these disorders. The incidence of WS was similar to that previously reported for Japan (Oka et al., 2001; Matsuo et al., 2001). In contrast, the incidence of EIEE was one-fortieth that of WS.

Since the clinical features of EIEE share those of early myoclonic encephalopathy (EME), which is characterized by neonatal onset, erratic myoclonia, myoclonic and partial seizures, and a suppression-burst pattern, distinguishing EIEE from EME is difficult in some patients. However, some clear differences exist between the two, including the etiology, main seizure pattern, and evolution of EEG changes, in which a suppression-burst pattern changes to hypsarrhythmia and later slow spike-waves or multifocal spikes in EIEE, whereas the suppression-burst pattern in EME occurs for life. Therefore, we believe that the incidence of EIEE is accurate.

The reported incidence and prevalence rates of WS for various countries are shown in Tables 1 and 2, respectively. The incidence of WS in infancy and early childhood ranged distributed from 0.06 to 0.45 per 1000 live births, as shown in Table 1. The first reason for the large differences in the incidence may be the inclusion criteria used in the studies. A study should clearly state the demography of the study population, the selection criteria, and the case ascertainment method (Kotsopoulos et al., 2002). The most reliable method for obtaining the incidence rate is a cohort study (Kotsopoulos et al., 2002), although no such study has analyzed the incidence of WS. However, if the above-mentioned conditions are met in full, one can determine the incidence with considerable accuracy by surveying medical records (Trevathan et al., 1999); this is deemed to be the cumulative incidence (Commission on Epidemiology and Prognosis, International League Against Epilepsy, 1993).

WS is a severe epileptic syndrome with very characteristic clinical seizure manifestations and EEG findings. Therefore, identifying the cases of WS and clarifying the incidence rate correctly is possible using hospital surveys in countries with advanced pediatric and child health care. However, some problems remain in determining the incidence of WS accurately because atypical cases do not fit the standard criteria for WS. Recent advances in EEG technology, such as long-term monitoring and simultaneous video-EEG-electromyography monitoring, have shown that a significant number of patients with WS have partial seizures in addition to spasms and that some patients develop spasms in series after age 1–2 years, which is considered late-onset WS (Ohtsuka, 1998). These advances in understanding WS may be the second reason for the large differences in the incidence rate.

The limitations of our study were that the study was not prospective, but was a survey; children who moved away from Miyagi Prefecture after birth, but before the diagnosis of WS or EIEE, could have resulted in an underestimation of the cumulative incidence of WS and EIEE; and some patients with late-onset WS may have been missed because the questionnaire was sent out 4 months after the end of the survey period. In addition, the cumulative incidence of EIEE may have been more underestimated compared to that of West syndrome in the present study, since pediatricians in clinics or institutions have little access to NICU, which might have resulted in making a diagnosis of West syndrome but not EIEE in children who evolved from EIEE to West syndrome. Therefore, our study provides a minimum estimate of the cumulative incidence of WS and EIEE. Nevertheless, the incidence rates appear to be appropriate, since the demographic and selection criteria for our study population were clearly described.

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Case report

Mild phenotype in Pelizaeus-Merzbacher disease caused by a *PLP1*-specific mutation

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Abstract

We present the case of a 26 year-old man who developed normally until he began having difficulty walking at age 12. He subsequently became unable to stand at 15 years old and exhibited mental regression and generalized tonic convulsions by age 20. Magnetic resonance imaging revealed incomplete myelination of cerebral white matter, which resembled that of Pelizaeus-Merzbacher disease. By sequencing the proteolipid protein 1 (*PLP1*) gene, we found a novel mutation (c.352_353delAG (p.Gly130fs)) in the latter half of exon 3 (exon 3B) that is spliced out in the DM20 isoform. Exon 3B mutations are known to cause a mild phenotype since they do not disturb DM20 production. Mutations that truncate PLP1 correlate with a mild phenotype by activating the nonsense-mediated decay mechanism that specifically detects and degrades mRNAs containing a premature termination codon. This attenuates the production of toxic mutant PLP1. The very mild presentation in the present case seems to be derived from the unique nature of the mutation, which preserves DM20 production and decreases mutant PLP1.

Keywords: Pelizaeus-Merzbacher disease; Proteolipid protein 1; *PLP1*

1. Introduction

Proteolipid protein (PLP) 1 and its splice isoform DM20 are encoded by the *PLP1* gene. PLP1/DM20 proteins are major components of myelin expressed in oligodendrocytes in central nervous system (CNS) [1]. PLP1/DM20 translated in the endoplasmic reticulum (ER) is transported to the cell surface and integrated into plasma membrane presumably via four membrane-spanning domains with both amino- and carboxy-terminal ends on cytoplasmic side. Owing to its strong hydrophobicity, PLP1/DM20 can form a stable compact myelin sheath in cooperation with other myelin proteins [1]. Expression of PLP1 and DM20 are spatially and temporally regulated. DM20 expresses preferentially in embryonic stages in a variety of cell types, whereas PLP1 expresses postnatally in oligodendrocytes. Both PLP1 and DM20 constitute the predominant protein in myelin [2].

Pelizaeus-Merzbacher disease (PMD) is a severe X-linked recessive disorder caused by mutations of the *PLP1* gene [1]. A deficiency in PLP1/DM20 at the cell

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membrane by PLP1 mutation leads to the arrest of myelination. Moreover, mutant PLP1 elicits a response in the ER, which attempt to refold the misfolded mutant PLP1/DM 20 protein [3]. However, if the level of misfolded protein exceeds the controllable limit within the ER quality control system, apoptotic signals are transduced from ER [3]. This cellular process is reflected in developmental regression and atrophy of the CNS.

Exonic or intron/exon boundary mutations are found in 20–30% of PMD patients and phenotypes are severer than other mutations such as total deletion and duplication of *PLP1*. An exception has been observed in the latter half of exon 3 (exon 3B) of *PLP1* [4–8]. Mutations within this region are predicted not to disturb DM20 expression and function. Mutations that truncate PLP1 are related to a mild phenotype presumably by activating the nonsense-mediated decay (NMD) pathway, a mechanism that specifically detects and degrades mRNAs containing a premature termination codon [9]. This attenuates the production of mutant PLP1 levels and thus likely lessens the ER stress responses. In this report, we present a PMD patient with a very mild phenotype. We identified a novel *PLP1* gene mutation that is predicted to preserve DM20 production and results in a frame-shifted mutant PLP1 protein.

2. Materials and methods

2.1. Patient

This 26 year-old boy was born uneventfully at full term to Japanese parents. He was born with a body weight of 3660 g and an Apgar score of 9/9 at 1 and 5 min. No stridor or nystagmus was noted. He gained head control at 4 months, could sit without support at 8 months, and could walk without assistance at 4 years. He was pointed out spasticity of lower limbs and EEG abnormalities at 1 year. He was treated with carbamazepine for 14 years. No seizures occurred during that period. He could speak a few words at 2 years of age. He attended a special class in normal elementary and junior high school. He had no difficulties in daily conversation and writings. The patient began having difficulty walking at 12 years of age and became unable to stand at 15 years. He showed frequent urination and was diagnosed as neurogenic bladder at 15 years. MRI taken at that time revealed only mild ventricular enlargement. Myelination was not evaluated because of the motion artifact. At age 20, he showed signs of mental regression and began speaking fewer words. He exhibited generalized tonic convulsions and was treated with valproic acid at 24 years. He was subsequently referred to a hospital for evaluation. He was not small for his ages with a height of 167 cm and a weight of 54 kg. He could conversate with combining two words. He showed no nystagmus and exhibited alternating outer-nystagmus and oculomoter apraxia. He could walk with assistance. His muscle tone was hypertonic in the upper limbs. Clumsiness was observed with all extremities displaying exaggerated tendon reflexes and bilateral extensor plantar responses. Speech was slurred and dysmetry with terminal oscillation and dysdiadochokinesis were observed. Routine laboratory examinations revealed no biochemical abnormalities in the level of serum ammonia, lactate and pyruvate, very long chain fatty acids, or arylsulfatase A. Nerve conduction velocities and electromyographic studies were all normal. Measurement of auditory evoked brain responses revealed only wave I. MRI revealed a completion of myelination in the T1 signal. Myelination in the white matter was incomplete in the T2 signal (Fig. 1).

2.2. Genomic DNA sequencing

Genomic DNA from this patient was prepared from white blood cells using the Wizard Genomic DNA puri-

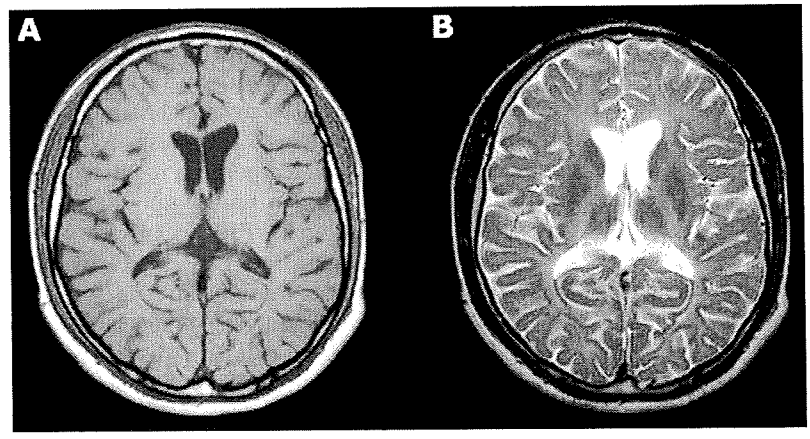

Fig. 1. Magnetic resonance imaging (MRI) at 26 year-old patient shows disappearance of contrast between cortex and white matter (A) on a T1-weighted image. T2-weighted image shows the incompletion of myelination in the white matter (B).

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fication kit (Promega, Madison, WI USA). PCR of seven exons and promoter regions of the *PLP1* gene was performed as previously described [10]. Subsequent sequencing analyses of the PCR fragments were performed by direct sequencing using the Big Dye Terminators v1.1 Cycle Sequencing kit (Applied Biosystems Foster City, CA). Duplication was screened by FISH as described [10].

3. Results

By direct sequencing of the patient's *PLP1* gene exons, exon/intron boundaries and a promoter region, we found a novel mutation in exon 3: c.352_353delAG (p.Gly130fs) (Fig. 2). No other sequence alterations were found and this mutation was not detected in more than 200 alleles. This two nucleotide deletion occurs in the latter half of exon 3 (exon 3B), which is not involved in *DM20* mRNA production (Fig. 3). FISH analysis showed normal copy numbers in this patient.

4. Discussion

Pelizaeus-Merzbacher disease belongs to leukodystrophies, one of a group of disorders that affect the white matter of the CNS. Genetic defects in PLP1/DM20, the most predominant myelin proteins, causes dual pathology: defects in CNS myelin formation (dysmyelination)

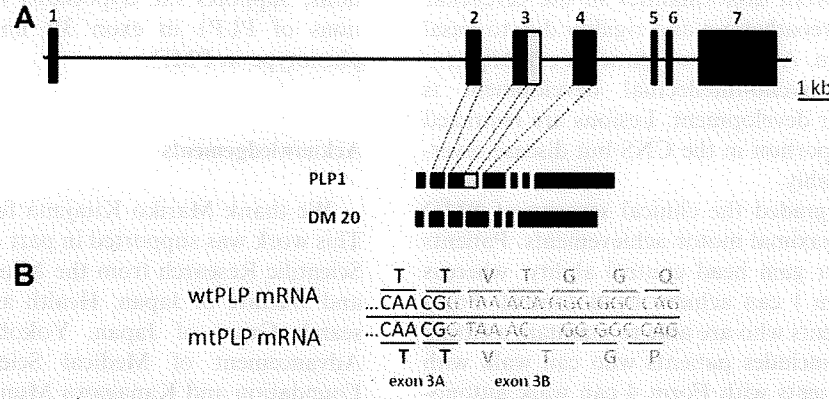

Fig. 2. Splicing the *PLP1* gene into *PLP1* mRNA and *DM20* mRNA. (A) Schematic presentation of *PLP1* gene structure. (upper panel) PLP1 gene is composed of seven exons. (lower panel) mRNA of PLP1/DM20 differs in only the latter half of exon 3 that is spliced out for the production of DM20 mRNA. (B) Two nucleotide deletion and subsequent frame shift in the Patient. Novel mutation in exon 3B, c.352_353delAG (p.Gly130fs), causes the frame shift in *PLP1* mRNA.

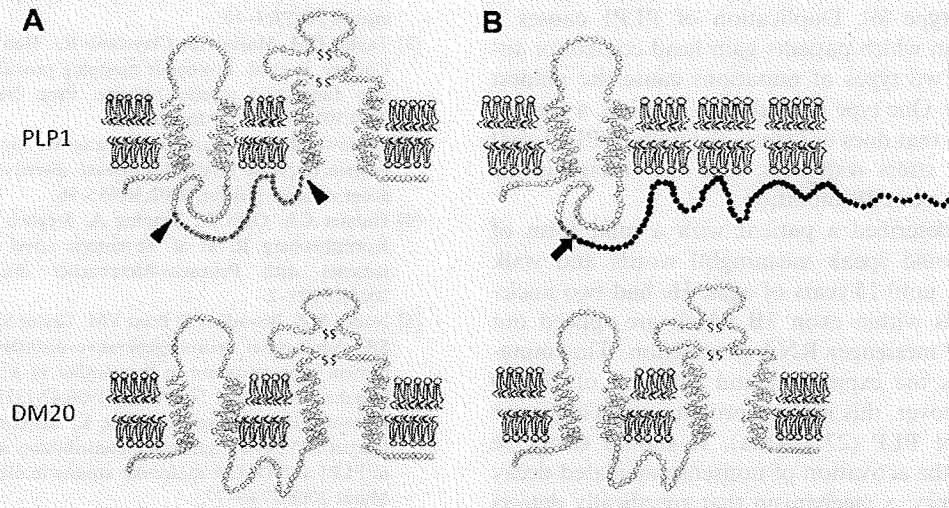

Fig. 3. Deduced PLP1 gene products; PLP1 and DM20. (A) Wild-type PLP1 (upper) and DM20 (lower) which are thought to include 4 membrane-spanning domains. Thirty-five intracellular amino acids (gray circle; between arrow head) are lacking in DM20. One circle corresponds to one amino acid. (B) PLP1 and DM20 of the patient. (upper) A two nucleotides deletion in exon 3B, c.352_353delAG (p.Gly130fs), causes a frame shift (arrow) and extension that are composed of 82 nonsense peptides. (lower) DM20 is identical to wild-type in this patient.

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and oligodendrocytes cell loss via apoptosis. PLP1 and DM20 are required for myelin compaction. Mutations in the *PLP1* gene, such as total deletion and truncation mutations, cause an inability to form normal myelin, which is easily revealed by diffuse high signals in all CNS white matter in T2-weighted MRI scans. Since PLP1/DM20 are constitute more than 50% of the protein in oligodendrocytes, mutant PLP1/DM20 cause the excessive ER stress responses and subsequent cell death that can be visualized by MRI/CT as brain atrophy.

Typically, patients with PMD show neonatal nystagmus and developmental delay that becomes apparent during infancy. Impairments of motor functions involve spastic paresis from the defect in the corticospinal tract, intention tremor from abnormalities in the cerebellar pathway, and choreoathetosis and rigidity due to basal ganglia dysfunction. Although all patients exhibit mental retardation, psycho-intellectual development is greater than motor development. Lesions are restricted in the myelinated portion in the CNS but disease severity varies considerably.

Cailloux et al. graded the clinical severity of PMD patients by their maximal motor achievements. Patients with Form 0 never gain head control ability, whereas patients with Form 1 can achieve head control. Form 2 includes the patients who are able to maintain a sitting position. Form 3 includes patients who can walk with support, while patients with Form 4 can walk autonomously. This last form overlaps the clinical phenotype of X-linked spastic paraplegia type 2, the allelic disease to PMD [8]. The patient described in the present case report belongs to Form 4, the mildest symptom group.

Amino acid substitutions, especially conserved amino acids in DM20/PLP1 within species, usually cause a severe phenotype [6]. Duplication of PLP1 causes a milder form, in which patients gain head control or sitting ability. Two types of mutations cause the mildest form of PMD. One type is total gene deletion, a truncation mutation that does not cause the mutant PLP1 that elicit the ER stress responses, and the second is the *PLP1*-specific exon 3B mutation.

Here, we described a patient with a mild form of PMD who could speak meaningful words and walk independently until 15 years of ages. He had two nucleotide deletions within exon 3B which are spliced out during *DM20* messenger RNA production. This mutation preserves the expression and function of DM20 protein. Moreover, this mutant protein is much shorter than wild-type PLP (277/241aa). It should easily be degraded via the activation of nonsense-mediated decay (NMD) pathway, a mechanism that specifically detects and degrades mRNAs containing a premature termination codon. The very mild phenotype observed is probably due to the dual effect of mutation: conservation of DM20 and the inability to elicit an ER stress response.

Thirteen different mutations have been reported in exon 3B (c.384C>G, 385C>T, 388C>T, 409C>G, 409C>T, 410delG, 418C>T, 430A>T, 434G>A, 441A>T, 442C>T, 446C>T). Twelve of them are one nucleotide changes and are predicted to preserve DM20 expressions. Clinical presentations are reported in 9 cases and 6 fit the criteria of Form 4, reinforcing the importance of DM20 function in addition to PLP1. Thus far, only one example of an exon 3B mutation that causes normal DM20 and truncated PLP1 has been reported (440delG; R137fsX8) [6]. This mutation caused two patients with Form 3 and one with Form 4. Our case is the second examples of an exon 3B mutation that produce normal DM20 and truncational PLP1. Our case, together with reports of other exon 3B mutations, supports the hypothesis that frame-shift mutations of PLP1 in exon 3B underlies the very mild phenotype in PMD.

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CASE REPORT

Brachmann-de Lange syndrome with congenital diaphragmatic hernia and *NIPBL* gene mutation

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ABSTRACT **We report herein a case of Brachmann-de Lange syndrome complicated with congenital diaphragmatic hernia in which a *NIPBL* gene mutation was identified. A female infant born at 37 weeks of gestation died 134 min after delivery, even though endotracheal intubation and resuscitation were performed immediately after the scheduled caesarean operation. We diagnosed the infant with Brachmann-de Lange syndrome from her physical characteristics. An abnormal peak at the 29th exon in the translation area of the *NIPBL* gene was detected using denaturing high-performance liquid chromatography. In addition, a mutation of cytosine to thymine (nonsense mutation) at the 5524th base was identified using the direct sequence method. This variation was likely the cause of the syndrome.**

Key Words: Brachmann-de Lange syndrome, congenital diaphragmatic hernia, denaturing high-performance liquid chromatography, direct sequence method, gene mutation

INTRODUCTION

Brachmann-de Lange syndrome (BDLS) is a multiple congenital anomaly syndrome characterized by growth and mental retardation, variable anomalies of the upper limbs and a peculiar face with hypertrichosis. A pediatrician named de Lange (1933) reported two cases of this disease while working at Amsterdam University in the Netherlands, and termed the disease Cornelia de Lange syndrome. It was subsequently revealed that Brachmann (1916) had reported on a patient exhibiting the same symptoms. As a result of these two reports, the condition is currently known as Brachmann-de Lange syndrome (Opitz 1985).

Brachmann-de Lange syndrome was originally thought to be related to 3q partial trisomic syndrome, as the clinical manifestations of the two diseases are relatively similar. More recently, Krantz *et al.* (2004) and Tonkin *et al.* (2004) reported a variation in the *NIPBL* gene in a BDLS patient, allowing the two diseases to be more easily distinguished.

We report herein a case of BDLS with congenital diaphragmatic hernia caused by a mutation in the *NIPBL* gene that was identified using denaturing high-performance liquid chromatography.

Case report

A 21-year-old woman delivered a female infant at 37 weeks and 2 days of gestation by scheduled caesarean operation due to intrauterine growth retardation and congenital diaphragmatic hernia diagnosed by fetal echography at a gestational age of 30 weeks and 2 days. The infant's birthweight was 1766 g (–2.6 SD) and her Apgar score was 1 at 1 min and 3 at 5 min. When the infant was born, her entire body was pale and she did not demonstrate spontaneous breathing patterns. Endotracheal intubation was immediately performed and artificial ventilation with high frequency oscillation (HFO) and nitric oxide inhalation therapy was initiated. Unfortunately, there was no improvement in her condition, even following the administration of resuscitative medication, including adrenaline and surfactant, and she died 134 min after birth.

We considered that the patient had BDLS due to her characteristic facial features, including synophrys, brachyrrhinia, long philtrum, thin lip, small mandible and short cervix, and the presence of hirsutism and a congenital diaphragmatic hernia. Although her limbs were small and short, and a bilateral single transverse palmar crease was recognized on each hand, the BDLS characteristics of syndactyly and limb reduction defects were not observed (Fig. 1).

At laboratory examination at birth, we identified slight acidosis; however, significant abnormal findings, including anemia and electrolyte imbalance in the cord blood were not observed. The infant's blood gas (venous blood) at 47 min after birth was also recognized as mixed acidosis of pH 6.763, PCO_2 188.0 mmHg, PO_2 3.2 mmHg and BE –16.5 mmol/L. Her hemoglobin was 6.8 g/dL, her C-reactive protein (CRP) was negative and there was no elevation in liver enzyme levels. Hypernatremia was observed in her electrolytes (Table 1). Amniotic fluid chromosomes were of a normal karyotype of 46, XX. X-ray of the entire body revealed a hanging bell-shaped thoracic cage, low pneumatization in the bilateral lungs and a stomach bubble in the middle thorax (Fig. 2).

Pathological autopsy of the infant was undertaken after we obtained informed consent from her parents. The placental weight was 190 g, which was small for the number of gestational weeks (our center average is 514 g), villi were immature and the umbilical cord contained a single umbilical artery. The left diaphragm was almost entirely defective and the liver, stomach, spleen, pancreas, small intestine and large intestine protruded into the intrathoracic area. Marked hypoplasia of the lungs was also recognized with a pulmonary weight ratio of 0.003 (normal is 0.012). In addition, the lungs were histologically immature. Bilateral hydroureter, annular pancreas and atrial septal defect were also observed. We did not examine the brain, as the parents did not consent to craniotomy.

After we obtained written informed consent from the parents for gene diagnosis, we extracted genomic DNA from the patient's blood and amplified the coding region (extending from the 2nd exon to the 47th exon) of the *NIPBL* gene using polymerase chain reaction (PCR). An abnormal peak in exon 29 was detected when analyzed using denaturing high-performance liquid

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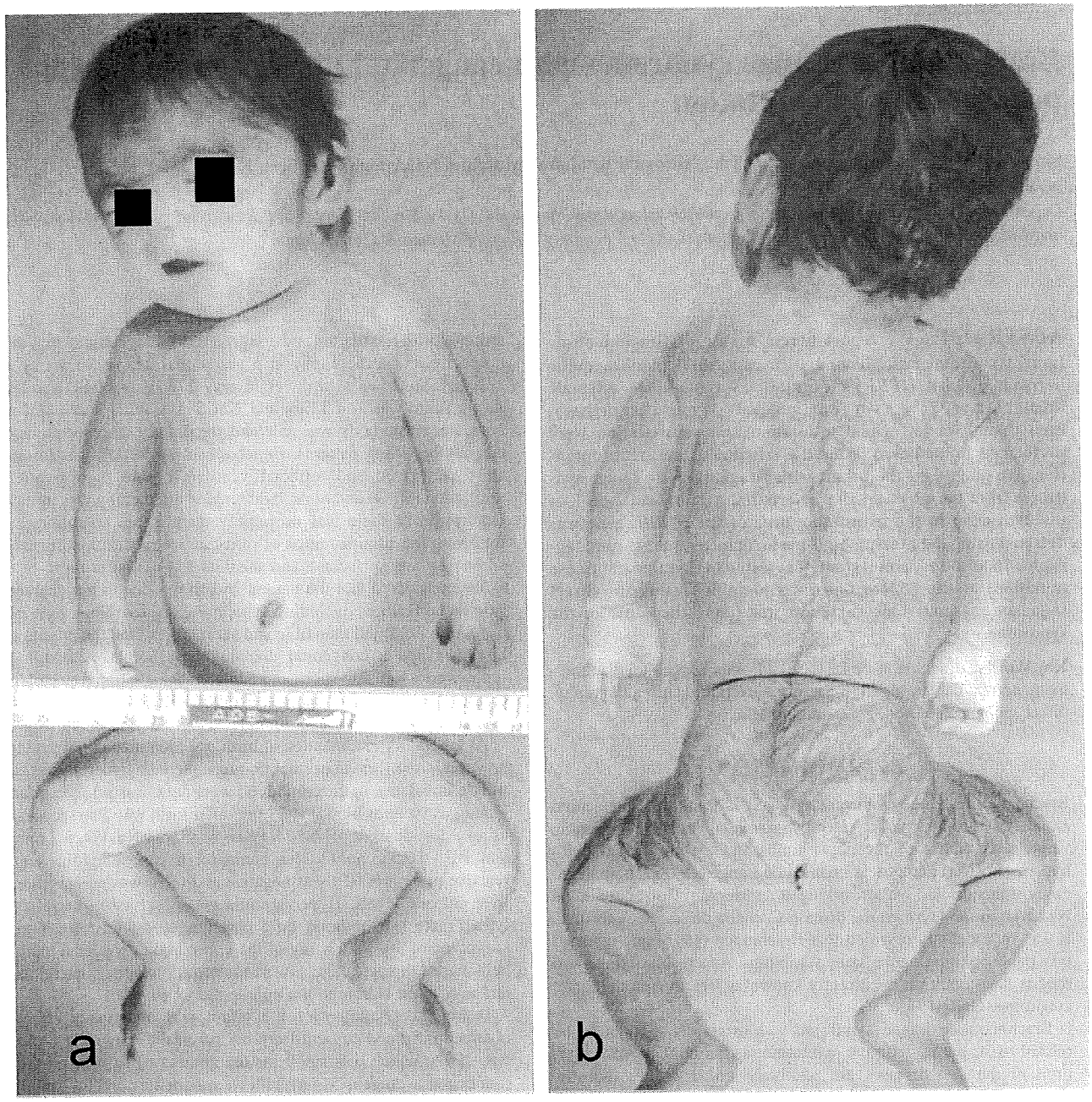

Fig. 1 Photographs highlighting the patient's symptoms (a,b) including hypertrichosis, short extremities, hypoplasia of the nipple and umbilicus, synophrys of the face, short and upturned nose or anteverted nostrils, long philtrum, thin lip, small mandible, short cervix and single bimanual palmar flexion curve without syndactyly or defects of the fingers.

chromatography (Fig. 3). Within the translation area of the *NIPBL* gene, a mutation of cytosine (C) to thymine (T) (nonsense mutation) at the 5524th base was identified using the direct sequence method. This amino acid change formed a stop codon, a result that we hypothesized would influence the complications in this patient.

DISCUSSION

Cornelia de Lange (1933) identified 10 traits, such as mental retardation, low birthweight, dwarfism, microbrachycephaly, heavy eyebrows meeting at the midline, long eyelashes, low-set ears, small hands and feet, proximal placed thumb and syndactyly of the toes in two patients while working at Amsterdam University. Beck (1976) later reported the original diagnostic standards of BDLS (Table 2) and suggested that patients with BDLS could be diagnosed if they exhibited eight of these 10 traits. In the current case, BDLS was not diagnosed in the fetal period, but was diagnosed after birth. The infant demonstrated nine of the traits described by de Lange and five of the traits described in the Beck standards. After confirming our findings with both the de Lange

Table 1 Examination of the umbilical cord and peripheral blood of the present case of Brachmann-de Lange syndrome with congenital diaphragmatic hernia and *NIPBL* gene mutation

	Cord blood	Patient's blood (47 min after birth)
Blood gas analysis		
pH	7.276	6.763
pCO_2	48.9 mmHg	188.0 mmHg
pO_2	20.5 mmHg	3.2 mmHg
Base excess	−4.3 mmol/L	−16.5 mmol/L
Blood cell counts		
White blood count	5900/μL	4900/μL
Platelet count	221 000/μL	93000/μL
Chemistry		
C-reactive protein	<0.05 mg/dL	<0.1 mg/dL
Sodium	140 mmol/L	183 mmol/L
Potassium	4.5 mmol/L	5.3 mmol/L
Calcium	9.5 mg/dL	8.3 mg/dL
Hemoglobin	13.6 g/dL	6.8 g/dL

Table 2 Findings in the present case of Brachmann-de Lange syndrome with congenital diaphragmatic hernia and *NIPBL* gene mutation

	This patient's findings
Cornelia de Lange (1933)	
Mental retardation	?
Low birthweight	+
Dwarfism	?
Microbrachycephaly	+
Heavy eyebrows meeting at the midline	+
Long eyelashes	+
Low ear insertion	−
Small hands and feet	+
Proximally placed thumb	−
Syndactyly of the toes	−
Beck (1976)	
Low hair line on forehead	+
Low hair line on neck	+
Long philtrum	+
Bushy eyebrows	+
Confluent eyebrows	+
Thick eyelashes	+
Antimongoloid eye slanting	−
Anteverted nostrils	+
Crescent-shaped mouth	+
Thin prolabium	+

+, present; ?, not detected due to early death.

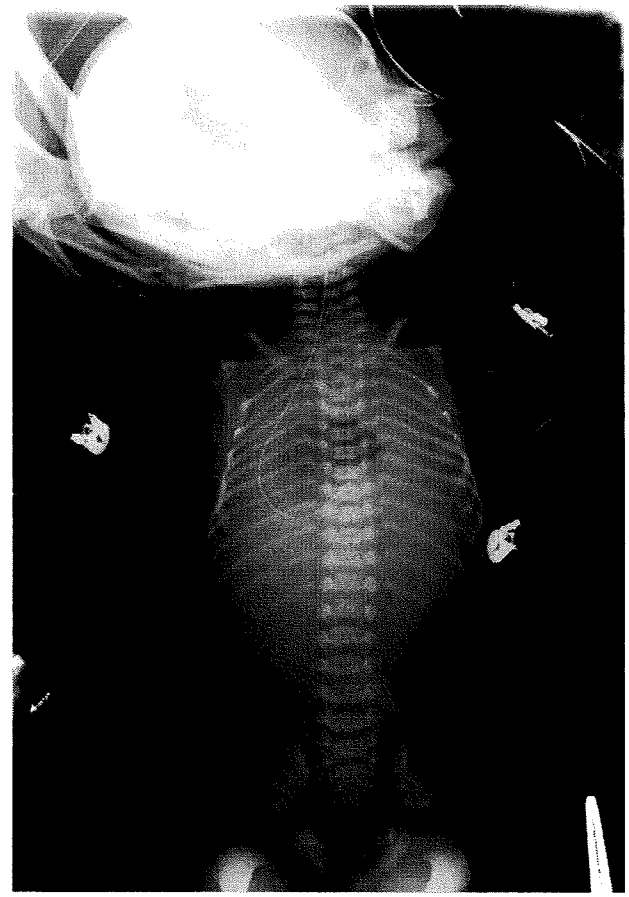

Fig. 2 X-ray of the entire body showing the hanging bell-shaped thoracic cage, low pneumatization in the bilateral lungs and a stomach bubble located in the middle thorax.

and Beck standards, we finally made a diagnosis based on the baby's physical characteristics.

This patient was also diagnosed based on the presence of intrauterine growth retardation and diaphragmatic hernia during the fetal period. Limb shortening was also observed. In the absence of abnormal karyotype or altered bone structures with limb shortening, BDLS is generally considered as a differential diagnosis (Beck and Fenger 1985; Kenneth 1988). Further, the placenta weighed only 190 g, which was low for the gestational period. This finding was consistent with the hypothesis that growth of not only the fetus, but also of the placenta is inadequate in cases of BDLS.

There have been only a few reports of BDLS with congenital diaphragmatic hernia in Japan (Kuroiwa *et al.* 1990; Suzuki *et al.* 1999). A small number of reports (e.g. Cunniff *et al.* (1993), Russel *et al.* (1993) and Marino *et al.* (2002)) have been described in other countries. The reports by these groups suggested that the prognosis was worse when the patient also exhibited congenital diaphragmatic hernia. The precise causes of congenital diaphragmatic hernia remain unknown. BDLS, Fryns syndrome, Goltz syndrome and Smith-Lemli-Opitz syndrome are all associated with congenital diaphragmatic hernia (Tibboel and Gaag 1996; Bianch *et al.* 2000). Recently, gene analysis of these various multiple malformation syndromes has been undertaken (Holder *et al.* 2007). Further gene

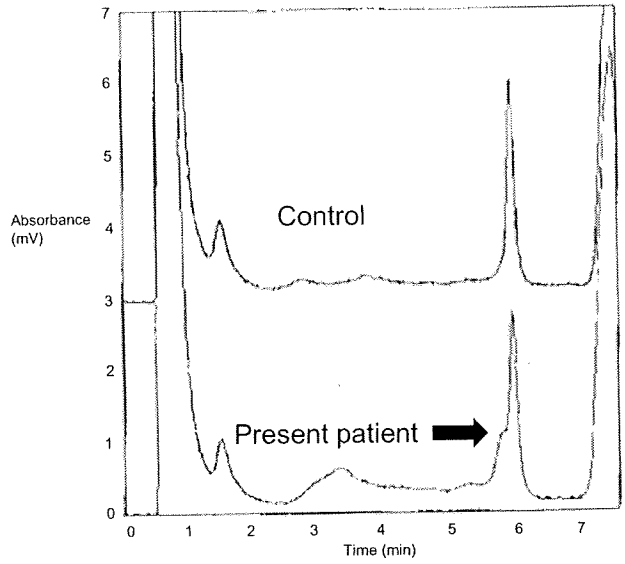

Fig. 3 Denaturing high-performance liquid chromatography of the 29th exon of the *NIPBL* gene (upper panel: control, lower panel: patient). Arrow shows the abnormal peak in the translation area (29th exon) of the *NIPBL* gene.

analyses in the various multiple malformation syndromes specifically associated with congenital diaphragmatic hernia are likely to shed light on which anomalies lead to diaphragmatic hernia.

In the present case, a mutation of C to T (nonsense mutation) at the 5524th base in the translation area of the *NIPBL* gene was identified. As a result, we concluded that this variation was likely to be the cause of the BDLS with diaphragmatic hernia. The *NIPBL* gene is located at 5p13.1 and contains 47 exons, and its transcription is thought to be related to Notch signal transmission. There have been many confirmed gene mutations, including deletion and insertion mutations, that are associated with BDLS (Gillis *et al.* 2004; Bhuiyan *et al.* 2006; Schoumans *et al.* 2007). Further, Musio *et al.* (2006) and Deardorff *et al.* (2007) have presented reports relating BDLS to both *SMC1* and *SMC3* gene mutations.

DNA analysis is important for confirming BDLS diagnosis. Analysis of gene mutations in genes such as *NIPBL* also represents a useful diagnostic method. With the accumulation of cases such as ours, further description of this disease will be possible.

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ARTICLE

BCOR analysis in patients with OFCD and Lenz microphthalmia syndromes, mental retardation with ocular anomalies, and cardiac laterality defects

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Oculofaciocardiodental (OFCD) and Lenz microphthalmia syndromes form part of a spectrum of X-linked microphthalmia disorders characterized by ocular, dental, cardiac and skeletal anomalies and mental retardation. The two syndromes are allelic, caused by mutations in the *BCL-6 corepressor* gene (*BCOR*). To extend the series of phenotypes associated with pathogenic mutations in *BCOR*, we sequenced the *BCOR* gene in patients with (1)

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OFCD syndrome, (2) putative X-linked ('Lenz') microphthalmia syndrome, (3) isolated ocular defects and (4) laterality phenotypes. We present a new cohort of females with OFCD syndrome and null mutations in *BCOR*, supporting the hypothesis that *BCOR* is the sole molecular cause of this syndrome. We identify for the first time mosaic *BCOR* mutations in two females with OFCD syndrome and one apparently asymptomatic female. We present a female diagnosed with isolated ocular defects and identify minor features of OFCD syndrome, suggesting that OFCD syndrome may be mild and underdiagnosed. We have sequenced a cohort of males diagnosed with putative X-linked microphthalmia and found a mutation, p.P85L, in a single case, suggesting that *BCOR* mutations are not a major cause of X-linked microphthalmia in males. The absence of *BCOR* mutations in a panel of patients with non-specific laterality defects suggests that mutations in *BCOR* are not a major cause of isolated heart and laterality defects. Phenotypic analysis of OFCD and Lenz microphthalmia syndromes shows that in addition to the standard diagnostic criteria of congenital cataract, microphthalmia and radiculomegaly, patients should be examined for skeletal defects, particularly radioulnar synostosis, and cardiac/laterality defects.
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Keywords: BCL-6 corepressor; oculofaciocardiodental syndrome; Lenz microphthalmia syndrome; mental retardation; ocular defects

Introduction

The X-linked microphthalmia syndromes, 11 of which are currently defined (Table 1), comprise a clinically and molecularly diverse group of disorders, a number of which overlap with X-linked mental retardation syndromes. Lenz microphthalmia syndrome has been recognized for more than 50 years and was previously assumed to be a unitary entity. However, clinical and linkage analyses suggest that it may be aetiologically heterogeneous, linked to both Xq27-q28[1] (MCOPS1; MIM 309800) or Xp11.4[2,3] (MCOPS2; MIM 300166). Specifically, the MCOPS2 form of Lenz microphthalmia syndrome has been shown to be caused by mutation of the BCL-6 corepressor gene (*BCOR*);[3] the genetic aetiology of MCOPS1 remains unknown. The original report of Lenz described a family with variable ocular manifestations, including anophthalmia, microphthalmia and coloboma. There were numerous extraocular anomalies including mental retardation, palatal and dental anomalies, congenital heart defects, skeletal defects (affecting the fingers and clavicles), unilateral renal aplasia and cryptorchidism;[4] this phenotype substantially overlaps with other X-linked microphthalmia syndromes (Table 1). Another X-linked microphthalmia-associated condition, oculofaciocardiodental (OFCD) syndrome, is characterized by ocular defects (congenital cataracts, microphthalmia), facial anomalies (septate nasal tip, high nasal bridge, midface hypoplasia, palatal anomalies), congenital cardiac defects (atrial/ventricular septal defects, other complex heart defects), dental irregularities (canine radiculomegaly, delayed and persistent dentition, hypodontia) and skeletal anomalies (syndactyly, hammer-type flexion deformities).[5–10] All affected individuals are female, with several incidences of mother-daughter transmission.

In 2004, a sequence variant was identified within the *BCOR* gene in a single family with Lenz microphthalmia syndrome.[3] The missense mutation (c.254C>T, p.P85L) cosegregated with the disease phenotype and was not identified in more than 450 control chromosomes. It represents the sole molecular cause of Lenz microphthalmia syndrome identified to date. In the same study, *BCOR* mutations were found in all tested patients with OFCD syndrome, suggesting the two conditions were allelic. The mutation types included nonsense, frameshift, deletion and splicing mutations, a finding subsequently replicated by others. Importantly, OFCD syndrome has been shown to encompass defects of laterality, including the heart and other viscera, suggesting that *BCOR* is necessary for left–right asymmetric development and that mutations in *BCOR* may represent a source of the reported male excess of laterality defects.[11,12]

This study aims to expand our understanding of the phenotypes associated with mutations in *BCOR*, supporting the hypothesis that OFCD syndrome is solely associated with null mutations in *BCOR* and to identify additional patients with Lenz microphthalmia syndrome who carry mutations in *BCOR*. We have demonstrated that mutations in *BCOR* may be responsible for apparently isolated ocular anomalies and tested the hypothesis that *BCOR* mutations cause non-syndromic cardiac/laterality defects. The continuous accumulation of patient data allows the frequency of non-cardinal phenotypes to be estimated and improve diagnosis and treatment of patients.

Materials and methods

Direct sequencing of the *BCOR* gene

The coding exons and flanking intronic sequences of the *BCOR* gene were amplified by PCR and directly sequenced as previously reported.[3]

Table 1 Defined X-linked microphthalmia syndromes

Syndrome nomenclature and alternative names	*MIM*	*Description*[a]	*Locus*	*Gene*
MCOPS1 Lenz microphthalmia syndrome (MAA)	309800	Micro/anophthalmia, dental anomalies, skeletal/digital anomalies, mental retardation, facial dysmorphia	Xq27-q28	
MCOPS2 Oculofaciocardiodental syndrome (MAA2)	300166	Micro/anophthalmia, congenital cataracts, radiculomegaly, cardiac defects, skeletal/digital anomalies, facial dysmorphia	Xp11.4	*BCOR*
MCOPS4	301590	Micro/anophthalmia, ankyloblepharon, mental retardation	Xq27-28	
MCOPS7 Midas syndrome MLS	309810	Microphthalmia, linear skin pigmentation defects	Xp22.2	*HCCS*
MCOPCT3	302300	Microphthalmia, congenital cataract		
MCOPCB1	300345	Microphthalmia, coloboma		
FDH (Focal dermal hypoplasia) Goltz syndrome	305600	Microphthalmia, coloboma, sclerocornea, linear skin lesions, digital anomalies, mental retardation		
BRESHECK syndrome	300404	Microphthalmia, CNS anomalies, skeletal anomalies, mental retardation, renal hypoplasia		
NHS (Nance–Horan syndrome)	302350	Microcornea, cataracts, dental anomalies	Xp22.13	*NHS*
ND (Norrie disease)	310600	Microphthalmia, neuroretinal degeneration, mental retardation, sensorineural deafness	Xp11.4	*NDP*
Not assigned; proposed as part of the Renpenning syndrome spectrum	300463	Microphthalmia, coloboma, skeletal/digital anomalies, mental retardation, facial dysmorphia	Xp11.23	*PQBP1*

MCOPS1, microphthalmia, syndromic 1; MAA, microphthalmia and associated anomalies; MCOPS2, microphthalmia, syndromic 2; MAA2, microphthalmia and associated anomalies 2; MCOPS4, microphthalmia, syndromic 4; MCOPS7, microphthalmia, syndromic 7; MCOPCT3, microphthalmia, isolated, with cataract 3; MCOPCB1, microphthalmia, isolated, with coloboma 1; BRESHECK, brain anomalies, retardation, ectodermal dysplasia, skeletal malformations, Hirschsprung disease, ear/eye anomalies, cleft palate/cryptorchidism, and kidney dysplasia/hypoplasia; CNS, central nervous system.
[a]Note the large phenotypic overlap of clinical features within this group of disorders.

Semi-quantitative multiplex fluorescent PCR analysis of the *BCOR* gene

Deletions within the *BCOR* gene were detected by semi-quantitative multiplex fluorescent PCR (QMF-PCR).[13,14] All exons of the *BCOR* gene and three control genes (*DSCR1* – chromosome 21, *CFTR* – chromosome 7 and *Factor IX* – chromosome X) were amplified in two duplicate multiplex reactions (primer sequences in Supplementary Table 1). One of each primer pair was labelled with the fluorescent phosphoramidite 6-FAM dye. Amplifications were performed in 25 μl reactions using the QIAGEN Multiplex PCR kit (Qiagen, France) with 75 ng of genomic DNA and a mix of primers (concentration range 1.3–8 μM). The reaction started with an initial denaturation of 15 min at 95°C followed by 23 cycles at 95°C for 30 s/60°C for 30 s/72°C for 45 s with an increment of 3 s per cycle. Final extension was at 72°C for 10 min. PCR products were purified and processed as previously described.[14] Two control DNAs (male and female) were included in each experiment. Results were analysed by superimposing fluorescent profiles of tested patients and controls.

Fluorescent *in situ* hybridization analysis of the *BCOR* gene

To determine the proportion of cells with a deletion in the *BCOR* gene, fluorescent *in situ* hybridization (FISH) was performed as previously described.[15] Metaphase spreads of peripheral leucocytes were obtained according to standard techniques. BAC clones used in FISH experiments were provided by The Wellcome Trust Sanger Institute (Cambridge, UK). Clones localized on chromosome Xp11.4 (RP11-320G24, RP11-330L22 and RP11-429N5) were directly labelled with Cy3. Chromosomes were counterstained with DAPI. The specific signal intensity and its sublocalization along the chromosome axis were analysed using a Leica fluorescence microscope equipped with the Visilog-6 program (Noesis, Les Ulis, France).

Ethics approval

The human subject research described here was reviewed and approved by ethics committees at St Mary's Hospital (Manchester, UK), the National Institutes of Health (Bethesda,

MD, USA), Baylor College of Medicine (Houston, TX, USA), and Hôpital Henri Mondor (Créteil, France).

Results

Sequence and copy number analysis of *BCOR* in patients with OFCD syndrome

From existing literature and previous work, we have identified 33 females with OFCD syndrome.[3,5–11,16–25] In this study, two of these patients are described in further clinical detail[11] and mutational analysis of *BCOR* completed in a family (mother and daughter).[25] In addition to these four previously reported patients, we have identified a further 31 females putatively diagnosed with OFCD syndrome and found mutations in *BCOR* in all families, bringing the cohort of patients described here to 35 cases. A summary of the phenotypes and mutations identified in patients with OFCD syndrome is given in Table 2. The positions of mutations in relation to the exon sequences are shown in Figure 1. The reference sequence used for mutation numbering is given in Supplementary Data 1. The 31 novel patients described here brings the total number of reported cases of OFCD syndrome to 64. We have also sequenced three females with features of OFCD syndrome who proved negative for *BCOR* mutations; however, the phenotypes were atypical for OFCD syndrome, with a notable lack of radiculomegaly in the dental phenotype in all cases.

The mutations identified here and in other studies involve deletions of significant portions of the *BCOR* coding sequence, alterations to conserved splice acceptor sites (predicted to lead to exon skipping with concomitant frameshifts) or small insertions/deletions which cause frameshifts and generate premature stop codons; it is predicted that such premature stop codons will trigger nonsense-mediated decay of the mRNA although the generation of C-terminally truncated protein species is also possible. The observation that, in all females tested to date, X-inactivation is grossly skewed in favour of the wild-type allele (data not shown) suggests that loss of wild-type BCOR protein confers significant selective disadvantage, certainly in haematological lineages. Females with OFCD syndrome are therefore functional mosaics, with cell populations and tissues expressing either wild-type BCOR (where lack of BCOR function is presumed lethal) or no BCOR/truncated BCOR (where lack of BCOR function can be supported). Amongst the pathogenic variants are two further instances of a previously reported c.2926C>T p.R976X nonsense mutation.[3]

In this study, we report for the first time two families with individuals mosaic for a mutation in *BCOR* (OFCD XVI and OFCD XVII). In OFCD XVI, the individuals mosaic for a *BCOR* mutation have the cardinal phenotype associated with OFCD syndrome. In contrast, the mosaic individual from OFCD XVII is asymptomatic. In both families, offspring present with the cardinal OFCD syndrome phenotype and are non-mosaic for heterozygous *BCOR* mutations.

OFCD XVI Individuals XVI.1 and XVI.2 are monozygotic twin sisters, both presenting with a classical OFCD syndrome phenotype. Individual XVI.3 (the daughter of individual XVI.1) also presented with an overlapping array of features. Using a quantitative fluorescent PCR method, a deletion of at least exons 4–15 was detected in patients XVI.1 and XVI.2. However, the decrease in PCR product amount derived from the *BCOR* gene was estimated at around 75% of control peak height, rather than the 50% expected for a non-mosaic deletion (Figure 2a and b). This result was recapitulated for all *BCOR* fragments amplified and strongly suggested somatic mosaicism of this deletion. By contrast, amplification was reduced to 50% of control peak height in patient XVI.3, suggesting non-mosaic distribution of the *BCOR* deletion (Figure 2c). FISH analysis was undertaken to confirm somatic mosaicism in individuals XVI.1 and XVI.2. FISH analysis on metaphase chromosomes from both patients demonstrated a deletion corresponding to BAC clone RP11-330L22 in 52/100 metaphase preparations for each patient, with only one signal from the X chromosome (Figure 2g; data from patient XVI.1 not shown). Two distinct signals were detected in the remaining 48 leucocyte preparations (Figure 2h; data from patient XVI.1 not shown). X chromosomes were identified using an X centromere marker and by banding pattern (not shown). These results supported the hypothesis that these two individuals are mosaic for the deleted region. For patient XVI.3, 100/100 metaphase preparations generated a single signal, confirming the non-mosaic nature of the deletion in this patient (Figure 2i). Two BAC clones flanking RP11-330L22, RP11-429N5 (proximal) encompassing exons 4–15 of BCOR and RP11-320G24 (distal) were tested in patient XVI.3. Two specific signals were detected in each metaphase preparation, suggesting that these regions are not deleted in this family (the proximal RP11-429N5 probe contains sufficient sequence outside of the *BCOR* gene to permit hybridization).

OFCD XVII The proband (XVII.2), a 14-month-old girl, presented with a classical OFCD syndrome phenotype. The mother was asymptomatic and had a normal panoramic dental X-ray. Quantitative fluorescent PCR detected a reduced amount of product corresponding to exons 13 and 14, suggesting a deletion of these exons in both females. Comparison of amounts of PCR product obtained suggested that the mother (XVII.1) was mosaic for this deletion, with the amount of PCR product at 75% of the control peak height

Table 2 Clinical data and identified mutations in *BCOR* for the patient cohort presented here

Family	*Case*	*Age*	*Ocular*	*Facial/cranial*	*Cardiac*	*Dental*	*Skeletal*	*Other*	*Mutation*
OFCD I	1[a] 2[a]	34 years 8 days	Congenital cataract Microphthalmia (2) Ptosis (1) Lens dislocation (2) Optic disc dysplasia (2)	Septate nasal cartilage High nasal bridge (2)	Heart murmur (1) ASD (2) VSD (2) Pulmonary valve stenosis (2)	Unerupted secondary teeth (1) Root radiculomegaly (1) Hypodontia (1) Not recorded (2)	Hammer toes (1) Radioulnar synostosis (1)	Poor feeding (2)	c.2926C>T p.R976X
OFCD II	1[b]	23 years	Congenital cataract Microphthalmia	Septate nasal cartilage	VSD PDA MI Dextrocardia	Persistant primary teeth Unerupted secondary teeth Root radiculomegaly Hypodontia	Hammer toes	Hearing impairment	c.1539_1540insG p.P514AfsX4
OFCD III	1 2 3 4 5 6[b]	69 years 65 years 43 years 40 years 21 years 4 years	Congenital cataract Microphthalmia Glaucoma (2, 4) Phthisis bulbi (1)	Septate nasal cartilage (1, 3, 4, 5) Cleft palate (6) Not affected (2)	ASD (6) VSD (6) DORV (6) Not recorded (1, 2, 3, 4, 5)	Delayed primary dentition (6) Persistent primary teeth (1, 3, 5) Unerupted secondary teeth (1, 2, 3, 4, 5) Root radiculomegaly (1, 2, 3, 5) Hypodontia (5)	Hammer toes (1, 2, 4, 5) Second-third toe syndactyly (3, 6) Radioulnar synostosis (4, 6)	Mental retardation (6) Asplenia (6)	c.4116delGinsCT p.E1372DfsX37
OFCD IV	1 2	37 years 11 years	Congenital cataract Microphthalmia Microcornea Iris synechia (1)	Septate nasal cartilage High nasal bridge Long/narrow face (1) High arched palate (2)	Aortic valve stenosis (1) Not affected (2)	Persistant primary teeth Unerupted secondary teeth Root radiculomegaly Hypodontia Fusion of teeth (1) Duplicated teeth (2)	Hammer toes (1) Second-third toe syndactyly Lordosis	Mild mental retardation (2) Hearing impairment Vomiting/ reflux	c.4488_4497del10 p.G1497PfsX68
OFCD V	1	31 years	Congenital cataract Microphthalmia	Septate nasal cartilage High nasal bridge	ASD	Persistant primary teeth Unerupted secondary teeth Root radiculomegaly	Hammer toes Radioulnar synostosis Lumbar scoliosis		c.4512_4514 delTTGinsA p.A1506X
OFCD VI	1 2 3 4	70 years 41 years 40 years 10 years	Congenital cataract Microphthalmia (2, 4) Iris synechia (2)	Septate nasal cartilage (2, 4) Submucosal cleft palate (4) Bifid uvula (4) Not recorded (1, 3)	ASD (4) Not affected (2, 3) Not recorded (1)	Persistent primary teeth (2, 4) Unerupted secondary teeth (2, 4) Root radiculomegaly (4) Hypodontia (2, 4) Fusion of teeth (4) Duplicated teeth (2, 4) Not recorded (1, 3)	Not recorded (1, 3) Hammer toes (2) Second-third toe syndactyly (2) Scoliosis (4)	Hearing impairment (1)	c.3621delA p.K1207NfsX31
OFCD VII	1	5 years	Congenital cataract Microphthalmia	Not affected	Not recorded	Persistent primary teeth	Second-third toe syndactyly		c.4303_4307delCCATG p.P1435LfsX24
OFCD VIII	1	12 years	Congenital cataract Microcornea	Septate nasal cartilage High nasal bridge Submucosal cleft palate	Pentalogy of Fallot	Delayed dentition Root radiculomegaly Hypodontia	Second-third toe syndactyly Radioulnar synostosis	Mild mental retardation Vesicoureteral reflux	c.4200delG p.P1401RfsX83
OFCD IX	1	7 years	Congenital cataract Microphthalmia Microcornea	Septate nasal cartilage Cleft palate	ASD	Persistent primary teeth Unerupted secondary teeth Root radiculomegaly Hypodontia	Hammer toes Bilateral radioulnar synostosis		c.3848-7_3865del25 Alters conserved splice acceptor site and predicts: p.delexon9 fsX18
OFCD X	1	2 years	Congenital cataract Microphthalmia Microcornea	Septate nasal cartilage High nasal bridge Submucosal cleft palate	ASD	Delayed primary dentition	Hammer toes	Mild mental retardation	c.2428C>T p.R810X
OFCD XI	1 2	42 years 13 years	Congenital cataract Microphthalmia Glaucoma (1) Retinal detachment (1)	Septate nasal cartilage Long/narrow face (1) Simple ears (2)	ASD VSD PDA Tricuspid valve insufficiency (2)	Root radiculomegaly Hypodontia	Hammer toes (2) Short fingers (1)	Cerebral atrophy (2) ADHD (2)	c.1276_1277delCT p.L426VfsX14
OFCD XII	1	17 years	Congenital cataract Glaucoma	Septate nasal cartilage High arched palate	ASD	Root radiculomegaly	Hammer toes Second-third toe syndactyly	Mild mental retardation	Large deletion encompassing at least exons 2-15
OFCD XIII	1	5 years	Congenital cataract	Not affected	ASD	Delayed primary dentition	Hammer toes Second-third toe syndactyly	Hearing impairment	c.3649_3667 dup19 p.A1224MfsX27
OFCD XIV	1	2 years	Congenital cataract Microphthalmia Coloboma	Septate nasal cartilage Simple ears	ASD VSD	Primary dentition unaffected	Second-third toe syndactyly Limited supination at wrist Partial fusion of vertebrae (C2-C3)		c.3427_3428 insA p.S1143LfsX4
OFCD XV	1	3 years	Congenital cataract Microphthalmia	Not affected	ASD	Primary dentition unaffected	Not recorded		c.3848-1G>C p.delexon9 fsX18
OFCD XVI	1 2 3	27 years 27 years 18 months	Congenital cataract Microphthalmia	Septate nasal cartilage (1, 2) High nasal bridge (1, 2) Long/narrow face (1, 2) Not affected (3)	ASD (3) VSD (1) Not affected (2)	Delayed primary dentition (3) Delayed dentition (1, 2) Persistent primary teeth (1, 2) Root radiculomegaly (1, 2)	Second-third toe syndactyly Radioulnar synostosis (2) Scoliosis (2)		Large deletion encompassing at least exons 4-15 Somatic mosaicism (1, 2) Heterozygous (3)
OFCD XVII	1 2	33 years 14 months	Congenital cataract (2) Microcornea (2) Not affected (1)	Septate nasal cartilage (2) Long/narrow face (2) Not affected (1)	Not affected (1, 2)	Delayed primary dentition (2) Not affected (1)	Second-third toe syndactyly (2) Not affected (1)		Small deletion encompassing exons 13 and 14 c.4742-141_4976+821del1410 p.DT581GfsX15 Somatic mosaicism (1) Heterozygous (2)
OFCD XVIII	1	20 years	Congenital cataract Microphthalmia Ptosis	Septate nasal cartilage High nasal bridge Long/narrow face Cleft palate	ASD	Root radiculomegaly Hypodontia	Second-third toe syndactyly Limited supination		c.4540C>T p.R1514X

Table 2 (*Continued*)

Family	*Case*	*Age*	*Ocular*	*Facial/cranial*	*Cardiac*	*Dental*	*Skeletal*	*Other*	*Mutation*
OFCD XIX	1	14 years	Congenital cataract Microphthalmia Ptosis	Septate nasal cartilage High nasal bridge Long/narrow face Simple ears	Not affected	Delayed dentition Root radiculomegaly Hypodontia Fusion of teeth	Not recorded	Mild mental retardation	c.570delC p.W191GfsX25
OFCD XX	1	12 years	Congenital cataract	Septate nasal cartilage High nasal bridge Long/narrow face	Not affected	Delayed dentition Persistent primary teeth Root radiculomegaly Hypodontia	Hammer toes Second-third toe syndactyly Scoliosis		c.863delC p.P288RfsX90
OFCD XXI	1	10 months	Congenital cataract Microphthalmia	Not recorded	ASD	Not recorded	Not recorded		c.2926C>T p.R976X
Lenz I	1	7 years	Microphthalmia	Narrow forehead Simple ears	ASD	Not affected	Multiple partial finger syndactyly Fifth finger clinodactyly Radioulnar synostosis	Mental retardation Hypospadias	c.254C>T p.P85L

Patients are represented by two numbers: the first indicates the family, the second indicates the individual case. Features specific to individuals within a family are indicated in parentheses ( ). Ages are at the time of examination.
ASD, atrial septal defect; VSD, ventricular septal defect; MI, mitral valve insufficiency; PDA, patent ductus arteriosus; DORV, double outlet right ventricle; ADHD, attention deficit hyperactivity disorder.
[a]Full phenotypes in McGovern *et al*.[25] *BCOR* mutation identified in this study.
[b]Brief phenotype and mutation in Hilton *et al*.[11] Full phenotype of individual and family members described in this study.

(Figure 2d). Amplification from exons 8 and 9 were at the control intensity. Amplification from proband XVII.2 was reduced to 50% for exons 13 and 14, suggesting a non-mosaic distribution of this deletion (Figure 2e). PCR amplification and sequencing of a region spanning the proposed deletion region was performed to refine the deletion to 1410 bp from intron 12–14 (Figure 2f).

Clinical features of OFCD syndrome

The cohort presented here comprises 31 new females and four previously described females with mutations in *BCOR* (35 patients). However, one female (XVII.1) is asymptomatic, despite proving mosaic for a *BCOR* mutation, and thus is excluded from the phenotypic evaluation, bringing the cohort of symptomatic females to 34. A summary of the frequency of phenotypes within the 34 symptomatic females is given in Table 3.

Ocular OFCD syndrome is associated with ocular defects and the cardinal manifestation of congenital cataract is present in each individual in the new cohort presented here (34/34). In addition to congenital cataract, patients displayed an array of eye anomalies, most frequently microphthalmia and/or microcornea in 82% of patients (28/34; Figure 3a and b).

Facial In our cohort, 31 patients were examined for facial anomalies and a facial phenotype was recorded in 26 patients. The most specific facial manifestation is septate nasal cartilage, observed in 96% (25/26) of affected patients (Figure 3a and b), often associated with a high nasal bridge and long and/or narrow face. Many of the patients had palatal anomalies, including cleft palate, high-arched palate and bifid uvula (31%; 8/26).

Cardiac Where examined, the majority of patients in the cohort presented here have a congenital heart defect (74%; 20/27), with 85% (17/20) presenting with a septal defect, most commonly an atrial septal defect. Further cardiac anomalies such as pentalogy of Fallot, double-outlet-right-ventricle, valve insufficiencies and patent ductus arteriosus are observed, suggesting a role for *BCOR* in multiple cardiac processes.

Dental In this cohort, 30 patients were examined for dental defects. Owing to age, data corresponding only to primary dentition were available for eight patients. Of these, 75% (6/8) have a dental phenotype of delayed primary dentition and/or persistent primary teeth. Two patients have thus far unaffected primary dentition but it is anticipated that secondary dentition will be affected. Data regarding both primary and secondary dentition are available for 22 patients in this cohort and all have a variety of dental anomalies associated with OFCD syndrome, that is, delayed/persistent dentition with multiple

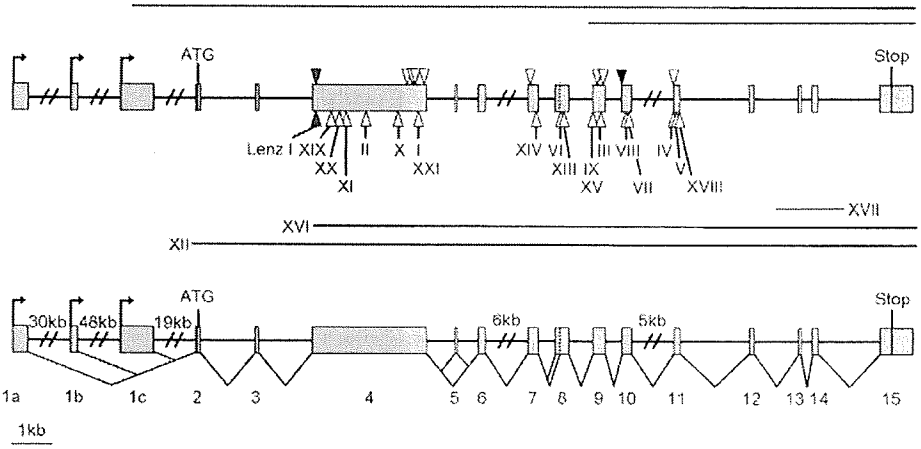

Figure 1 Location of reported mutations within the *BCOR* gene. Arrowheads above the diagram indicate mutations described in previous reports. Mutations indicated below the diagram are those identified in this study (see Table 2). Arrowheads in white indicate mutations corresponding to females with OFCD syndrome. Arrowheads in grey indicate mutations corresponding to males with Lenz microphthalmia syndrome. Arrowhead in black indicates an in-frame deletion occurring in a female with OFCD syndrome. Deletions are indicated by solid horizontal lines.

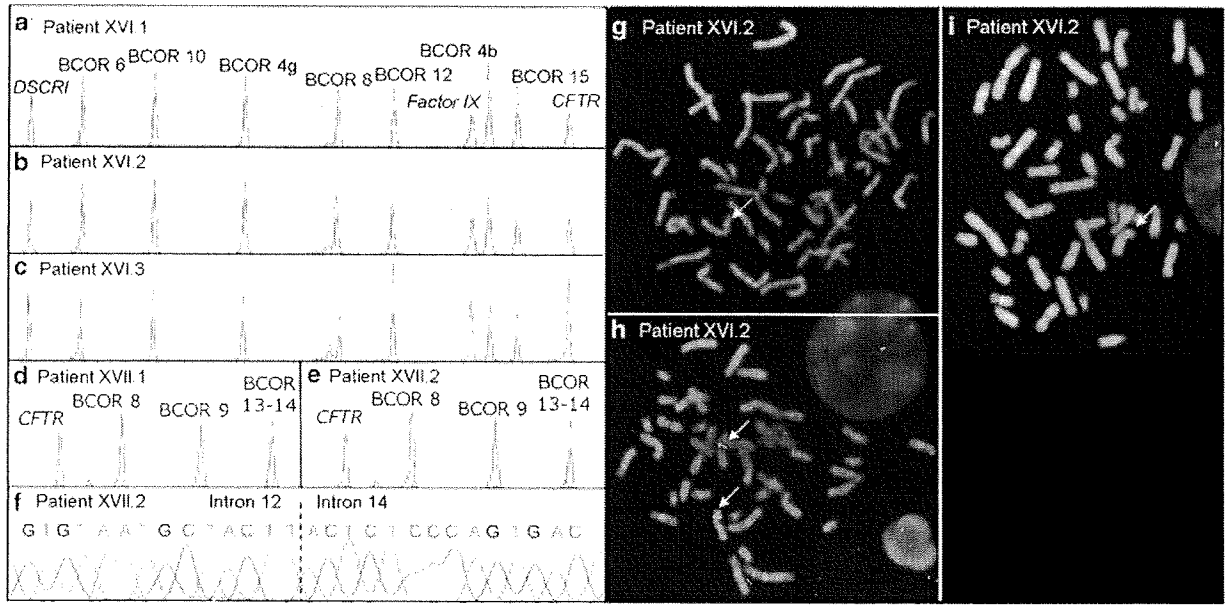

Figure 2 Evidence for somatic mosaicism in individuals XVI.1, XVI.2 and XVII.1. (**a**–**e**) QMF-PCR fluorescent spectra obtained for fragments of the *BCOR* gene and control genes, with patient peaks in red and control subject peaks in blue. Patient XVI.1 (**a**), patient XVI.2 (**b**) and patient XVII.1 (**d**) display diminished peak intensity to approximately 75% of the control peak intensity, suggesting mosaicism of the deleted region. Patient XVI.3 (**c**) and patient XVII.2 (**e**) display diminished peak intensity to approximately 50% of the control peak intensity, suggesting a non-mosaic deletion. (**f**) Sequence chromatogram of patient XVII.2, confirming a deletion between introns 12 and 14, resulting in loss of exons 13 and 14. (**g**–**i**) FISH analysis using BAC clone RP11-330L22, encompassing the *BCOR* gene, with signals indicated by white arrows. Data for patient XVI.1 are equivalent to patient XVI.2. Patient XVI.2 (**g**, **h**) displays a single signal in approximately 50% of metaphase preparations (**g**) whereas the remaining preparations show two specific signals (**h**), confirming that these individuals are mosaic for a deletion of the *BCOR* gene. For patient XVI.3 (**i**), a specific single signal was observed in 100% of metaphase preparations, confirming that the deletion of the *BCOR* gene is non-mosaic and present in all cells.

unerupted teeth, root radiculomegaly and absent/duplicated/fused teeth (100%; 22/22; Figure 3c). Root radiculomegaly, a unique and cardinal diagnostic feature of OFCD syndrome, is present in 91% (20/22) of patients, with diagnosis limited in the remaining two patients by the unavailability of a dental X-ray.

Table 3 Phenotypes associated with OFCD syndrome in patient cohort

Feature	*Occurrences*	*% Affected*
Females with *BCOR* mutation	35	
Females with OFCD syndrome phenotype	34/35	
Ocular		
Recorded	34/34	
Affected	34/34	100
Congenital cataract	34/34	100
Microphthalmia/microcornea	28/34	82
Coloboma	1/34	3
Ptosis	3/34	9
Secondary glaucoma	4/34	12
Lens dislocation	1/34	3
Optic disc dysplasia	1/34	3
Phthisis bulbi	1/34	3
Iris synechia	2/34	6
Retinal detachment	1/34	3
Facial		
Recorded	31/34	
Affected	26/31	84
Septate nasal cartilage	25/26	96
High nasal bridge	11/26	42
Long narrow face	8/26	31
Palate/uvula anomalies	8/26	31
Simple ears	2/26	8
Cardiac		
Recorded	27/34	
Affected	20/27	74
Unresolved heart murmur	1/20	5
Septal defects	17/20	85
Patent ductus arteriosus	3/20	15
Valve incompentency	4/20	20
Pentalogy of Fallot	1/20	5
Dextrocardia	1/20	5
Double outlet right ventricle	1/20	5
Dental		
Recorded	30/34	
Primary dentition only	8/30	
Primary and secondary dentition	22/30	
Affected	22/22	100
Delayed/persistent/unerupted dentition	18/22	82
Root radiculomegaly (secondary teeth)	20/22	91
Hypodontia/duplication/fusion (secondary teeth)	14/22	63
Skeletal		
Recorded	29/34	
Affected	28/29	97
Hammer toes	15/28	54
Second-third toe syndactyly	16/28	57
Radioulnar synostosis/limited supination at wrist	9/28	32
Lordosis/scoliosis/vertebral fusion	7/28	25
Short fingers	1/28	4
Other		
Mental retardation	6/34	18
Cerebral atrophy	1/34	3
ADHD	1/34	3
Hearing impairment	5/34	15
Poor feeding/vomiting/reflux	3/34	9
Asplenia	1/34	3
Vesicoureteral reflux	1/34	3

ADHD, attention deficit hyperactivity disorder.

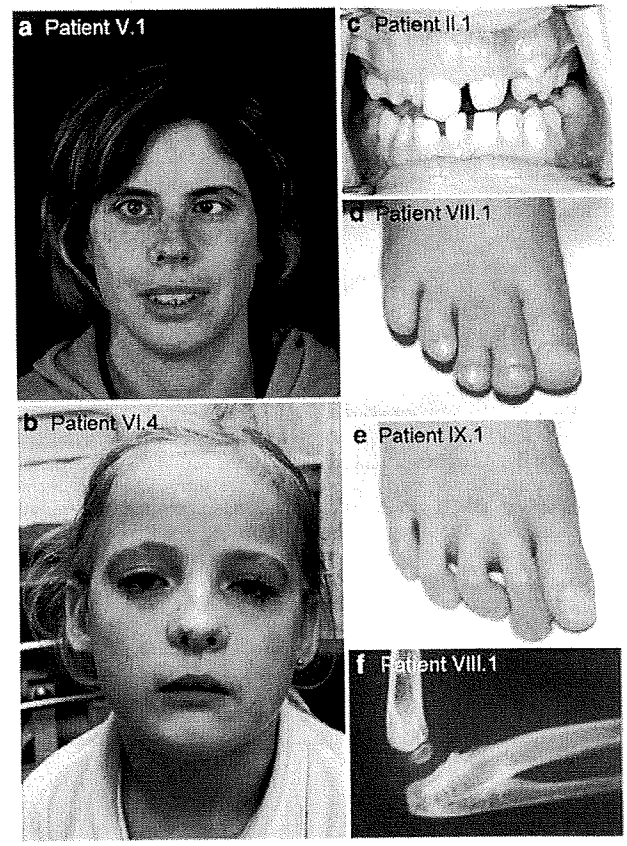

Figure 3 OFCD syndrome. (**a**, **b**) Typical facial features of OFCD syndrome include microphthalmia (left eye of each patient), long narrow facies and septate nasal cartilage. (**c**) The dental phenotype of OFCD syndrome includes hypodontia and tooth fusion and duplication. (**d**, **f**) Digit anomalies in OFCD syndrome include second-third toe syndactyly (**d**) and hammer toes (**e**). (**f**) Radioulnar synostosis in a patient with OFCD syndrome.

Skeletal A number of abnormalities of the skeleton have been reported to be associated with OFCD syndrome and in 29 patients examined, we observe skeletal anomalies in 28 cases. Hammer toes are present in 54% (15/28) of affected patients (Figure 3e) and second-third toe syndactyly in 57% (16/28; Figure 3d); 93% (26/28) of patients have at least one of these phenotypes. Radioulnar synostosis is more recently recognized as a skeletal feature of OFCD syndrome and was detected in 25% (7/28) of affected patients of the cohort described here (Figure 3f), with a further two patients reporting limited supination.

Other Developmental problems have been observed in patients with OFCD syndrome and in the cohort presented here, 18% (6/34) of patients demonstrate a level of mental retardation, although in most cases this is mild. Hearing loss, both conductive and sensorineural, is

observed in 15% (5/34) of patients. Patients with OFCD syndrome may manifest gastrointestinal problems, possibly as part of a laterality phenotype, and we report feeding difficulties and repeated vomiting and reflux in 9% (3/34) of the cohort presented here. A further manifestation of defective lateral patterning, asplenia, is observed in one patient.

Sequence analysis of *BCOR* in males with presumed X-linked ('Lenz') microphthalmia syndrome

To date, a single family in which males are affected by X-linked microphthalmia remain the sole patients with 'Lenz' microphthalmia syndrome to have been shown to carry a *BCOR* mutation.[3] We have sequenced the *BCOR* gene in 21 males with a putative diagnosis of Lenz microphthalmia syndrome, based on an ocular phenotype presenting with additional features of Lenz microphthalmia syndrome, including mental retardation, hearing impairment and skeletal/digital anomalies. In the majority of cases (20/21), there was no evidence of a family history.

We identified a missense mutation in one patient with Lenz microphthalmia syndrome, c.254C>T, predicting p.P85L substitution at the protein level (Lenz I; Table 2). This missense change is identical to the mutation previously described to be associated with Lenz microphthalmia syndrome.[3] The patient presented with bilateral microphthalmia, mild mental retardation, atrial septal defect and a typical array of skeletal and digital abnormalities. Interestingly, the patient presented with right radioulnar synostosis, a phenotype not previously associated with Lenz microphthalmia syndrome but strongly linked to OFCD syndrome.

We did not find a mutation in *BCOR* in 20 of the male patients, suggesting that (1) the diagnosis of Lenz microphthalmia syndrome is disputed or (2) mutations in *BCOR* are not the major cause of Lenz microphthalmia syndrome, a finding replicated by others.[22]

Sequence analysis of *BCOR* in patients with unspecified ocular developmental anomalies

To investigate the contribution of mutations in *BCOR* in non-specific forms of ocular and mental retardation syndromes, we analysed the *BCOR* gene in a panel of 96 patients with isolated microphthalmia, coloboma and/or mental retardation. We identified a single mutation in *BCOR* in a female patient with what had been recorded as isolated bilateral cataract and unilateral microphthalmia (OFCD VII.1). The proband's mother had a similar phenotype. The mutation was c.4303_4307delCCATG p.P1435LfsX24. On further questioning, it was found that the patient had numerous primary teeth removed in teenage years and had second-third toe syndactyly, suggesting that this female has a mild OFCD syndrome phenotype.

Sequence analysis of *BCOR* in patients with unspecified lateral specification defects

We have previously identified defects in lateral specification as part of the clinical spectrum of OFCD syndrome.[11] We therefore hypothesize that mutations in *BCOR* may account for non-syndromic laterality defects, with heart defects representing the most common manifestation of defects in lateral determination. This hypothesis is supported by the observation that in population studies, there is a ~2:1 male predominance of patients with laterality defects not attributable to mutations in *ZIC3*;[12,26] further X-linked genes, such as *BCOR*, may therefore be involved in human lateral specification. We analysed the *BCOR* gene in a panel of 96 patients with a variety of cardiac/laterality defects. We identified sequence alterations in three patients: a female with *situs inversus* and dextrocardia (heterozygous, c.2288G>T, p.R763L), a female with mesocardia, VSD, partial anomalous pulmonary venous return and intestinal malrotation (heterozygous, c.3974A>G, p.K1325R) and a male with transposition of great arteries, pulmonary atresia, VSD and asplenia (hemizygous, c.3974A>G, p.K1325R). These sequence alterations do not appear in SNP databases or within the available EST sequences. However, these changes were also identified in three sequences from a panel of 171 ethnically matched unaffected controls (one female heterozygous for c.2288G>T, p.R763L; two females heterozygous for c.3974A>G, p.K1325R), suggesting that they are rare polymorphisms rather than pathogenic changes.

Discussion

In this study, we have focused on the precise clinical features of OFCD and Lenz microphthalmia syndromes, allelic disorders caused by mutation of the *BCOR* gene, as examples of X-linked syndromic microphthalmia-associated conditions. Given the heterogeneity amongst X-linked microphthalmia syndromes, extension of our knowledge of the mechanism by which *BCOR* mutation is associated with OFCD and Lenz microphthalmia syndromes may be pertinent to other disorders. To that end, we searched for mutations in the *BCOR* gene in patients with OFCD syndrome, presumed X-linked recessive ('Lenz') microphthalmia syndrome, isolated ocular defects and lateral specification defects.

Amongst pathogenic sequence variants that cause OFCD syndrome and Lenz microphthalmia syndromes, the mutations presented here confirm the sharply demarcated genotype/phenotype correlation whereby hemizygous males that carry a missense mutation in *BCOR* have Lenz microphthalmia syndrome and heterozygous females carrying a null allele have OFCD syndrome. The phenotype in female patients may be variable, not only due to differential X-inactivation effects, but also due to somatic

mosaicism, a novel finding in three females in the cohort presented here. To date, mutations in *BCOR* are the sole molecular cause of OFCD syndrome and all mutations identified are null alleles. It is notable that females with putative diagnoses of OFCD syndrome in the absence of radiculomegaly prove to be negative for mutations in *BCOR*, suggesting a strong association of this cardinal phenotype with mutations in *BCOR*. The mutation we found in a female patient with apparently isolated microphthalmia and cataract suggests that OFCD syndrome may be under diagnosed and, at the mild end of the spectrum, may have relatively insignificant non-ocular features.

Three patients with OFCD syndrome with manifestations consistent with defective lateral patterning (dextrocardia, asplenia and intestinal malrotation) have been described.[11] The clinical features of two of these patients (II.1 and III.6), who display dextrocardia and asplenia respectively, are described in more detail in this report. As it has been demonstrated that BCOR is necessary in the frog embryo to confer correct lateral organization of heart and intestinal tract, it is hypothesized that defects of lateral patterning, particularly of internal organs, are a feature of OFCD syndrome. The association of cardiac septal defects and correct laterality specification is well documented and it is likely that the high frequency of cardiac septal defects in OFCD syndrome is the most common manifestation of aberrant laterality specification. Defects of the midline are aetiologically linked with lateral patterning and it is possible that the facial clefting and septate nasal cartilage observed in OFCD syndrome also result from defective lateral patterning.

Amongst the skeletal features associated with OFCD syndrome it is of note that radioulnar synostosis, a more recently recognized feature, was detected in 25% of the patients with a skeletal phenotype described here. This was also a feature of the patient with Lenz microphthalmia syndrome with the c.254C>T, p.P85L missense mutation. This is identical to what was previously described in Lenz microphthalmia syndrome,[3] supporting the pathogenicity of this sequence variant and also suggesting that radioulnar synostosis is an important marker of *BCOR* mutation.

The genetic causes of Lenz microphthalmia syndrome thus remain largely undefined. Subsequent to the first report by Lenz (1955),[4] there have been a large number of reports of X-linked recessive microphthalmia syndrome.[27–41] These reports show broad phenotypic variability in males with multiple congenital anomalies. However, in some cases, the absence of a proven X-linked family history must call into question the validity of the diagnosis. It is likely that some reported cases of 'sporadic Lenz microphthalmia syndrome' are in fact misdiagnosed and represent other forms of 'syndromic microphthalmia'. As a result, the exact incidence of Lenz microphthalmia syndrome remains uncertain although the number of families with proven X-linked inheritance is very small. Unfortunately, a genotypic diagnosis of Lenz microphthalmia syndrome is currently impossible due to lack of genetic information. Lenz microphthalmia syndrome has been associated with at least two genetic loci, one at Xq27-q28[1] and the second occupied by the *BCOR* gene.[2,3] After exclusion of a *BCOR* mutation in all except one of our male cohort, we examined other candidate genes which might be associated with X-linked microphthalmia/mental retardation, specifically *PQBP1* (mutations in which cause microphthalmia and mental retardation)[42] and *BCOR-like 1* (based on homology to *BCOR* and chromosomal location at Xq26.1, in the region of the MCOPS1 locus). No mutations were found in either gene (data not shown), demonstrating that neither is a major cause of X-linked microphthalmia syndromes. Combined with the low incidence of mutations in *BCOR* in male microphthalmia patients, these data suggest that the major locus for X-linked microphthalmia/mental retardation remains unidentified.

In summary, we have undertaken careful phenotypic analysis of both females with OFCD syndrome and male patients with MCOPS2. Apart from ocular defects, facial dysmorphia, congenital heart defects and dental anomalies, we find a high incidence of skeletal defects. Although both congenital heart defects and defects of laterality determination may be associated with OFCD, we did not find *BCOR* sequence variants amongst those with unspecified lateral specification defects. Nonetheless we recommend that laterality defects should be investigated, both as cardiac manifestations and intestinal problems in patients with OFCD syndrome. Finally, we suggest that the skeletal phenotype of radioulnar synostosis – which we have seen to be associated with both OFCD and Lenz microphthalmia syndrome – as a further clinical predictor of *BCOR* mutation.

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