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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. *European Journal of Human Genetics* (2009) 17, 1325–1335; doi:10.1038/ejhg.2009.52; published online 15 April 2009

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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-q28¹ (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 extra-ocular 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

<i>Syndrome nomenclature and alternative names</i>	<i>MIM</i>	<i>Description^a</i>	<i>Locus</i>	<i>Gene</i>
MCOPS1 Lenz microphthalmia syndrome (MAA)	309800	Micro/anophthalmia, dental anomalies, skeletal/digital anomalies, mental retardation, facial dysmorphism	Xq27-q28	
MCOPS2 Oculofaciocardiodental syndrome (MAA2)	300166	Micro/anophthalmia, congenital cataracts, radiculomegaly, cardiac defects, skeletal/digital anomalies, facial dysmorphism	Xp11.4	<i>BCOR</i>
MCOPS4	301590	Micro/anophthalmia, ankyloblepharon, mental retardation	Xq27-28	
MCOPS7 Midas syndrome MLS	309810	Microphthalmia, linear skin pigmentation defects	Xp22.2	<i>HCCS</i>
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	<i>NHS</i>
ND (Norrie disease)	310600	Microphthalmia, neuroretinal degeneration, mental retardation, sensorineural deafness	Xp11.4	<i>NDP</i>
Not assigned; proposed as part of the Renpenning syndrome spectrum	300463	Microphthalmia, coloboma, skeletal/digital anomalies, mental retardation, facial dysmorphism	Xp11.23	<i>PQBP1</i>

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.

^aNote 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 (*DSCRI* – 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 μ l 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 μ M). 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 detail¹¹ 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	34 years	Congenital cataract	Septate nasal cartilage	Heart murmur (1)	Unruptured secondary teeth (1)	Hammer toes (1)	Poor feeding (2)	c.2926C>T	
	2 ^a	8 days	Microphthalmia (2)	High nasal bridge (2)	ASD (2) VSD (2)	Hypodontia (1)	Radicular synostosis (1)		p.R976X	
OFCD II	1 ^b	23 years	Leisnig dysplasia (2)	Septate nasal cartilage	Pulmonary valve stenosis (2)	Persistent primary teeth	Hammer toes	Hearing impairment	c.1539_1540insG	
			Congenital cataract		PDA	Unruptured secondary teeth			p.P514Asx4	
			Microphthalmia		MI	Root radiculomegaly				
					Dextrocardia	Hypodontia				
					ASD (6)	Delayed primary dentition (6)	Hammer toes (1, 2, 4, 5)	Mental retardation (6)	c.4116delGinsCT	
					VSD (6)	Persistent primary teeth	Second-third toe syndactyly (3, 6)	Asplenia (6)	p.E1372Dfsx37	
	DOOR (6)	Persistent primary teeth	Radicular synostosis (4, 6)							
OFCD III	1 2 3 4 5 6 ^b	69 years 65 years 43 years 40 years 21 years 4 years	Congenital cataract	Septate nasal cartilage	Not recorded	Unruptured secondary teeth (1, 2, 3, 4, 5)	Hammer toes (1, 2, 4, 5)	Mental retardation (6)		
			Microphthalmia	Cl ₁ , 3, 4, 5)		Root radiculomegaly (1, 2, 3, 5)				
			Chaucoma (2, 4)	Not affected (2)						
			Phthisis bulbi (1)							
OFCD IV	1 2	37 years 11 years	Congenital cataract	Septate nasal cartilage	Aortic valve stenosis (1)	Hypodontia (5)	Hammer toes (1)	Mild mental retardation (2)	c.4488_4497del10	
			Microphthalmia	High nasal bridge	Not affected (2)	Persistent primary teeth	Second-third toe syndactyly	Hearing impairment	p.G1497fsx68	
OFCD V	1	31 years	Microphthalmia	High nasal bridge		Unruptured secondary teeth	Lordosis	Vomiting/ reflux		
			Microphthalmia	High arched palate (2)		Hypodontia				
OFCD VI	1 2 3 4	70 years 41 years 40 years 10 years	Congenital cataract	Septate nasal cartilage	ASD	Duplicated teeth (2)	Hammer toes	Hearing impairment (1)	c.4512_4514 delTTGinsA	
			Microphthalmia	High nasal bridge		Persistent primary teeth	Radicular synostosis		p.A1506X	
			Microphthalmia (2, 4)	Submucosal cleft palate (4)	ASD (4)	Unruptured secondary teeth	Lumbar scoliosis			
			Iris synechia (2)	Blind uvula (4) Not recorded (1, 3)	Not affected (2, 3)	Persistent primary teeth (2, 4)	Hammer toes (1, 3)	Hearing impairment (1)	c.3621delA	
OFCD VII	1	5 years	Congenital cataract	Not affected	Not recorded	Unruptured secondary teeth (2, 4)	Second-third toe syndactyly		p.K1207Nfsx31	
			Microphthalmia			Root radiculomegaly (4)				
OFCD VIII	1	12 years	Congenital cataract	Septate nasal cartilage	Pentalogy of Fallot	Hypodontia (2, 4)	Second-third toe syndactyly		c.4303_4307delCCATG	
			Microphthalmia	High nasal bridge		Fusion of teeth (4) Duplicated teeth (2, 4)				
OFCD IX	1	7 years	Congenital cataract	Septate nasal cartilage	ASD	Delayed dentition	Second-third toe syndactyly		p.P1435Lfsx24	
			Microphthalmia	Submucosal cleft palate		Root radiculomegaly	Radicular synostosis	Vesicoureteral reflux	c.4200delG	
OFCD X	1	2 years	Congenital cataract	Septate nasal cartilage	ASD	Hypodontia	Hammer toes. Bilateral radicular synostosis		p.P1401Rfsx83	
			Microphthalmia	High nasal bridge		Unruptured secondary teeth				
OFCD XI	1 2	42 years 13 years	Congenital cataract	Septate nasal cartilage	ASD	Persistent primary teeth	Hammer toes	Mild mental retardation	c.3848-7_3865del25	
			Glaucoma (1)	Long/narrow face (1)	PDA	Root radiculomegaly		Alters conserved splice acceptor site and predicts: p.deletion9 fsx18		
OFCD XII	1	17 years	Congenital cataract	Septate nasal cartilage	Tricuspid valve insufficiency (2)	Hypodontia	Hammer toes	Mild mental retardation	p.R810X	
			Glaucoma	High arched palate	ASD	Delayed primary dentition				
OFCD XIII	1	5 years	Congenital cataract	Not affected		Root radiculomegaly	Hammer toes (2)	Cerebral atrophy (2)	c.1276_1277delCT	
			Microphthalmia	Simple ears		Hypodontia	Short fingers (1)		ADHD (2)	p.L426Vfsx14
OFCD XIV	1	2 years	Congenital cataract	Septate nasal cartilage		Root radiculomegaly	Hammer toes	Mild mental retardation		
			Coloboma	Simple ears		Delayed primary dentition				
OFCD XV	1	3 years	Congenital cataract	Not affected		Primary dentition unaffected	Hammer toes	Hearing impairment	c.3649_3667 dup19	
			Microphthalmia	Septate nasal cartilage (1, 2)	ASD (3) VSD (1)	Delayed primary dentition (3)	Second-third toe syndactyly			
			Congenital cataract	High nasal bridge (1, 2)	Not affected (2)	Delayed dentition (1, 2)	Limited supination at wrist			
OFCD XVI	1 2 3	27 years 27 years 18 months	Congenital cataract	Long/narrow face (1, 2)		Root radiculomegaly (1, 2)	Radicular synostosis (2)	Somatic mosaicism (1, 2)	c.3427_3428 insA	
			Microphthalmia	Not affected (3)		Delayed primary dentition (2)	Scoliosis (2)	Heterozygous (3)		
OFCD XVII	1 2	33 years 14 months	Congenital cataract (2)	Septate nasal cartilage (2)	Not affected (1, 2)	Not affected (1)	Second-third toe syndactyly (2)	Small deletion encompassing exons 13 and 14	c.4742	
			Microphthalmia (2)	Long/narrow face (2)		Not affected (1)	Not affected (1)			
OFCD XVIII	1	20 years	Congenital cataract	Septate nasal cartilage	ASD	Root radiculomegaly	Second-third toe syndactyly	Limited supination	p.D1381Gfsx15	
			Microphthalmia	High nasal bridge		Hypodontia				Somatic mosaicism (1)

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.W191GfX25
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 Not recorded	Hammer toes Second-third toe syndactyly Scoliosis		c.863delC p.P288RfsX90
OFCD XXI	1	10 months	Congenital cataract Microphthalmia Microphthalmia	Not recorded	ASD	Not affected	Not recorded	Mental retardation Hypospadias	c.2926C>T p.R976X
Lenz I	1	7 years		Narrow forehead Simple ears	ASD		Multiple partial finger syndactyly Fifth finger clinodactyly Radioulnar synostosis		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.

^aFull phenotypes in McGovern *et al.*²⁵ *BCOR* mutation identified in this study.

^bBrief 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

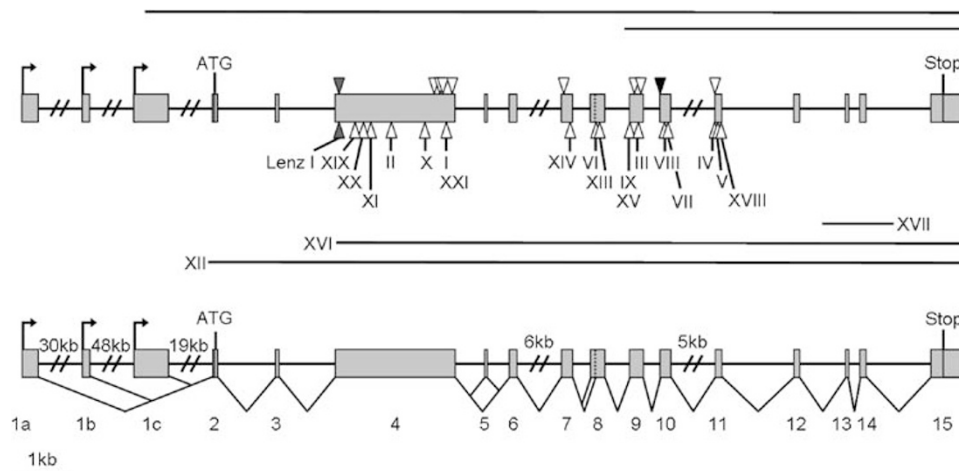


Figure 1 Location of reported mutations within the *BCOR* gene. Arrowheads above the diagram indicate mutations described in previous reports. Mutations indicated below the diagram are those identified in this study (see Table 2). Arrowheads in white indicate mutations corresponding to females with OFCD 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.

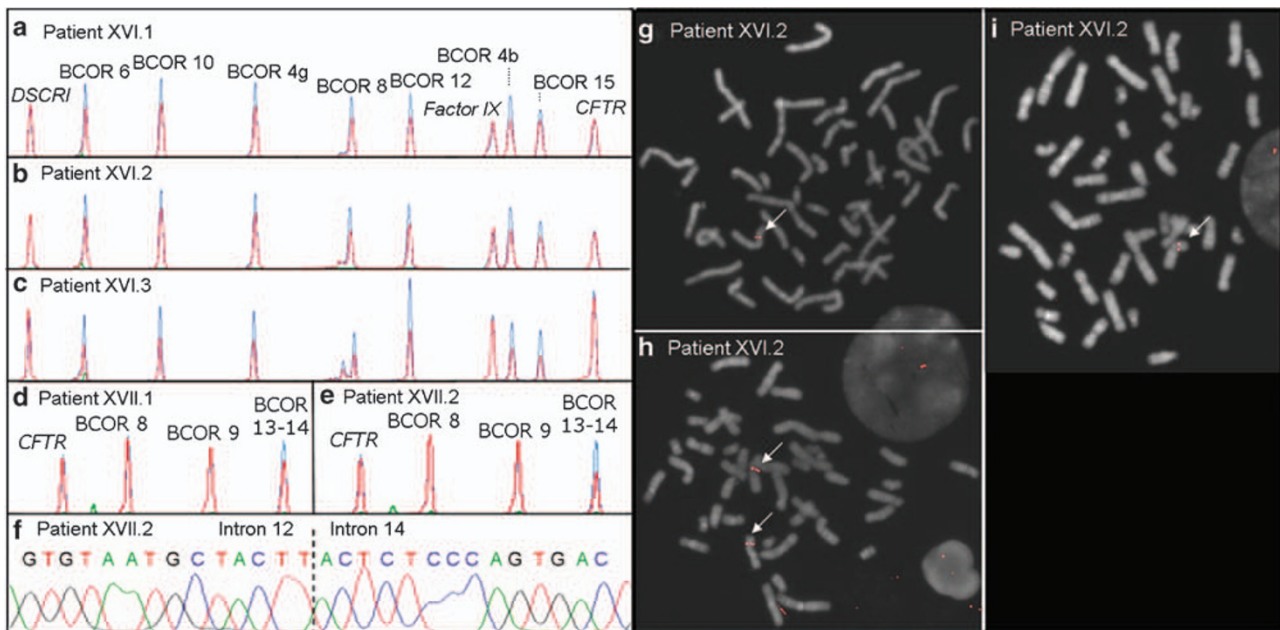


Figure 2 Evidence for somatic mosaicism in individuals XVI.1, XVI.2 and XVII.1. (a–e) QMF-PCR fluorescent spectra obtained for fragments of the *BCOR* gene and control genes, with patient peaks in red and control subject peaks in blue. Patient XVI.1 (a), patient XVI.2 (b) and patient XVII.1 (d) display 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 <i>BCOR</i> mutation	35	
Females with OFCD syndrome phenotype	34/35	
<i>Ocular</i>		
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
<i>Facial</i>		
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
<i>Cardiac</i>		
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 incompetency	4/20	20
Pentalogy of Fallot	1/20	5
Dextrocardia	1/20	5
Double outlet right ventricle	1/20	5
<i>Dental</i>		
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
<i>Skeletal</i>		
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
<i>Other</i>		
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-q28¹ 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 dysmorphism, 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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