Mutations in FLNB cause boomerang dysplasia
L S Bicknell, T Morgan, L Bonafé, M W Wessels, M G Bialer, P J Willems, D H Cohn, D Krakow, S P Robertson


Boomerang dysplasia (OMIM #112310) is a lethal skeletal dysplasia characterised by disorganised skeletal ossification. Characteristic radiological findings include irregular ossification of the limb bones and vertebral, underdevelopment of the acetabulum, and hypoplastic, boomerang shaped femora from which the disorder derives its name. At least eight cases have been described in the literature to date. A similar lethal skeletal dysplasia, Piepkorn dysplasia, has been proposed to be allelic to boomerang dysplasia. Cartilage histology demonstrates both disorganised cartilage anlagen of the developing long bone and frequent multinucleated chondrocytes in the reserve zone of the growth plate. Other developmental abnormalities beyond the skeleton have been described, including omphalocele and encephalocoele.

Similarities in the radiographic and histological findings of boomerang dysplasia and atelosteogenesis I (AOI; OMIM #108720) and III (AOIII; OMIM #108721) have suggested allelism for these disorders. In all three entities, radiographic findings include deficient and irregular ossification of bones and vertebral bodies with distal humeral hypoplasia and joint dislocations in addition to craniofacial abnormalities. Additionally, multinucleated chondrocytes have been observed in both AOI and boomerang dysplasia. Boomerang dysplasia is distinguished from AO on the basis of a more severe defect in mineralisation, with complete absence of ossification in some limb elements and vertebral segments. A preponderance of males among reported cases of boomerang dysplasia and some phenotypic similarities to the otopalatodigital syndrome spectrum disorders caused by mutations in the FLNA gene suggested an X linked aetiology to some authors.

Recently we described the association of mutations in the gene encoding the 280 kDa cytoskeletal protein filamin B with several disorders characterised by skeletal defects and joint dislocations. Filamin B is a modular protein. An actin binding domain composed of two calponin homology domains (CH1 and CH2) is located at the amino terminus, followed by a rod domain composed of 24 structurally homologous repeats and terminating in a carboxyterminal dimerisation domain. The actin binding domain in filamin B is similar to those found in other cytoskeletal proteins, such as β-spectrin, dystrophin, and α-actinin-4.

Four distinct conditions have been attributed to mutations in FLNB: AOI, AOIII, recessively inherited spondylcarpotarsal syndrome (OMIM #272460), and a dominant form of Larsen syndrome (OMIM #150250). Individuals with AOI, AOIII, and dominantly inherited Larsen syndrome were found to be heterozygous for missense mutations in FLNB. The majority
of the mutations leading to AOI or AOIII lead to substitutions localised to the CH2 domain of the actin binding domain of filamin B. The sole exception is the mutation 2251G→C, located in exon 15, which is predicted to lead to the substitution G751R in repeat 6, found in an individual with AOII.\(^{14}\) During the work that identified the group of disorders resulting from mutations in \textit{FLNB}, one individual with boomerang dysplasia was assessed for mutations by direct sequencing, but no mutation was identified.\(^{15}\)

Given the radiographic, phenotypic, and histological evidence suggesting that boomerang dysplasia is related to disorders caused by mutations in genes encoding filamins, we examined two individuals with boomerang dysplasia for mutations in \textit{FLNA} and \textit{FLNB} using denaturing high performance liquid chromatography (DHPLC) analysis as the screening technique.

Individual 1 was the case previously examined by direct sequencing. This male fetus was the first child of non-consanguineous, healthy parents. Second trimester ultrasound scanning revealed thoracic hypoplasia and marked shortening of all limbs with irregular ossification of multiple skeletal elements. The fetus was delivered at 22 weeks’ gestation. Post-mortem analysis demonstrated marked thoracic hypoplasia, short limbs, and the characteristic facial dysmorphism associated with boomerang dysplasia. There was no encephalocele or omphalocele present. The radiographic findings were typical for boomerang dysplasia (fig 1).

Individual 2 was a fetus of 17 weeks’ gestation, previously reported in detail by Wessels \textit{et al.}\(^{14}\) This fetus demonstrated severe micromelia with two of the three long tubular bones missing in all four limbs. On radiographic examination, the remaining radii were boomerang shaped, the tibiae were segment-like, the spine was poorly ossified, and the thorax was hypoplastic. Histologically, the cartilage matrix was hypocellular with multinucleated giant chondrocytes present.

The coding regions and intron–exon splice junctions of both \textit{FLNA} and \textit{FLNB} were amplified from genomic DNA obtained from both cases using intronic primers described previously\(^{15,16}\) (reaction conditions and primer sequences are available on request). For analysis of \textit{FLNA}, amplicon products were visualised by agarose gel electrophoresis and then mixed with equal amounts of PCR amplified products from an unrelated healthy male control before heteroduplex formation. Samples were subjected to DHPLC on a WAVE DNA fragment analysis system (Transgenomic Inc.) according to the manufacturer’s instructions. Amplicons demonstrating anomalous trace forms were re-amplified and cycle sequenced on an ABI 3100 sequencer. Identified mutations were confirmed by restriction enzyme digest of amplified DNA, and parentage was confirmed as declared for case 1 by the segregation of six unlinked microsatellite markers. Parental samples were unavailable for case 2. This study was approved by the Otago ethics committee.

Examination of \textit{FLNB} (exons 1–46) revealed mutations in both subjects. In case 1, the individual was shown to be heterozygous for the mutation 512T→C, which is predicted to lead to the substitution L171R. This mutation creates a site for the restriction enzyme \textit{HpaII}, enabling the demonstration that it had arisen de novo in this family (fig 2), providing genetic evidence of pathogenicity. In retrospect, this mutation was present in the original analysis, but it was overlooked. The second mutation, 703T→C, was found in exon 4 in case 2, and predicts the substitution S235P (fig 2). Parental samples were not available, but this mutation was not present in 100 control chromosomes. Both mutations are predicted to alter residues within the CH2 region of the actin binding domain of filamin B. These residues are highly evolutionarily conserved among vertebrate filamins (fig 3). The mutations suggest that these amino acids play a critical role in actin binding domain function. For the 703T→C mutation, the same substitution has been observed at the analogous residue in \textit{ACTN4}, a gene that encodes the cytoskeletal adapter protein \(\alpha\)-actinin-4 (fig 3), in a patient with focal and segmental glomerulosclerosis.\(^{16}\)

Disease associated substitutions within the actin binding domain of filamin B lead to a diversity of phenotypes. Previously we have reported substitutions leading to AOI (\(n=3\)), AOIII (\(n=1\)), and Larsen syndrome (\(n=2\)).\(^{13}\) All of these phenotypes are associated with defects in skeletogenesis and joint formation. The identification of mutations leading to substitutions within the actin binding domain of filamin B in two patients with boomerang dysplasia confirms that this condition is allelic to these disorders reflecting previous clinical suspicions of relatedness.\(^{1,2}\)

A proportion of mutations that cause the otopalatodigital spectrum of skeletal disorders lies within the analogous CH2 region in \textit{FLNA}. These mutations also lead to a broad spectrum of phenotypic severity.\(^{13}\) There is no simple correlation between the location of these mutations and the severity of the resultant defects in skeletal ossification. While otopalatodigital syndrome type 1 manifests more mildly than otopalatodigital syndrome type 2, these conditions can result
Mutations in FLNB cause boomerang dysplasia

The process of altered cell cleavage leading to both the spread expression of FLNB within the growth plate and most notably in the cleavage furrow of dividing chondrocytes. These appearances could result from defective cell cleavage during the proliferation of chondrocytes within the epiphyseal growth plate. Previously we have demonstrated widespread expression of FLNB within the growth plate and most notably in the cleavage furrow of dividing chondrocytes. The process of altered cell cleavage leading to both the defective skeletal patterning and mineralisation in the boomerang dysplasia/atelosteogenesis group of disorders remains to be elucidated.

The identification of mutations causing boomerang dysplasia in FLNB confirms boomerang dysplasia as part of the chondrodysplasia spectrum including AOI and III, and non-lethal Larsen syndrome. Phenotypic overlap between boomerang dysplasia and AO, as reported here and previously, and the colocalisation of mutations underlying these diseases in exons encoding the actin binding domain of FLNB is indicative of a clinopathological spectrum for these disorders. This further broadening of the phenotypic spectrum associated with mutations in FLNB highlights the importance of a functional actin cytoskeleton for many normal morphogenetic processes, including skeletogenesis.

ACKNOWLEDGEMENTS

We are grateful to the families for their participation in this study.

Authors' affiliations
L S Bicknell, T Morgan, S P Robertson, Department of Paediatrics and Child Health, University of Otago, Dunedin, New Zealand
L Bonafe, Division of Molecular Paediatrics, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
M W Wessels, Department of Clinical Genetics, Erasmus University and University Hospital, Rotterdam, The Netherlands
M G Bialer, Department of Pediatrics, Schneider Children’s Hospital at North Shore/NYU Medical Center, Manhasset, NY, USA
P J Willems, Gendia, Antwerp, Belgium
D H Cohn, Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
D Krakow, Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
S Robertson is supported by the Child Health Research Foundation of New Zealand.

Competing interests: none declared

Correspondence to: Professor S Robertson, Department of Paediatrics and Child Health, Dunedin School of Medicine, PO Box 913, Dunedin, New Zealand; stephen.robertson@stonebow.otago.ac.nz

Received 12 December 2004
Revised version received 7 February 2005
Accepted 18 February 2005

REFERENCES
dysplasia in a fetus with manifestations of atelosteogenesis I and Boomerang dysplasia.


Mutations in FLNB cause boomerang dysplasia

L S Bicknell, T Morgan, L Bonafé, M W Wessels, M G Bialer, P J Willems, D H Cohn, D Krakow and S P Robertson

J Med Genet 2005 42: e43
doi: 10.1136/jmg.2004.029967

Updated information and services can be found at:
http://jmg.bmj.com/content/42/7/e43

These include:

References
This article cites 24 articles, 8 of which you can access for free at:
http://jmg.bmj.com/content/42/7/e43#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Calcium and bone (307)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/