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ARTICLE

Postzygotic mutation and germline mosaicism in the otopalatodigital syndrome spectrum disorders

Stephen P Robertson*,[1], Sarah Thompson[1], Timothy Morgan[1], Muriel Holder-Espinasse[3], Véronique Martinot-Duquenoy[4], Andrew OM Wilkie[2] and Sylvie Manouvrier-Hanu[3]

[1]*Department of Paediatrics and Child Health, Dunedin School of Medicine, Otago University, Dunedin, New Zealand;* [2]*Weatherall Institute of Molecular Medicine, Oxford University, Oxford, UK;* [3]*Department of Clinical Genetics, University Hospital, Lille, France;* [4]*Department of Plastic Surgery, University Hospital, Lille, France*

The otopalatodigital syndrome (OPD) spectrum disorders are a heterogeneous group of skeletal dysplasias caused by mutations in the X-linked gene, *FLNA*. All OPD spectrum disorders (otopalatodigital syndromes types 1 and 2, frontometaphyseal dysplasia and Melnick-Needles syndrome) exhibit significant interfamilial variability in their expressivity, especially in female subjects. Factors contributing to this may include allelic heterogeneity, variation in the degree of skewing of X inactivation or, conceivably, mosaicism for the underlying causative mutation. We report here monozygotic twin sisters who are discordant for the severe phenotype, Melnick-Needles syndrome, associated with the heterozygous mutation, 3596C > T. We also describe two brothers with otopalatodigital syndrome type 1 due to the *FLNA* mutation 620G > A. The mutation is not detectable in the blood leucocytes of their clinically unaffected mother, indicating that she is a germline mosaic for the condition. The description of somatic mutations and germline mosaicism in *FLNA* has implications for clinical and molecular diagnosis, phenotypic expression and genetic counseling of families with these disorders.
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Introduction

The otopalatodigital syndrome (OPD) spectrum disorders are a phenotypically heterogeneous group of conditions characterized by a skeletal dysplasia and variable anomalies in the brain, craniofacial structures, cardiac, genitourinary and gastrointestinal systems.[1] The four principal constituent syndromes within this group are otopalatodigital syndromes types 1 and 2 (OPD1, OMIM 311300:[2] OPD2, OMIM 304120[3]), frontometaphyseal dysplasia (FMD, OMIM 305620[4]) and Melnick-Needles syndrome (MNS, OMIM 309350[5]). Male subjects with OPD1 exhibit the mildest phenotype consisting of cleft palate, deafness and a skeletal dysplasia. Individuals with OPD2 are more severely affected and have a variety of extraskeletal anomalies. Apart from a skeletal dysplasia, male subjects with FMD have a propensity to develop stenoses of the subglottic region, ureters and urethra. The most severe end of the phenotypic spectrum is represented by MNS. This condition presents primarily in the female subjects with a skeletal dysplasia that can limit respiratory function and mobility. Males born to female subjects with MNS are usually lethally affected in the embryonic or fetal period, although some survive beyond infancy.[1,6]

Missense mutations or small in-frame deletions in the X-linked gene encoding filamin A (*FLNA*) give rise to all four OPD spectrum phenotypes.[7] All male individuals with OPD1 and all female individuals with MNS reported to date

*Correspondence: Professor SP Robertson, Department of Paediatrics and Child Health, Dunedin School of Medicine, Otago University, PO Box 913, Dunedin, New Zealand.
Tel: +64 3 4797469; Fax: +64 3 4797469;
E-mail: stephen.robertson@stonebow.otago.ac.nz
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have had identifiable mutations in *FLNA*. However, some male and female individuals with phenotypes consistent with OPD2 and FMD have not had mutations identified using highly sensitive screening modalities, suggesting that genetic heterogeneity exists for these conditions.

Putative loss-of-function mutations in *FLNA* lead to disorders that are phenotypically distinct from the OPD spectrum group and are characterized by abnormalities of neuronal migration – periventricular nodular heterotopia (PVNH, OMIM 300049[8]) and PVNH-Ehlers Danlos syndrome.[9] The PVNH disorders characteristically present in female individuals, with germline mutations in male individuals assumed to lead to embryonic lethality.

The female phenotypes associated with the OPD spectrum disorders demonstrate variable expressivity.[10,11] Although a component of this is undoubtedly due to allelic heterogeneity, some intrafamilial variability in phenotypic expression has been associated with differences in the degree of skewing of X inactivation,[12] whereas other instances cannot be explained on this basis.[13] Recently, missense mutations and somatic mosaicism have been shown to underlie milder phenotypes in the PVNH spectrum and confer survivability in male individuals who present with these conditions.[14] Most male individuals who were demonstrated to be mosaic for mutations in *FLNA* had attenuated phenotypes as compared to female individuals with the same mutation.

We report here two instances of postzygotic mutation in *FLNA* that has led on both occasions to an OPD spectrum disorder. In one instance, we demonstrate the presence of the mutation 3596C>T in *FLNA*, leading to MNS in one of a pair of monozygotic twins. In the second instance, a clinically unaffected female individual has produced two sons with OPD1. These findings confirm that postzygotic mutations in *FLNA* do occur and could influence the phenotypic variability of the OPD spectrum disorders. The demonstration of germline mosaicism for OPD1 indicates that caution should be exercised in counseling recurrence risks for these conditions upon presentation of an isolated case.

Materials and methods

Case reports

Family 1 Twin girls were born at term to a healthy nonconsanguineous couple. There was one older male sibling who was well. Sonograms throughout the pregnancy were unremarkable apart from the demonstration of separate amniotic sacs within a single chorion. Delivery was at 36 weeks. The first twin had a birthweight of 2.7 kg; the second twin weighed 2.6 kg. Twin 1 had recurrent respiratory obstruction during the first 18 months owing to significant tracheobronchomalacia. Clinical and radiographic evaluation led to the observation of thoracic hypoplasia and bowing of the upper and lower limbs with a subsequent diagnosis of Melnick-Needles syndrome at 17 months of age. Additional anomalies included a large atrial septal defect, multiple ventricular septal defects and bilateral hydronephrosis owing to ureteric stenosis that necessitated surgical intervention on the right. Subsequently, multiple orthopaedic surgical procedures have been required, including chest expansion surgery, to retain mobility and improve respiratory function. A requirement for ambulatory oxygen supplementation developed at age 11 years. Her height at the age of 18 years is 145 cm (−3.1 SD) and her weight is 37 kg (−2.7 SD) (Figure 1a). Her twin sister (Figure 1b) has remained healthy with no significant illnesses. She is nondysmorphic. Her height is 163 cm (+0 SD). Characterization of the mutation underlying the phenotype observed in twin 1 has previously been reported.[7]

Family 2 The first affected male in this family was the second of four sons born to nonconsanguineous healthy parents after an unremarkable pregnancy. In particular, the mother did not exhibit any dysmorphism or hearing loss. There was no family history of cleft palate, digital anomalies or deafness. At birth, the child was noted to have broad thumbs, bilateral duplicated great toes and a cleft soft palate. At 11 years of age, he was reassessed. His height was 149 cm (+1 SD), his weight was 49 kg (+3 SD), and his OFC was 56 cm (+1.5 SD). He had a flat midface, hypertelorism, downslanting palpebral fissures and esotropia. Bilateral II–III toe syndactyly was once again noted in addition to his digital anomalies (Figure 2a). He had conductive deafness (a 40 dB loss in the low-frequency range). A CT scan showed bilateral fusion of the malleus to the attic of the middle ear. His school progress was below average but was attributed to a delayed diagnosis of his deafness. Skull radiographs showed absent frontal sinuses and hand radiographs showed poorly modeled metacarpals

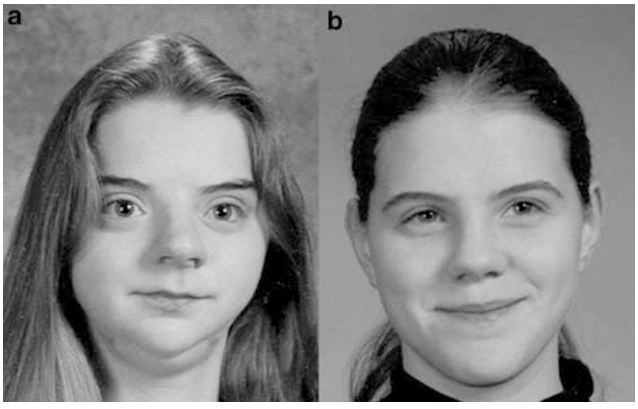

Figure 1 Clinical appearances of the twin sisters. (**a**) Twin 1 has exorbitism, supraorbital hyperostosis and micrognathia consistent with her diagnosis of MNS. (**b**) Twin 2 demonstrates no manifestations of the condition.

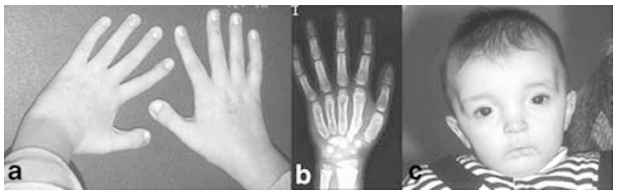

Figure 2 Clinical photographs and radiographs of two brothers with OPD type 1. (**a**) The hands of the affected elder brother demonstrate proximally set thumbs, which are also broad and spatulate. (**b**) Radiographs of the hands show undermodelling of all metacarpals and phalanges, pronounced broadening and shortening of the first metacarpal and an accessory ossification centre at the base of the second metacarpal. (**c**) Clinical appearance of the affected younger brother male demonstrating hypertelorism, downslanting palpebral fissures, flat midface and micrognathia.

and phalanges, a notably shortened and broadened first metacarpal and a secondary ossification centre at the base of the second metacarpal (Figure 2b). Radiographs of the feet showed short, broad metatarsals. At 14 years of age, he underwent an unsuccessful surgical procedure to treat the middle ear malformation. At 17 years of age, his height was 170 cm (0 SD), and his weight was 90 kg (+4 SD).

The fourth son of this same couple was born after an unremarkable pregnancy.

At birth, Pierre Robin sequence with glossoptosis, micrognathia and cleft hard palate were noted in addition to broad thumbs and great toes. He required respiratory support until 4 months of age. He was fed partially via a gastrostomy tube from 5 months to 2 years of age owing to swallowing difficulties. At 7 months of age, a pectus excavatum was noticed. Radiographs showed delayed bone maturation, broad first metacarpals and metatarsals and an increased mandibular angle. At 9 months of age, the cleft palate was surgically repaired. At 18 months, deafness was identified and a CT scan revealed ossicular malformations with dysplastic stapes and hypoplastic round windows bilaterally.

At the age of 7 years, his growth (height 122 cm (+0 SD), weight 23 kg (+0 SD), OFC 53.5 cm (+1SD)) and cognitive development are normal. He has dysmorphic features (hypertelorism, downslanting palpebral fissures, fullness of the lateral supraorbital ridges) and pectus excavatum (Figure 2c). Hand radiographs show broad first metacarpals and phalanges, shortened distal phalanges, a pseudoepiphysis at the base of proximal phalanx II bilaterally and abnormal carpal bones (a transverse capitate, preaxially relocated trapezoid and one supernumerary carpal bone near the lunate bone).

Molecular analysis

Ethical approval was obtained from the Otago Ethics Committee and informed consent was obtained from each participating individual or their guardian. DNA was extracted from blood leucocytes and cultured skin fibroblasts using standard procedures. Examination of *FLNA* for pathogenic mutations was performed by amplification of the coding regions and direct sequencing using an ABI3100 automated sequencer as previously described.[7] Primers for amplifying and sequencing exons 3 and 22 of *FLNA* are available on request. Zygosity analysis was performed at the Australian Genome Resource Facility and consisted of genotyping of 30 unlinked polymorphic microsatellite repeats with published heterozygosity values >0.75, in both twin sisters. The prior probability of two same-sex twin siblings being monozygotic was assigned as 0.5.

The relative degree of mosaicism for the 3596C>T mutation in *FLNA* was quantified by the use of an assay using the restriction enzyme *Hyp*188I, which has a recognition site ablated by the 3596C>T mutation. Exon 22 of *FLNA* was amplified using genomic DNA (40 ng) from leucocytes from both twin sisters, a normal female control, an unrelated germline heterozygote for the 3596C>T mutation and from skin fibroblasts obtained from the affected twin. The forward primer for this PCR was fluorescently labeled. Reaction conditions were an initial denaturing step of 10 min at 96°C followed by 24 cycles of 30 s at 60°C, 30 s at 72°C and 60 s at 96°C followed by a final extension step of 7 min at 72°C. The resultant PCR products were digested with *Hyp*188I at 37°C and resolved on an ABI 377 analyser. The bands corresponding to the mutant and wild-type alleles were quantified using GeneScan 2.1 software, expressed as a percentage and normalized to results obtained from the germline control.

Results

Family 1

Amplification and sequence analysis of exon 22 of *FLNA* were performed on DNA extracted from both whole blood and skin fibroblasts from the affected twin from family 1. As previously reported, a single heterozygous mutation, 3596C>T, that predicts the substitution S1199L in repeat 10 of filamin A, was identified in DNA extracted from both tissues (case ID 2239).[7] The mutation was shown to be absent in DNA extracted from blood leucocytes from her twin sister and mother (Figure 3a). Zygosity analysis of the two twins revealed that identical alleles were shared by both individuals at all 30 microsatellite marker loci analysed indicating that the posterior probability of the twins being dizygotic is <0.001.

Quantitation of the degree of mosaicism in the affected twin was obtained by utilizing a restriction fragment length polymorphism created by the mutation for the enzyme *Hyp*188I (Figure 4a). The ratio of mutant to wild-type allele in both leucocytes and skin fibroblasts from the affected twin was not significantly different from that measured in an unrelated female subject who was an obligate heterozygote for the same mutation (Figure 4b).

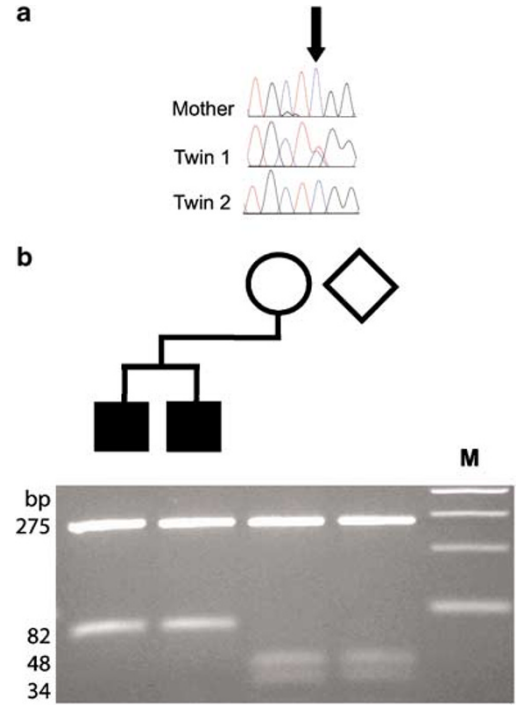

Figure 3 (**a**) Sequence chromatograms showing presence of the 3596C>T mutation in one twin sister but absence of the same mutation in her monozygotic twin and mother. (**b**) *Hpa*II restriction enzyme digest of a 357 bp PCR-amplified DNA product from exon 3 of *FLNA* from the brothers affected with OPD1 and their clinically unaffected mother. M: DNA markers.

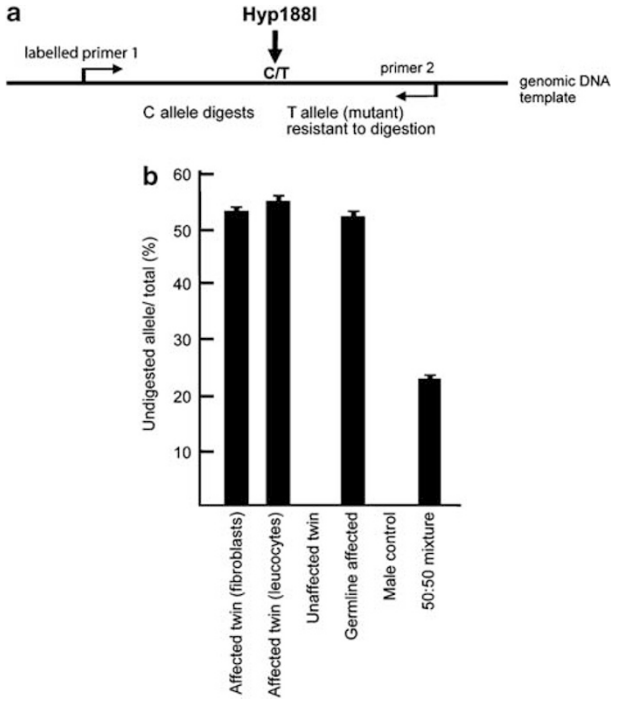

Figure 4 Quantitation of the degree of mosaicism for the 3596C>T mutation. (**a**) Fluoresently labeled PCR products from exon 22 of *FLNA* were digested with *Hyp*188I and the amount of mutant allele (that fraction of the PCR product resistant to digestion) was expressed as a percentage of the total yield of the reaction. DNA from a healthy male control individual was used as a control to verify complete digestion of the template. (**b**) Genomic DNA was derived from leucocytes (affected and unaffected twin, germline control, healthy male control) and fibroblasts (affected twin). The 50:50 mixture denotes results using DNA consisting of an equal amount of genomic DNA from an affected germline individual for the 3596C>T mutation and an unrelated healthy female control. Depicted are the results (±SEM) from six independent replicates.

Family 2

Exon 3 of *FLNA* was sequenced in both brothers from family 2, with a clinical diagnosis of OPD1 and their clinically unaffected mother. A single transition, 620C>T, that predicts the substitution P207L within the actin-binding domain of filamin A, was found in both brothers but was shown to be absent in leucocyte DNA obtained from the mother (Figure 3b). Sample misassignment was excluded by demonstrating segregation of several microsatellite markers in a pattern consistent with the declared parental relationship. The pathogenicity of this mutation has been previously established in two families segregating an OPD1 phenotype.[2,7,15] DNA samples were not available from the two unaffected siblings.

Discussion

The demonstration of mosaicism for pathogenic mutations has important ramifications for the diagnosis of conditions and counseling offered to families. Somatic mutations can lead to attenuated presentations of phenotypes, segmental disease and tissue-restricted expression of disease states.[16] Germline mosaicism is well documented in a wide range of genetic diseases with the phenomenon being commonly encountered in conditions such as osteogenesis imperfecta and Duchenne muscular dystrophy.[17] Many individuals with germline mosaicism have also been shown to be low level somatic mosaics. In the instance described here (family 2), there were no clinical manifestations evident in the mother of the two male individuals with OPD1, but no tissues other than blood were available to examine for the presence of the mutation.

This report also describes the demonstration of a postzygotic mutation in one of a pair of monozygotic twins who were disconcordant for the MNS phenotype. Molecular verification of disconcordance between monozygotic twins for a Mendelian phenotype is rare but has been previously reported. Examination of 30 polymorphic microsatellite markers in both sisters indicated that they shared identical alleles at all loci, establishing the odds that these two siblings represent monozygotic twins as greater than 1000:1. The affected twin exhibited pronounced features of the disorder. Her phenotype, with disease manifestations in multiple organ systems (craniofacial dysmorphism, height less than −3 SD, tracheobronchomalacia,

requirement for ambulatory oxygen from early teenage years, congenital heart disease, bilateral hydronephrosis), is as severe as some individuals who have the same mutation in their germline.[18] Further analysis of the degree of mosaicism in this individual in two tissues indicated that her mutational load was indistinguishable from that of a female individuals, who is a germline heterozygote for the identical mutation (Figure 4b), an observation that is consistent with her severe clinical phenotype. Although this individual must have sustained the 3596C>T mutation at a very early stage postcleavage and as a consequence does not present a milder phenotype, there clearly remains the possibility that attenuated phenotypes could result from somatic mutations that occur later in development. This hypothesis is supported by the recent demonstration of mosaicism for mutations in *FLNA* in male individuals with PVNH. Germline transmission of these alleles from female individuals leads to embryonic lethality in the male individual, whereas in male individuals with mosaicism, a phenotype similar to, or milder than, that found in female individuals with PVNH has been observed.[14,19]

Disconcordance between monozygotic twins for mutations leading to a Mendelian disorder has been reported previously.[20] Such occurrences underscore that postzygotic mutation is one of the multiple mechanisms that can contribute to make monozygotic twins genetically non-identical.

Mosaicism may also account for some reports of clinical diagnoses of MNS in male individuals.[6] An over-representation of female individuals in reports on this phenotype and the description of a male phenotype characterized by a severe skeletal dysplasia and multiple visceral, craniofacial and central nervous system anomalies has led to the suggestion that germline mutations associated with MNS in the female individuals lead to a perinatal lethal phenotype in the hemizygous state.[21] Most male subjects reported in the literature as with Melnick-Needles syndrome can be categorized into one or the other of two groups. A very severe, usually lethal, MNS phenotype presents in male individuals born to mothers manifesting MNS.[22] In contrast, milder male phenotypes are reported in sons born to unaffected women.[6,23] Although at least one exception to this rule has been reported more recently indicating that mechanisms other than mosaicism contribute to variable expressivity in male subjects with this condition,[1] it is conceivable that mildly affected male subjects are mosaic for MNS-causing mutations.

The description of germline mosaicism for the mutation 620C>T leading to OPD1 in two brothers has also important implications for genetic counseling in these conditions. Our observation indicates that the recurrence risk for an OPD spectrum disorder in a family in whom a proband presents as an isolated case and the mother is not a germline carrier is higher than the new mutation rate. A precise estimate of this risk cannot be arrived at until large cohorts of families have been analysed, and the frequency of germline mosaicism empirically measured. Although germline mosaicism has not been reported for PVNH, the report of postzygotic mutations leading to somatic mosaicism[14] would suggest that this is possible. In the case of PVNH and OPD1 in particular, constitutional heterozygotes for pathogenic mutations can have minimal clinical manifestations. Only 85% of female subjects with PVNH manifest epilepsy despite having periventricular neuronal heterotopia apparent on MRI.[8,24] Obligate carriers of OPD1 mutations can similarly demonstrate little or no clinical craniofacial or digital anomalies.[10] In the latter condition, establishing the identity of the underlying mutation will aid in differentiating a recurrence risk of an obligate carrier from that associated with germline mosaicism for the mutant allele.

The observation of somatic mosaicism in MNS may explain some of the variable expressivity in this condition. Additionally, the possibility of germline mosaicism must now be considered in counseling recurrence risks for families who present with a single isolated individual with an OPD spectrum disorder.

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References

1 Verloes A, Lesenfants S, Barr M *et al*: Fronto-otopalatodigital osteodysplasia: clinical evidence for a single entity encompassing Melnick-Needles syndrome, otopalatodigital syndrome types 1 and 2, and frontometaphyseal dysplasia. *Am J Med Genet* 2000; **90**: 407–422.
2 Dudding BA, Gorlin RJ, Langer LO: The oto-palato-digital syndrome. A new symptom-complex consisting of deafness, dwarfism, cleft palate, characteristic facies, and a generalized bone dysplasia. *Am J Dis Child* 1967; **113**: 214–221.
3 Fitch N, Jequier S, Papageorgiou A: A familial syndrome of cranial, facial, oral and limb anomalies. *Clin Genet* 1976; **10**: 226–231.
4 Gorlin RJ, Cohen Jr MM: Frontometaphyseal dysplasia. A new syndrome. *Am J Dis Child* 1969; **118**: 487–494.
5 Melnick JC, Needles CF: An undiagnosed bone dysplasia. A 2 family study of 4 generations and 3 generations. *Am J Roentgenol Radium Ther Nucl Med* 1966; **97**: 39–48.
6 van der Lely H, Robben SG, Meradji M, Derksen-Lubsen G: Melnick-Needles syndrome (osteodysplasty) in an older male – report of a case and a review of the literature. *Br J Radiol* 1991; **64**: 852–854.
7 Robertson SP, Twigg SR, Sutherland-Smith AJ *et al*: Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nat Genet* 2003; **33**: 487–491.
8 Fox JW, Lamperti ED, Eksioglu YZ *et al*: Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* 1998; **21**: 1315–1325.
9 Sheen VL, Jansen A, Chen MH *et al*: Filamin A mutations cause periventricular heterotopia with Ehlers-Danlos syndrome. *Neurology* 2005; **64**: 254–262.

10 Gorlin RJ, Poznanski AK, Hendon I: The oto-palato-digital (OPD) syndrome in females. *Oral Surg Oral Med Oral Pathol* 1973; **35**: 218–224.
11 Robertson S, Gunn T, Allen B, Chapman C, Becroft D: Are Melnick-Needles syndrome and oto-palato-digital syndrome type II allelic? Observations in a four-generation kindred. *Am J Med Genet* 1997; **71**: 341–347.
12 Robertson SP, Walsh S, Oldridge M, Gunn T, Becroft D, Wilkie AO: Linkage of otopalatodigital syndrome type 2 (OPD2) to distal Xq28: evidence for allelism with OPD1. *Am J Hum Genet* 2001; **69**: 223–227.
13 Kristiansen M, Knudsen GP, Soyland A, Westvik J, Orstavik KH: Phenotypic variation in Melnick-Needles syndrome is not reflected in X inactivation patterns from blood or buccal smear. *Am J Med Genet* 2002; **108**: 120–127.
14 Guerrini R, Mei D, Sisodiya S *et al*: Germline and mosaic mutations of FLN1 in men with periventricular heterotopia. *Neurology* 2004; **63**: 51–56.
15 Le Marec B, Odent S, Bracq E, Bulard MB, Bourdiniere J, Babut JM: [Oto-palato-digital type I syndrome in five generations. Relationship to the type II form]. *Ann Genet* 1988; **31**: 155–161.
16 Youssoufian H, Pyeritz RE: Mechanisms and consequences of somatic mosaicism in humans. *Nat Rev Genet* 2002; **3**: 748–758.
17 Zlotogora J: Germ line mosaicism. *Hum Genet* 1998; **102**: 381–386.
18 Krajewska-Walasek M, Kozlowski K: Melnick-Needles syndrome. *Australas Radiol* 1994; **38**: 146–147.
19 Parrini E, Mei D, Wright M, Dorn T, Guerrini R: Mosaic mutations of the FLN1 gene cause a mild phenotype in patients with periventricular heterotopia. *Neurogenetics* 2004; **5**: 191–196.
20 Kondo S, Schutte BC, Richardson RJ *et al*: Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nat Genet* 2002; **32**: 285–289.
21 Krajewska-Walasek M, Winkielman J, Gorlin RJ: Melnick-Needles syndrome in males. *Am J Med Genet* 1987; **27**: 153–158.
22 Donnenfeld AE, Conard KA, Roberts NS, Borns PF, Zackai EH: Melnick-Needles syndrome in males: a lethal multiple congenital anomalies syndrome. *Am J Med Genet* 1987; **27**: 159–173.
23 Theodorou SD, Ierodiaconou MN, Gerostathopoulos N, Grivas T: Osteodysplasty (Melnick-Needles syndrome) in a male. *Prog Clin Biol Res* 1982; **104**: 139–142.
24 Sheen VL, Dixon PH, Fox JW *et al*: Mutations in the X-linked filamin 1 gene cause periventricular nodular heterotopia in males as well as in females. *Hum Mol Genet* 2001; **10**: 1775–1783.