha-haptoglobin locus associated with a deletion 16q22-16qter. *Cytogenet Cell Genet* 1978;**22**:513.
14 Fujiwara M, Yoshimoto T, Morita Y, Kamada M. Interstitial deletion of chromosome 16q: 16q22 is critical for 16q- syndrome. *Am J Med Genet* 1992;**43**:561-4.
15 Fryns JP, Melchior S, Jaeken J, Van den Berghe H. Partial monosomy of the long arm of chromosome 16 in a malformed newborn: karyotype 46,XX,del(16)(q21). *Hum Genet* 1977;**38**:343-6.
16 Fryns JP, Proesmans W, Van Hoey G, Van den Berghe H. Interstitial 16q deletion with typical dysmorphic syndrome. *Ann Genet* 1981;**24**:124-5.
17 Hoo JJ, Lowry RB, Lin CC, Haslam RHA. Recurrent *de novo* interstitial deletions of 16q in two mentally retarded sisters. *Clin Genet* 1985;**27**:420-5.
18 Krauss CM, Caldwell D, Atkins L. Interstitial deletion and ring chromosome derived from 16q. *J Med Genet* 1987;**24**:308-12.
19 Lin CC, Lowry RB, Snyder FF. Interstitial deletion for a region in the long arm of chromosome 16. *Hum Genet* 1983;**65**:134-8.
20 Monaghan KG, Van Dyke DL, Wiktor A, Feldman GL. Cytogenetic and clinical findings in a patient with a deletion of 16q23.1: first report of bilateral cataracts and a 16q deletion. *Am J Med Genet* 1997;**73**:180-3.
21 Naritomi K, Shimora N, Izumikawa Y, Sameshima K, Ohdo S, Hirayama K. 16q21 is critical for the 16q deletion syndrome. *Clin Genet* 1988;**33**:372-5.
22 Natt E, Westphal EM, Toth-Fejel SE, *et al*. Inherited and *de novo* deletion of the tyrosine aminotransferase gene locus at 16q22.1-q22.3 in a patient with tyrosinemia type II. *Hum Genet* 1987;**77**:352-8.
23 Natt E, Magenis RE, Zimmer J, Mansouri A, Scherer G. Regional assignment of the human loci for uvomorulin (UVO) and chymotrypsin B (CTRB) with the help of two overlapping deletions on the long arm of chromosome 16. *Cytogenet Cell Genet* 1989;**50**:145-8.
24 Rivera H, Vargas-Moyeda E, Möller M, Torres-Lamas A, Cantú JM. Monosomy 16q: a distinct syndrome. *Clin Genet* 1985;**28**:84-6.
25 Schuffenhauer S, Callen DF, Seidel H, Shen Y, Lederer G, Murken J. *De novo* interstitial deletion 16(q12.1q13) of paternal origin in a 10-year-old boy. *Clin Genet* 1992;**42**:246-58.
26 Taysi K, Fishman M, Sekhons GS. A terminal long arm deletion of chromosome 16 in a dysmorphic infant: 46,XY,del(16)(q22). *Birth Defects* 1978;**XIV**(6C):343-7.
27 Trautmann U, Pfeiffer RA, Seufert-Satomi U, Tietze HU. Simultaneous *de novo* interstitial deletion of 16q21 and intercalary duplication of 19q in a retarded infant with minor dysmorphic features. *J Med Genet* 1993;**30**:330-1.
28 Werner W, Kraft S, Callen DF, Bartsch O, Hinkel GK. A small deletion of 16q23.1-16q24.2 (del(16)(q23.1q24.2).ish del(16)(q23.1q24.2) (D16S395+, D16S348-, P5432)) in a boy with iris coloboma and minor anomalies. *Am J Med Genet* 1997;**70**:371-6.
29 Witt DR, Lew SP, Mann J. Heritable deletion of band 16q21 with normal phenotype relationship to late replicating DNA. *Am J Hum Genet* 1988;**43**:A127.
30 Kohlhase J, Wischermann A, Reichenbach H, Froster U, Engel W. Mutations in the *SALL1* putative transcription factor gene cause Townes-Brocks syndrome. *Nat Genet* 1998;**18**:81-3.
31 Townes PL, Brocks ER. Hereditary syndrome of imperforate anus with hand, foot, and ear anomalies. *J Pediatr* 1972;**81**:321-6.
32 Schröck E, Thiel G, Lozanova T, *et al*. Comparative genomic hybridization of human malignant gliomas reveals multiple amplification sites and nonrandom chromosomal gains and losses. *Am J Pathol* 1994;**144**:1203-17.
33 Kohlhase J, Taschner PE, Burfeind P, *et al*. Molecular analysis of *SALL1* mutations in Townes-Brocks syndrome. *Am J Hum Genet* 1999;**64**:435-45.
34 ISCN 1995. In: Mitelman F, ed. *An international system for human cytogenetic nomenclature*. Basel: S Karger, 1995.
35 Wischermann A, Holschneider AM. Townes-Brocks-Syndrom. *Monatsschr Kinderheilkd* 1997;**145**:382-6.
36 Powell CM, Michaelis RC. Townes-Brocks syndrome. *J Med Genet* 1999;**36**:89-93.
37 De Pina-Neto J. Phenotypic variability in Townes-Brocks syndrome. *Am J Med Genet* 1984;**18**:147-52.
38 Rossmiller DR, Pasic TR. Hearing loss in Townes-Brocks syndrome. *Otolaryngol Head Neck Surg* 1994;**111**:175-80.
39 Newman WG, Brunet MD, Donnai D. Townes-Brocks syndrome presenting as end stage renal failure. *Clin Dysmorphol* 1997;**6**:57-60.
40 O'Callaghan M, Young ID. The Townes-Brocks syndrome. *J Med Genet* 1990;**27**:457-61.
41 Cameron TH, Lachiewicz AM, Aylsworth AS. Townes-Brocks syndrome in two mentally retarded youngsters. *Am J Med Genet* 1991;**41**:1-4.
42 Ishikiriyama S, Kudoh F, Shimojo N, Iwai J, Inoue T. Townes-Brocks syndrome associated with mental retardation. *Am J Med Genet* 1996;**61**:191-2.
43 Friedman P, Rao K, Aylsworth A. Six patients with the Townes-Brocks syndrome including five familial cases with a pericentric inversion of chromosome 16. *Am J Hum Genet Suppl* 1987;**41**:A60.
44 Serville F, Lacombe D, Saura R, Billeaud C, Sergent, MP. Townes-Brocks syndrome in an infant with translocation t(5;16). *Genet Couns* 1993;**4**:109-12.
45 Hersch JH, Jaworski M, Solinger RE, Weisskopf B, Donat J. Townes syndrome: a distinct multiple malformation syndrome resembling VACTERL association. *Clin Genet* 1986;**25**:100-2.
46 Parent P, Bensaid M, LeGuern H, *et al*. Clinical heterogeneity of Townes-Brocks syndrome. *Arch Pediatr* 1995;**2**:551-4.
47 Marlin S, Toublanc JE, Petit C. Two cases of Townes-Brocks syndrome with previously undescribed anomalies. *Clin Dysmorphol* 1998;**7**:295-8.
48 Gabrielli O, Bonifazi V, Offidani AM, Cellini A, Coppa GV, Giorgi PL. Description of a patient with difficult nosological classification: Goldenhar syndrome or Townes-Brocks syndrome. *Minerva Pediatr* 1993;**45**:459-62.
49 Johnson JP, Poskanzer L, Sherman S. Three-generation family with resemblance to Townes-Brocks syndrome and Goldenhar/oculo-auriculo-vertebral spectrum. *Am J Med Genet* 1996;**61**:134-9.
50 Moeschler J, Clarren SK. Familial occurrence of hemifacial microsomia with radial limb defects. *Am J Med Genet* 1982;**12**:371-5.
51 Fraser FC, Cooper AR. Micropthalmia as part of the Townes (sic) syndrome. *Proc Greenwood Genet Centre* 1985;**4**:129.

J Med Genet 2000;**37**:392–394

Oculocutaneous albinism type 2 with a *P* gene missense mutation in a patient with Angelman syndrome

EDITOR—Oculocutaneous albinism type 2 (OCA2) is an autosomal recessive disorder characterised by defective melanin production of the skin, hair, and eyes,[1] which is caused by mutations of the *P* gene.[2 3] The specific function of *P* has not been clarified, although it is likely to act as a transporter in the melanosomal membrane.[2 4]

The *P* gene is located in 15q11-q13, which is deleted in the majority of patients with Angelman syndrome (AS) and Prader-Willi syndrome (PWS).[2 5] The *P* gene is not imprinted and both alleles are expressed. PWS and AS patients with typical deletions are thus hemizygous for *P*. It is also well established that AS and PWS deletion patients usually show hypopigmentation of the skin and hair, and *P* is suggested to be responsible for this hypopigmentation as well,[6 7] although the mechanism has not yet been established.

A small intragenic deletion and a V443I missense mutation of the *P* gene were identified in the maternally inherited alleles of two PWS plus OCA2 patients who had a paternally inherited deletion of 15q11-q13.[2 3] Here we describe the first evidence that a *P* gene mutation is responsible for OCA2 associated with AS.

The male patient was born at 38 weeks' gestation to unrelated Japanese parents. There were no complications of pregnancy or delivery, but the birth weight, 1850 g, was small for gestational dates. Generalised albinism was noted at birth. Motor development was delayed, and he had a generalised tonic-clonic convulsion at the age of 18 months. He was admitted to hospital at the age of 19 months because of non-convulsive epileptic status, as reported previously.[8] Physical examination showed the