Original Article

Familial Ebstein Anomaly

Whole Exome Sequencing Identifies Novel Phenotype Associated With *FLNA*

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Background—Familial Ebstein anomaly is a rare form of congenital heart disease. We report 7 individuals among 2 generations of 1 family with Ebstein anomaly. This family was first reported in 1991 by Balaji et al in which family members were also reported to have a mild skeletal phenotype. The most likely mechanism of inheritance was concluded to be autosomal dominant. We sought to identify the genetic pathogenesis in this family using a next generation sequencing approach.

Methods and Results—Whole exome sequencing was performed in 2 cousins in this family using the Agilent SureSelect Human all Exon 51 Mb version 5 capture kit. Data were processed through an analytic in-house pipeline. Whole exome sequencing identified a missense mutation in *FLNA* (Filamin A), an actin-binding protein located at Xq28, mutations in which are associated with the skeletal phenotypes Frontometaphyseal dysplasia, Otopalatodigital, and Melnick–Needles syndrome, with X-linked periventricular nodular heterotopia and FG syndrome (Omim, 305450). Review of the phenotypes of those with the mutation in this family shows increased severity of the cardiac phenotype and associated skeletal features in affected males, consistent with X-linked inheritance.

Conclusions—Although congenital heart disease is reported in families with mutations in *FLNA*, this is the first report of individuals being affected by Ebstein anomaly because of a mutation in this gene and details the concurrent skeletal phenotype observed in this family. **(*Circ Cardiovasc Genet*. 2017;10:e001683. DOI: 10.1161/CIRCGENETICS.116.001683.)**

Key Words: Ebstein anomaly ■ exome ■ filamin A ■ frontometaphyseal dysplasia ■ X-linked

Ebstein anomaly (EA) is a rare form of congenital heart disease (CHD) that occurs in ≈1 per 200 000 births.[1] In those with EA, there is malformation of the tricuspid valve with adherence of the septal and posterior valve leaflets to the myocardium, whereas the anterior leaflet is redundant, perforated, or tethered. This is associated with apical displacement of the valve annulus and concomitant atrialization and enlargement of the right ventricle.[1] An associated interatrial communication can also be identified in the majority of cases with this anomaly.

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Genetic factors are recognized in the pathogenesis of this condition as it is more common in those with a family history of (any) CHD,[2] but families in which there are multiple cases of EA occurring in a Mendelian pattern of inheritance are rare.[1] The only genes implicated to date are *NKX2-5*, identified in association with a variety of structural congenital heart defects with phenotypes including EA[3] and the sarcomeric gene β-myosin heavy chain (*MYH7*). Mutations in *MYH7* have been found in association with EA, initially in 4 individuals within the same family with this lesion and in 6% of a second cohort of unrelated individuals with this type of CHD.[4]

Over the last decade, next generation DNA sequencing has become widely available. The techniques used allow capture of the whole exome, and massively parallel DNA sequencing offers a valuable means to identify genes underlying Mendelian disorders.[5] Next generation DNA sequencing has been widely applied to study familial and sporadic forms of CHD and helped to identify several genes underlying these conditions.[6,7] Although these findings have helped understanding of CHD, they do not resolve the cause of most CHD cases.[7] Although the majority of CHD is of unknown pathogenesis, next generation sequencing has led to the discovery of *NR2F2*, a pleiotropic transcription factor, mutations in which are associated with nonsyndromic atrioventricular septal defects.[8]

Here, we report a familial case of EA(Figure 1)[9] manifesting across at least 2 generations, caused by a novel missense

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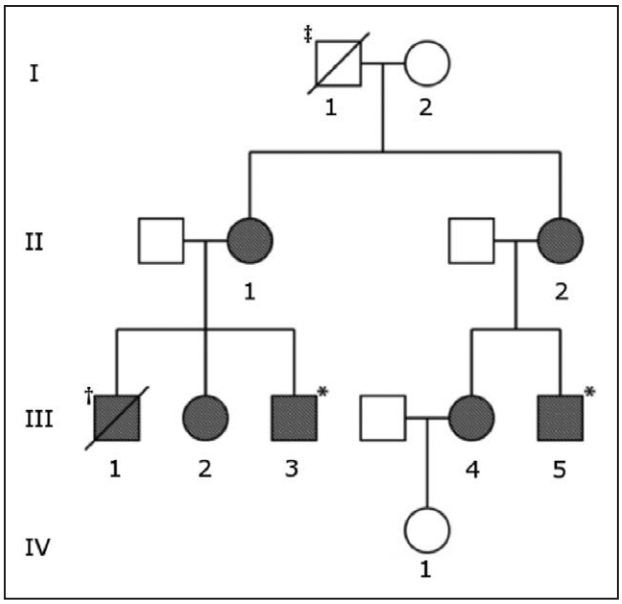

Figure 1. Family pedigree showing segregation of Ebstein anomaly. Affected individuals are shown in dark symbols. * indicates patient selected for whole exome sequencing analysis. †Individual III (1) died at 10 d of age from cardiac failure. ‡Individual I (1) is designated as likely affected after family description of surgical mitral valve replacement, keloid scarring, stiff joints, and wide-based gait.

variant identified through whole exome sequencing (WES) of an affected cousin pair. The clinical manifestation of this family was reported previously but the cause was not identified.[9]

Subjects and Methods

The family was ascertained through the Wessex Clinical Genetics Service, having presented for genetic counseling about the likely recurrence risk of EA after the death of the male proband. Twenty-five years after their original presentation, we obtained consent for detailed genetic investigations in surviving members of the family. The proband, male III (1) was severely affected; he was born at 33 weeks of gestation and died at 10 days of age. Unfortunately, no genetic material was available from him. WES was conducted on DNA samples from individuals III (3) and III (5). Six affected surviving members of the family were examined by a clinical geneticist and their echocardiograms were reviewed by 2 cardiologists. Detailed phenotyping was completed after the identification of a likely causal mutation. Targeted characterization of phenotypic features associated previously with the likely causal gene was undertaken.

DNA Extraction

Genomic DNA was extracted from peripheral venous blood samples collected in EDTA. DNA concentration was estimated using the Qubit 2.0 Fluorometer and A 260:280 ratio calculated using a nanodrop spectrophotometer. The average DNA yield obtained was 150 µg/mL, and ≈20 µg of DNA was used for next generation sequencing for each patient.

WES Data Generation and Data Analysis

WES was performed using the Agilent SureSelect Human all Exon 51 Mb version 5 capture kit. As described previously,[10,11] default parameters were applied: fastq raw data generated from Illumina paired-end sequencing were aligned against the human reference genome (hg19) using Novoalign (novoalign/2.08.02). SAMtools[12] (samtools/0.1.19) was used to call variation and ANNOVAR (annovar/2013Feb21)[13] was applied for variant annotation against a database of RefSeq transcripts. A bespoke script was used to assign individual variants as novel if they were not reported previously in the dbSNP137 databases,[14] 1000 Genomes Project,[15] the Exome Variant Server of European Americans of the NHLI-ESP project with 6500 exomes (http://evs.gs.washington.edu/EVS/), in 46 unrelated human subjects sequenced by Complete Genomics,[16]or in the Southampton database of reference exomes (n=329).

Resultant variant files for each individual were subjected to further in-house quality control tests to detect DNA sample contamination and ensure sex concordance by assessing autosomal and X-chromosome heterozygosity. Variant sharing between all pairs of individuals was assessed to confirm sample relationships. Sample provenance was confirmed by independent genotyping of a validated single nucleotide polymorphism panel, developed specifically for exome data.[17]

Tiered Selection

Tier 1

MYH7 and *NKX2-5* are the 2 candidate genes known to be involved in EA. These 2 genes were tested previously by Sanger sequencing within the family with negative findings. Nevertheless, these genes were selected as top priority to confirm negative results from this alternative sequencing resource.

Tier 2

The Human Gene Mutation Database Professional 2013.4 (BIOBASE Biological Databases)[18] was interrogated using the inclusive term heart disease to retrieve an extensive list of causal genes known to be involved in heart conditions. The query returned 338 genes from which 219 were selected as associated with clinical phenotypes similar to congenital heart defects using the following terms: for example, congenital heart defects, heart valve defect, atrial septal defects and postaxial hexadactyly, and cardiomyopathy and hypertrophic (Table I in the Data Supplement for complete list).

Tier 3

Using WES on the affected cousin pair, their exomes were compared to identify all genetic variants shared between them. All shared variants were removed that were observed (in various states of zygosity) within the local Southampton control cohort of exomes (n=329). Of the remaining variants, synonymous variants with low likelihood to impact protein function, splicing variants with a MaxEnt score of <3, and variants with low conservation across species (PhyloP <0.99) were removed. The known phenotypic effects of the remaining variants were examined for relevance to cardiac anomalies.

Ethical Approval

This study meets the standards expected by the governance structure of the National Health Service and a clear and informed consent was taken from the family about the potential benefits and limitations of such work.

Results

Quality Control Analysis

Exome data were high quality defined by 80% of mappable bases of the Gencode defined exome represented by coverage of at least 20 reads. The average depth of coverage was 60.52 and 54.81 for patients III.3 and III.5 respectively, Table II in the Data Supplement. The 2 samples from the affected cousins selected for exome analysis exhibited expected variant sharing for third degree relatives and no excess of sharing with any other sample on same dispatch DNA plate. Autosomal and X-chromosome heterozygosity were consistent with sex and did not indicate any sample contamination. VerifyBamID[19] did not indicate any presence of contamination, and the application of a single nucleotide polymorphism tracking panel[17] confirmed sample provenance.

Tiered Analysis

In the Tier 1, no coding variation was found in *MYH7* and *NKX2-5* consistent with previous Sanger sequencing results.

Tier 2, the analysis was extended to the 219 genes prioritized from Human Gene Mutation Database. A total of 429 variants were called in either affected cousin across the 219 genes. Given the apparent dominant mode of inheritance evidenced by the pedigree segregation and further supported by literature findings,[20,21] 401 mutations seen in heterozygous form in a local reference database of 329 individual without heart defects were excluded. Of the remaining 28 variants, a further 27 variants were excluded from downstream analysis as they were not observed in both affected cousins and occurred in 1 individual only. A single variant on the X-chromosome in the *FLNA* (Filamin A) gene remained (NM_001110556, c.G4660A, p.G1554R). This variant was novel by our assessment and consistently predicted to be deleterious by several in silico software annotation tools (SIFT, Polyphen2, Mutation Taster, and Gerp=5.67).

Finally, for the Tier 3 analysis, we identified 16 543 variants shared between the cousins. These were filtered to yield a final list of 9 variants (Table III in the Data Supplement). Comprehensive literature review excluded 8 variants within genes functionally irrelevant to phenotype (Table III in the Data Supplement). The novel nonsynonymous *FLNA* variant (c.G4660A, p.G1554R) represented the only outstanding candidate variant. The variant was confirmed using Sanger sequencing. We confirmed the segregation of the genotypic variant assumed to be causal to be consistent with the inheritance pattern of the family on the 6 living affected blood relatives diagnosed with EA. The variant was confirmed as carried in heterozygous state in all affected females and in hemizygous state in all affected males. Patient IV(1) was not tested for the variant.

Mutation Within *FLNA*

The p.G1554R mutation segregating in our family with EA sits within the 14th repeated rod-domain (Figure 2). The mutation replaces a nonpolar amino acid with a polar amino acid and is likely to impact tertiary structure as indicated by PolyPhen2 and GERP scores.

Clinical Phenotype

A summary of the cardiac, musculoskeletal, and facial phenotypes of the family members available for clinical examination is shown in the Table.

Cardiac Phenotypes

Patient I(1)

Medical notes were not available but there was a history, given by surviving relatives of this individual having had a mitral valve replacement in middle age.

Patient I (2)

Limited information was available but was reported by her daughter, II(2), to have no symptoms of cardiac disease at the age of 93 years. She also had a son and a daughter with a different partner and neither child, or in turn, any of their 9 children have presented with cardiac disease.

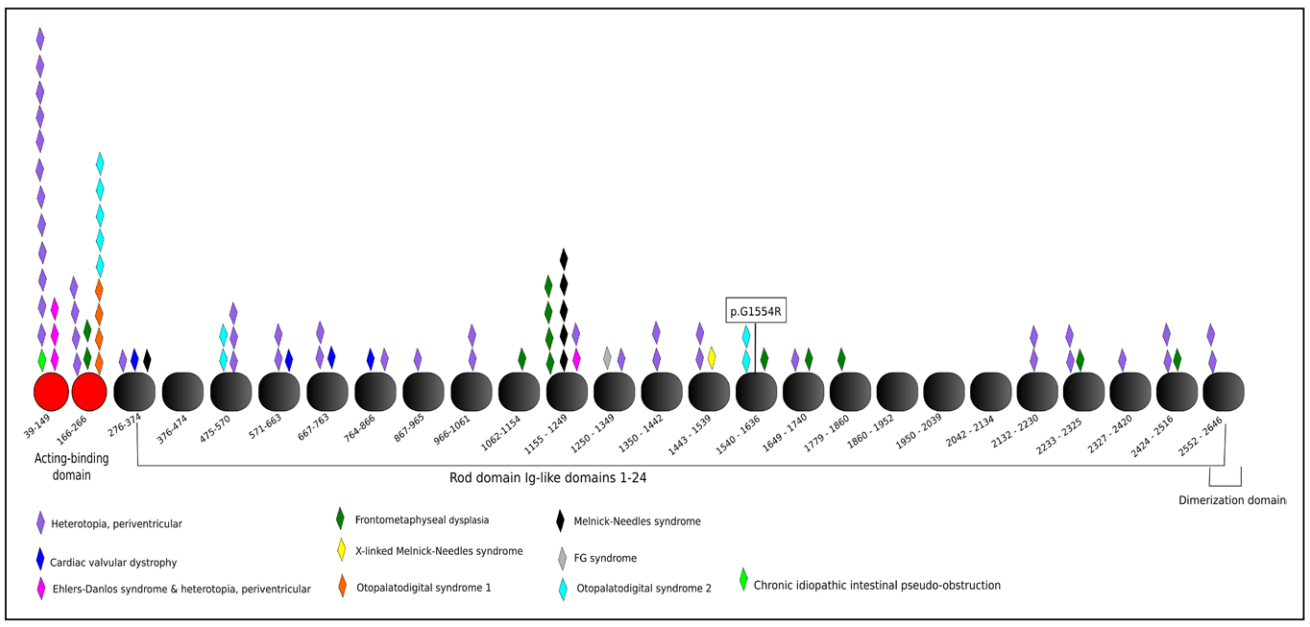

Figure 2. Schematic representation of filamin A protein. Filamin A actin-binding protein is encoded across 48 exons of the *FLNA* gene and acts as a regulator of the actin cytoskeleton.[20,21] The protein contains an N-terminal actin-binding domain (ABD) and a region comprised of 24 repeated immunoglobulin-like (Ig-like) subdomains.[22] The predicted tertiary structure of these repeated domains is 7 antiparallel β sheets of 3 and 4 β strands.[23,24] The Ig-like domains are required for protein interactions with other Ig-like domains via their β-sheets.[25,26] Mutations within *FLNA* have been associated with multiple conditions: cardiac valvular dysplasia, FG syndrome, frontometaphyseal dysplasia, periventricular heterotopia, Melnick–Needles syndrome, otopalatodigital syndrome, type I, otopalatodigital syndrome, type II, and terminal osseous dysplasia.[24] The 107 different known disease causal mutations reported in Human Gene Mutation Database (HGMD) are herein represented by diamonds (eg, the 5 different mutations associated with Melnick–Needles Syndrome fall within the tenth Ig-like domain, whereas mutations associated with periventricular heterotopia are spread across multiple Ig-like domains and the actin-binding domains. The G1554R mutation identified in the current family is shown. All known *FLNA* causal disease mutations, extracted from HGMD in January 2014, are in Table 4 in the Data Supplement.

Table. Cardiac, Musculoskeletal, and Facial Phenotype for Family Members Available for Clinical Examination

	Male		Female			
	III(5)*	III(3)*	II(2)	II(1)	III(4)	III(2)
Age at examination, y	31	27	57	59	34	35
Skeletal features						
Head circumference	60.0 cm (91–98 centile)	60.5 cm (91–98 centile)	58.5 cm (98–99 centile)	54 cm (9–25 centile)	55.5 cm (50 centile)	56.2 cm (50–75 centile)
Limb musculature	↓↓	↓↓	↓	–	–	–
Hypertelorism	++	++	–	+	–	–
Prominent supraorbital ridges	++	++	–	–	–	+
Proptosis	++	++	–	+	–	+
Elbow supination	↓	↓	↓	–	↓	–
Elbow stiffness	++	++	+	+	–	–
Finger stiffness	–	+	+	+	–	+
Keloid scarring	++	++	+	+	+	–
Knee stiffness	++	++	+	–	–	–
Intellectual delay	–	–	–	–	–	–
Cardiac features						
Aortic regurgitation	Mild	–	–	–	–	–
Aortic root aneurysm	–	–	–	–	–	–
Aortic valve insufficiency	Mild	–	–	–	–	–
Atrial septal defect	+	–	–	–	–	–
Bicuspid aortic valve	+	–	–	–	–	–
Cardiomyopathy	–	–	–	–	–	–
Dysplastic pulmonary valve	–	–	–	–	–	–
Left ventricular noncompaction	–	–	–	–	–	–
Mitral regurgitation	Mild	Severe	–	–	–	–
Mitral stenosis	+	+	–	–	–	–
Mitral valve cleft	–	–	–	–	–	–
Mitral valve dysplasia	+	Severe	–	–	–	–
Mitral valve prolapse	–	–	–	–	–	–
Myxomatous valvular dystrophy	+	–	–	–	–	–
Patent ductus arteriosus	–	–	–	–	–	–
Patent foramen ovale	–	–	+	–	–	–
Pulmonary regurgitation	+	+	–	–	–	Mild
Pulmonary stenosis	–	–	–	–	–	–
Surgical intervention	–	Monocuspid tricuspid valve repair in 2006	Ebstein repair in 2006	–	–	–
Tricuspid regurgitation	Mild	Moderate	Residual post Ebstein repair	Mild	Mild	Mild
Tricuspid stenosis	+	+	–	–	–	–
Tricuspid valve displacement	2.0 cm	Surgical repair	Surgical repair	1.4 cm	1.3 cm	1.3 cm
Ventricular septal defect	–	–	–	–	–	–

– indicates not present; +, present; ↓, reduced; and NA, not available.
*Exome sequenced patient.

Patient II (1)
The echocardiogram demonstrated mild apical displacement of the tricuspid valve with a large antero-superior leaflet. There was no mitral valve anomaly and no mitral stenosis or regurgitation; the aortic valve was normal. The left atrium was mildly dilated, consistent with the age of the patient.

Patient II (2)
In this description, the following findings also relate to those found at surgery (repair of EA and placement of a 32 mm tricuspid annuloplasty ring). There was a large anterior leaflet of the tricuspid valve that was partially tethered. There was a partially tethered posterior leaflet and a fully Ebsteinized septal leaflet of the tricuspid valve.

Patient III (1)
The infant was born at 33 weeks gestation with hydrops fetalis. Echocardiography showed severe EA with absent flow into the pulmonary arteries. Despite optimum medical treatment, including with ventilation, dopamine, and prostaglandin infusions, he died at 10 days of age.

Patient III (2)
The findings were similar to III(4) above with mild apical displacement of the septal leaflet of the tricuspid valve (1.3 cm) and mild associated tricuspid regurgitation.

Patient III (3)
The following findings relate to those found at surgery (repair of EA and replacement of the mitral valve) and on preoperative 3-dimensional echocardiography. The tricuspid valve was typical of EA with a large mobile anterior-superior leaflet fused to the posterior leaflet that was partially tethered. There were some secondary cords to the back of both antero-superior and posterior leaflets, with complete tethering of the final portion of the posterior leaflet and Ebsteinization of the whole of the septal leaflet. There was a funnel-like opening into the right ventricle with associated tricuspid stenosis and regurgitation. The mitral valve was stenotic with a fixed orifice (1 cm^2) and heavy calcification of the posterior leaflet and a parachute arrangement of the mitral chordae that were fibrosed and fused.

Patient III (4)
There was isolated mild apical displacement of the tricuspid valve (1.3 cm) with mild associated tricuspid regurgitation.

Patient III (5)
On examination of echocardiogram findings, the tricuspid valve was dysplastic with apical displacement of the septal leaflet (2 cm), mild tricuspid regurgitation, and mild tricuspid stenosis (peak gradient 7, mean 5 mm Hg) with E/A reversal. The mitral valve was also dysplastic with a large antero-lateral papillary muscle and 3 small postero-medial papillary muscles instead of 1 well-formed structure. Mild mitral regurgitation and mild mitral stenosis (peak gradient 14, mean 4 mm Hg) were seen with a dilated left atrium (length 8 cm, volume 34 mL, and area 18.1 cm^2). There was a small amount of calcification on the anterior leaflet. An effectively bicuspid aortic valve was seen with 2 low commissures and a small right coronary cusp. There was mild aortic regurgitation but no aortic stenosis; there was no pulmonary regurgitation. A moderate secundum atrial septal defect with an associated left to right shunt was evident.

Patient IV (1)
Neonatal clinical examination, including detailed cardiovascular examination was normal. At the time of assessment of other family members, patient IV(1)'s parents declined an echocardiogram, genetic testing, or further clinical assessment.

Noncardiac Phenotypes

Although certain mutations in *FLNA* are associated with a neurological phenotype (periventricular nodular heterotopia), there was no history of seizures or unsteadiness in any members of the family. Cerebellar examination was normal in all. There was no history of learning difficulties in any family member examined. Systematic enquiry about urologic diagnoses and deafness was also negative. One family member III(4) had a diagnosis of irritable bowel syndrome but no other abnormalities of bowel motility were reported. About the skeletal phenotype, all family members examined gave a history of stiff joints with both surviving males having fixed flexion of the knees and ankles. A history of joint stiffness was also reported by the family to have been present in family member I(1); anecdotally his gait was also reported to be the same as that observed by the family in both III(3) and III(5). All patients examined had proximally placed and externally rotated fifth toes; male III(5) also had short thumbs with hypoplastic distal phalanges. Both males and 2 of the females had limited supination of the elbows. A dental phenotype was observed in III(5) who had oligodontia with incomplete eruption of secondary dentition. Keloid scarring, noted in all members of the family who were examined was also reported to have been present in I(1; Table for summary).

Although not examined as part of this study, individual IV(1) was described by the family as having no joint limitation, clearly demonstrated to them by her enthusiasm for exercise and general flexibility. The ease of movement in IV(1) was described as a direct contrast to the joint restriction seen in members of generation III during childhood. Patient I(1) was deceased at the time of the study; he died from bowel cancer at the age of 75 years.

Discussion

EA is a rare condition and even less frequently observed segregating in families. We describe a family with evidence for this rare cardiac defect over at least 2 generations. This family was described previously, but genetic pathogenesis remained elusive.

In this study, we have applied contemporary sequencing technology and effective filtering of a cousin pair and identified a single variant within an X-linked gene known to cause cardiac developmental defects but not associated previously with EA. We have confirmed the mutation using traditional Sanger techniques and demonstrated heterozygous and hemizygous carrier status for all female and male affected family members respectively.

The single base substitution (G>A) causes a missense mutation in which a nonpolar Glycine amino acid residue is replaced with a positively charged Arginine. The variant was within a highly conserved (Polyphen=0.999) residue region of the protein that encodes for the 14th Ig-like domain. The tertiary structure of the Ig-like repeated domain consists in 7 antiparallel β

sheets and therefore it might be expected that the p.G1554R mutation causes a conformational change in the β strands.

We have found no previous evidence of this variant in our local or public repository databases. However, mutations within the Ig-Rod domain are causal of a variety of congenital heart defects.

The phenotypic spectrum of disease associated with mutations in *FLNA* is broad and includes several conditions with a predominantly skeletal phenotype: FG syndrome, Frontometaphyseal dysplasia, Melnick–Needles syndrome,[27] Otopalatodigital syndrome, types I and II,[27] and terminal osseous dysplasia.[28] It also includes individuals with a predominantly neurological phenotype (periventricular nodular heterotopia)[29] and those with cardiac manifestations of disease (X-linked cardiac valvular dysplasia).[30] Its causality is further underscored by the missense and loss of function variants presented in ExAC.[31] The family identified were examined for any features of the above conditions. Macrocephaly, seen in FG syndrome and was found in both of the males and 1 female, however, this condition also includes mental retardation, not present in this family. Survival to adulthood noted in this family is not typical in males affected by Melnick–Needles syndrome or Otopalatodigital syndrome type II. Skeletal features including joint contractures, prominent supraorbital ridges, and proptosis observed in members of the family described are typically associated with fibromuscular dysplasia;[27] however, typical of this condition is also conductive and sensorineural hearing loss, neither of which were present in this family. In addition, while cardiac anomalies are described in fibromuscular dysplasia, EA is not.

The data available for the 6 cases described above (based on a combination of the echocardiogram and magnetic resonance imaging findings and the operative findings in the 2 patients who underwent surgery) clearly identify varying degrees of severity of EA in these cases. The phenotype varied between mild septal displacement of the septal leaflet of the tricuspid valve with mild associated tricuspid regurgitation, to severe EA with a combination of tricuspid stenosis and regurgitation. In 3 of the 4 females (II(1), III(4), and III(2)), the anomaly was subtle with isolated mild displacement of the tricuspid valve and only mild tricuspid regurgitation. Only in female patient II (2) was the anomaly more severe and required surgery. This more severe phenotype could be explained by a hypothesized skewed pattern of X-chromosome inactivation that in many other X-linked disorders has been considered as the cause of the phenotype in female carriers.

The males III (3) and III (5) had more severe cardiac presentation with both tricuspid and mitral valves involvement requiring surgery. It is unusual for patients with EA to have mitral valve disease: this is congenital in nature but has also been associated with calcification that was progressive in at least 1 of them. The patient III (3) developed severe mitral valve stenosis and this was an important factor in the timing of surgery. The other, III (5), had a dysplastic mitral valve with only mild calcification of the anterior leaflet and only mild stenosis and regurgitation. Only 1 patient had an atrial septal defect and 1 patient (operated) had a patent foramen ovale. However, it is not possible to exclude small patent foramen ovales in the other patients as they have not had a contrast echocardiogram.

The finding of mitral valve disease in addition to EA and abnormalities of the pulmonary and aortic valves in members of this family illustrates a phenotypic overlap with X-linked valvular dysplasia.[23] The variability of the phenotype comparing affected males with each other (and similarly, comparing the affected females with each other) is consistent with that observed among individuals who harbor pathogenic variants in other genes that predispose to the manifestation of a cardiac phenotype, such as *GATA4*.[32]

In conclusion, the absence of a neurological phenotype, survival of some males to adulthood, and the presence of EA in multiple members of this family mean that the *FLNA* mutation described is associated with the unique phenotype seen in this family and furthermore, it represents a novel cause for familial EA. However, because of the presence of additional features segregating with EA in this family, there is little evidence that mutations in *FLNA* will be a common cause of sporadic, nonsyndromic EA.

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Disclosures

None.

References

1. Attenhofer Jost CH, Connolly HM, Dearani JA, Edwards WD, Danielson GK. Ebstein's anomaly. *Circulation*. 2007;115:277–285. doi: 10.1161/CIRCULATIONAHA.106.619338.
2. Ferencz C, Rubin JD, McCarter RJ, Brenner JI, Neill CA, Perry LW, et al. Congenital heart disease: prevalence at livebirth. The Baltimore-Washington Infant Study. *Am J Epidemiol*. 1985;121:31–36.
3. Benson DW, Silberbach GM, Kavanaugh-McHugh A, Cottrill C, Zhang Y, Riggs S, et al. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. *J Clin Invest*. 1999;104:1567–1573. doi: 10.1172/JCI8154.
4. Postma AV, van Engelen K, van de Meerakker J, Rahman T, Probst S, Baars MJ, et al. Mutations in the sarcomere gene MYH7 in Ebstein anomaly. *Circ Cardiovasc Genet*. 2011;4:43–50. doi: 10.1161/CIRCGENETICS.110.957985.
5. Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, et al. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet*. 2011;12:745–755. doi: 10.1038/nrg3031.
6. Arrington CB, Bleyl SB, Matsunami N, Bonnell GD, Otterud BE, Nielsen DC, et al. Exome analysis of a family with pleiotropic congenital heart disease. *Circ Cardiovasc Genet*. 2012;5:175–182. doi: 10.1161/CIRCGENETICS.111.961797.
7. Zaidi S, Choi M, Wakimoto H, Ma L, Jiang J, Overton JD, et al. De novo mutations in histone-modifying genes in congenital heart disease. *Nature*. 2013;498:220–223. doi: 10.1038/nature12141.
8. Al Turki S, Manickaraj AK, Mercer CL, Gerety SS, Hitz MP, Lindsay S, et al; UK10K Consortium. Rare variants in NR2F2 cause congenital heart defects in humans. *Am J Hum Genet*. 2014;94:574–585. doi: 10.1016/j.ajhg.2014.03.007.
9. Balaji S, Dennis NR, Keeton BR. Familial Ebstein's anomaly: a report of six cases in two generations associated with mild skeletal abnormalities. *Br Heart J*. 1991;66:26–28.
10. Andreoletti G, Ashton JJ, Coelho T, Willis C, Haggarty R, Gibson J, et al. Exome analysis of patients with concurrent pediatric inflammatory bowel disease and autoimmune disease. *Inflamm Bowel Dis*. 2015;21:1229–1236. doi: 10.1097/MIB.0000000000000381.

11. Christodoulou K, Wiskin AE, Gibson J, Tapper W, Willis C, Afzal NA, et al. Next generation exome sequencing of paediatric inflammatory bowel disease patients identifies rare and novel variants in candidate genes. *Gut*. 2013;62:977–984. doi: 10.1136/gutjnl-2011-301833.
12. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25:2078–2079. doi: 10.1093/bioinformatics/btp352.
13. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38:e164. doi: 10.1093/nar/gkq603.
14. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*. 2001;29:308–311.
15. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012;491:56–65.
16. Drmanac R, Sparks AB, Callow MJ, Halpern AL, Burns NL, Kermani BG, et al. Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science*. 2010;327:78–81. doi: 10.1126/science.1181498.
17. Pengelly RJ, Gibson J, Andreoletti G, Collins A, Mattocks CJ, Ennis S. A SNP profiling panel for sample tracking in whole-exome sequencing studies. *Genome Med*. 2013;5:89. doi: 10.1186/gm492.
18. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas NS, et al. Human Gene Mutation Database (HGMD): 2003 update. *Hum Mutat*. 2003;21:577–581. doi: 10.1002/humu.10212.
19. Jun G, Flickinger M, Hetrick KN, Romm JM, Doheny KF, Abecasis GR, et al. Detecting and estimating contamination of human DNA samples in sequencing and array-based genotype data. *Am J Hum Genet*. 2012;91:839–848. doi: 10.1016/j.ajhg.2012.09.004.
20. Schunkert H, Bröckel U, Kromer EP, Elsner D, Jacob HJ, Riegger GA. A large pedigree with valvuloseptal defects. *Am J Cardiol*. 1997;80:968–970.
21. Sinkovec M, Kozelj M, Podnar T. Familial biventricular myocardial noncompaction associated with Ebstein's malformation. *Int J Cardiol*. 2005;102:297–302. doi: 10.1016/j.ijcard.2004.05.033.
22. de Castro E, Sigrist CJ, Gattiker A, Bulliard V, Langendijk-Genevaux PS, Gasteiger E, et al. ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. *Nucleic Acids Res*. 2006;34(web server issue):W362–W365. doi: 10.1093/nar/gkl124.
23. Robertson SP, Jenkins ZA, Morgan T, Adès L, Aftimos S, Boute O, et al. Frontometaphyseal dysplasia: mutations in FLNA and phenotypic diversity. *Am J Med. Genet A*. 2006;140:1726–1736. doi: 10.1002/ajmg.a.31322.
24. Robertson SP. Filamin A: phenotypic diversity. *Curr Opin Genet Dev*. 2005;15:301–307. doi: 10.1016/j.gde.2005.04.001.
25. Bork P, Holm L, Sander C. The immunoglobulin fold. Structural classification, sequence patterns and common core. *J Mol Biol*. 1994;242:309–320. doi: 10.1006/jmbi.1994.1582.
26. Halaby DM, Poupon A, Mornon J. The immunoglobulin fold family: sequence analysis and 3D structure comparisons. *Protein Eng*. 1999;12:563–571.
27. Robertson SP, Twigg SR, Sutherland-Smith AJ, Biancalana V, Gorlin RJ, Horn D, et al; OPD-spectrum Disorders Clinical Collaborative Group. Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nat Genet*. 2003;33:487–491. doi: 10.1038/ng1119.
28. Sun Y, Almomani R, Aten E, Celli J, van der Heijden J, Venselaar H, et al. Terminal osseous dysplasia is caused by a single recurrent mutation in the FLNA gene. *Am J Hum Genet*. 2010;87:146–153. doi: 10.1016/j.ajhg.2010.06.008.
29. Fox JW, Lamperti ED, Ekşioğlu YZ, Hong SE, Feng Y, Graham DA, et al. Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* 1998;21:1315–1325.
30. Kyndt F, Gueffet JP, Probst V, Jaafar P, Legendre A, Le Bouffant F, et al. Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy. *Circulation*. 2007;115:40–49. doi: 10.1161/CIRCULATIONAHA.106.622621.
31. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–91.
32. Garg V, Kathiriya IS, Barnes R, Schluterman MK, King IN, Butler CA, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. *Nature*. 2003;424:443–447. doi: 10.1038/nature01827.

CLINICAL PERSPECTIVE

This study details the finding of a novel missense variant in *FLNA* gene (filamin A) identified through whole exome sequencing in a case of familial Ebstein anomaly (EA). These results provide evidence for a highly penetrant identifiable genetic cause for this condition in the 6 living affected blood relatives diagnosed with EA. Though rare, familial EA has been reported previously, including in association with mutations in *MYH7* and *NKX2.5*, both of which are inherited in an autosomal dominant way. *FLNA* is located on the X-chromosome and the X-linked inheritance of this novel variant is consistent with the phenotypes observed in this family. An X-linked pattern of inheritance for EA was not reported previously in the literature. Appreciation of the different inheritance patterns of familial EA is important to give accurate counseling on the recurrence risks for this condition. In addition, the clinical details in this report expand the phenotypic spectrum associated with mutations in *FLNA*. The recognition of these additional features will allow the clinician to conduct comprehensive clinical evaluation when assessing individuals with pathogenic variants in this gene. Such features may also act as diagnostic clues when assessing those with EA and a possible underlying genetic cause.

Familial Ebstein Anomaly: Whole Exome Sequencing Identifies Novel Phenotype Associated With FLNA

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Supplementary Material

Supplementary Table 1. Prioritised genes (n=219) selected from the HGMD

Gene name	Associated heart disease	% of coverage on Agilent V5 capture kit
ABCA1	HDL deficiency	97.03
ABCC9	Cardiomyopathy, dilated	89.75
ABCG1	Ischaemic heart disease, increased risk	90.72
ACADVL	Very long chain acyl-CoA dehydrogenase deficiency	100.00
ACE	Reduced expression	88.02
ACTC1	Cardiomyopathy, hypertrophic	94.02
ADAMTS10	Weill-Marchesani syndrome	83.05
ADORA1	Ischaemic cardiomyopathy, infarct size	92.43
AGER	Heart disease, reduced risk	14.29
ALAS2	Severe iron overload	100.00
ALOX15	Carotid plaque, in coronary heart disease	100.00
ANXA5	Myocardial infarction, reduced risk	91.87
APOA1	Apolipoprotein A1 deficiency	100.00
APOB	Increased cholesterol levels	100.00
AQP5	Variable RAAS response to salt-loading	86.93
ATM	Ataxia telangiectasia	96.30
BBS1	Bardet-Biedl syndrome	100.00
BBS2	Bardet-Biedl syndrome	87.31
BBS4	Bardet-Biedl syndrome	100.00
BCL9	Congenital heart disease	92.34
BCOR	Oculofaciocardiodental syndrome	91.77
BMPR2	Pulmonary hypertension, primary	93.58
C6orf105	Coronary heart disease, increased risk	0
CAMTA1	Impaired episodic memory performance	78.41
CASQ2	Ventricular tachycardia, polymorphic	100.00
CAV3	Muscular dystrophy, limb girdle	100.00
CD36	CD36 deficiency	41.20
CETP	Altered CETP activity	100.00
CFC1	Congenital heart defects	72.49
CHD7	Congenital heart disease, protection, asssociation with	88.42
CITED2	Congenital heart defects	80.21
CNDP1	Carnosinase deficiency	100.00
CNR1	Increased waist circumference in obese men, association	93.70
COX15	Hypertrophic cardiomyopathy, early onset	50.66
CPT2	Carnitine palmitoyltransferase 2 deficiency	98.38
CREB1	Heart rate response to submaximal exercise	76.49
CRP	Increased CRP level	100.00
CSRP3	Cardiomyopathy, hypertrophic	85.59
CUL3	Congenital heart disease	100.00
CXCL16	Coronary heart disease, increased risk	64.22
CYBA	Coronary artery disease, increased risk	100.00
CYP2C9	Warfarin sensitivity	99.79
CYP3A4	Coronary heart disease, increased risk	79.43
DAPK3	Congenital heart disease	84.32
DMD	Cardiomyopathy, dilated	97.39
DSC2	Arrhythmogenic right ventricular dysplasia/cardiomyopathy	100.00
DSP	Cardiomyopathy, arrhythmogenic right ventricular	100.00
EFTUD2	Microcephaly, Tetralogy of Fallot, heart defect & cleft soft palate	73.22
EHMT1	Mental retardation	100.00
ELN	Supravalvular aortic stenosis	100.00
EMD	Muscular dystrophy, Emery-Dreifuss	100.00
ENG	Pulmonary hypertension, primary	100.00
ESR1	Left ventricular hypertrophy	92.35
EVC	Ellis-van Creveld syndrome	97.48

EYA1	Cayler's cardiofacial syndrome	86.90
EYA4	Sensorineural deafness	93.22
F2R	Coronary heart disease	96.48
F7	Reduced plasma F7 levels	100.00
FABP4	Hypertriglyceridaemia, reduced risk	100.00
FBN1	Marfan syndrome	98.17
FBN2	Congenital heart disease	99.51
FGB	Increased plasma fibrinogen levels	58.66
FLNA	Heterotopia, periventricular	98.44
FOXC1	Axenfeld anomaly, with atrial septal defect	100.00
FOXC2	Hypoplastic left heart syndrome	100.00
FOXF1	Alveolar capillary dysplasia / misalignment of pulmonary veins	100.00
FOXH1	Congenital heart defects	100.00
FOXL1	Hypoplastic left heart syndrome	100.00
FOXP1	Congenital heart defects	84.90
FXN	Friedreich ataxia	34.67
G6PC3	Neutropaenia with cardiac and urogenital malformations	68.80
GAA	Glycogen storage disease 2	77.07
GATA3	Hypoparathyroidism, deafness and renal dysplasia	94.15
GATA4	Congenital heart defects	97.15
GATA6	Congenital heart disease	93.63
GCH1	Decreased expression	100.00
GDF1	Congenital heart defects	98.20
GFM1	Combined oxidative phosphorylation deficiency	100.00
GJA1	Heart malformations	92.52
GLA	Fabry disease	100.00
GNAQ	Increased promoter activity	99.27
GNAS	Reduced expression	94.68
GNPTAB	Mucolipidosis II	97.92
HADHA	Mitochondrial trifunctional protein deficiency	100.00
HAND1	Heart hypoplasia	100.00
HAND2	Congenital heart disease	100.00
HCN4	Ventricular tachycardia	100.00
HFE2	Haemochromatosis	89.52
HOXA1	Bosley-Salih-Alorainy syndrome, BSAS	100.00
HSD11B2	Apparent mineralocorticoid excess	100.00
HSPA8	Coronary heart disease, decreased risk	95.22
HUWE1	Congenital heart disease	97.28
INSIG2	Obesity	97.37
INVS	Nephronophthisis 2	93.31
IRF8	Coronary heart disease in SLE	97.90
IRX4	Congenital heart disease	100.00
JAG1	Alagille syndrome	99.55
JUP	Naxos disease	97.22
KCNH2	Long QT syndrome	94.28
KCNJ2	Andersen syndrome	28.33
KCNQ1	Long QT syndrome	100.00
KDM5A	Congenital heart disease	99.85
KDM5B	Congenital heart disease	100.00
KDR	Coronary heart disease	98.61
KIF5B	Reduced promoter activity	57.43
LAMP2	Glycogen storage disease 2b	56.16
LDHB	Lactate dehydrogenase deficiency	73.72
LDLR	Hypercholesterolaemia	97.65
LEFTY2	Left-right axis malformation	100.00
LMNA	Heart-hand syndrome	91.34
LPA	Lp(a) deficiency	59.10
LPL	Lipoprotein lipase deficiency	51.85
LRP2	Congenital heart disease	100.00
MAP2K1	Cardio-facio-cutaneous syndrome	99.88
MAP2K2	Cardio-facio-cutaneous syndrome	99.48
MC4R	Obesity, autosomal dominant	100.00
MED13L	Congenital heart defect	100.00

MED20	Congenital heart disease	100.00
MID1	Opitz G/BBB syndrome	79.57
MKKS	McKusick-Kaufman syndrome	72.67
MLL2	Congenital heart disease	100.00
MMP2	Torg-Winchester syndrome	97.81
MMP3	Atherosclerosis progression	100.00
MTHFR	Coronary heart disease, increased risk	95.30
MTRR	Coronary heart disease, increased risk	100.00
MTTP	Abetalipoproteinaemia	100.00
MYBPC3	Cardiomyopathy, hypertrophic	94.81
MYH6	Congenital heart defects	99.02
MYH7	Cardiomyopathy, hypertrophic	98.16
MYL2	Cardiomyopathy, hypertrophic	100.00
MYL3	Cardiomyopathy, hypertrophic	100.00
MYLK2	Cardiomyopathy, hypertrophic	100.00
MYOCD	Decreased left ventricular mass	97.64
MYPN	Cardiomyopathy, dilated	90.12
NAA15	Congenital heart disease	100.00
NF1	Congenital heart disease	92.85
NKX2-5	Congenital heart disease, non-syndromic	100.00
NODAL	Congenital heart disease	88.21
NOS1AP	Cardiac repolarisation	100.00
NOS3	Coronary spasm	94.45
NOTCH1	Hypoplastic left heart syndrome	100.00
NPHP3	Situs inversus	83.83
NPPA	Atrial fibrillation	100.00
NPR1	Reduced expression	100.00
NSD1	Sotos syndrome	98.51
NUB1	Congenital heart disease	100.00
OAZ1	Coronary heart disease	82.72
PBX3	Congenital heart defects	95.97
PBX4	Congenital heart defects	100.00
PCSK9	Hypocholesterolaemia	100.00
PDLIM3	Cardiomyopathy, dilated	66.91
PHKG2	Phosphorylase kinase deficiency	74.29
PIGL	Colobomas, heart defects, ichthyosiform dermatosis, mental retardation & ear anomalies	100.00
PITX2B	Congenital heart disease	0
PKNOX1	Congenital heart defects	96.46
PLN	Cardiomyopathy, modifier of	93.12
PRKAG2	Cardiomyopathy, hypertrophic	88.08
PRKAR1A	Carney complex	33.44
PTCH1	Congenital heart disease	90.95
PTPN11	Atrioventricular septal defect	37.60
RAB10	Congenital heart disease	95.69
RAC2	Anthracycline-induced cardiotoxicity	47.55
RNF20	Congenital heart disease	98.38
RPL11	Diamond-Blackfan anaemia	100.00
RPL36	Diamond-Blackfan anaemia	61.95
RPL5	Diamond-Blackfan anaemia	96.90
RPS17	Diamond-Blackfan anaemia	100.00
RPS19	Diamond-Blackfan anaemia	56.18
RPS7	Diamond-Blackfan anaemia	86.68
RYR2	Arrhythmogenic right ventricular cardiomyopathy	100.00
SALL4	IVIC syndrome	100.00
SCN5A	Longer QT interval	96.86
SCO2	Cytochrome c oxidase deficiency	82.85
SGCB	Muscular dystrophy, limb girdle	96.73
SHBG	Hyperandrogenism	97.18
SLC12A1	Bartter syndrome	99.38
SLC12A3	Gitelman syndrome	79.07
SLC29A3	H syndrome	100.00
SMAD2	Congenital heart disease	93.44
SOS1	Cardio-facio-cutaneous syndrome	100.00

SRI	Cardiomyopathy, hypertropic	100.00
STK39	Hypertension	100.00
STRA6	Anophthalmia syndrome	63.05
SUPT5H	Congenital heart disease	94.10
SUV420H1	Congenital heart disease	97.01
TAB2	Congenital heart defects	92.45
TAZ	Left ventricular noncompaction	100.00
TBX1	Ventricular septal defect	98.47
TBX20	Congenital heart disease	100.00
TBX3	Ulnar-mammary syndrome	100.00
TBX5	Atrial septal defects & postaxial hexodactyly	73.29
TCAP	Cardiomyopathy, hypertrophic	100.00
TDGF1	Congenital heart defects	95.53
TFAP2B	Char syndrome	34.63
TGFBR2	Loeys-Dietz aortic aneurysm syndrome	100.00
THBD	Myocardial infarction	100.00
THBS2	Myocardial infarction, protection, association	96.11
TLL1	Atrial septal defect	93.56
TMEM121	Congenital heart disease	97.50
TNNI3	Cardiomyopathy, hypertrophic	98.81
TNNT2	Cardiomyopathy, hypertrophic	100.00
TPM1	Cardiomyopathy, hypertrophic	100.00
TRIM37	Mulibrey nanism	73.29
TSC1	Tuberous sclerosis	98.24
TSC2	Tuberous sclerosis	99.53
TTN	Cardiomyopathy, dilated	97.96
TTR	Amyloidosis	100.00
UBE2B	Congenital heart disease	100.00
UBR1	Johanson-Blizzard syndrome	100.00
UGDH	Heart valve defect	90.67
USP34	Congenital heart disease	100.00
USP44	Congenital heart disease	91.81
VNN1	HDL cholesterol concentration	83.84
WDR5	Congenital heart disease	96.60
WFS1	Wolfram syndrome	95.74
ZEB2	Mowat-Wilson syndrome	46.23
ZIC3	Cardiac malformation	90.18
ZNF202	Atherosclerosis	92.53

Supplementary Table 2. Summary statistics for exome sequencing - mapping and coverage

Sample ID	Number of sequenced reads	Total no. aligned reads	Total no. unique align.	Mapped to target reads +/- 150bp (%)	Mapped to target reads (%)	Target bases with coverage >1 (%)	Target bases with coverage >5 (%)	Target bases with coverage >10 (%)	Target bases with coverage >20 (%)	Mean coverage	% X Heterozygosity	Gender based upon the % of X heterozygous
III.3	51354316	51054667	50308899	88.29	75.89	99.66	98.23	95.41	86.99	60.52	14.32	Male
III.5	46266178	45972807	45324773	89.53	75.87	99.64	98.2	95.26	85.92	54.81	14.45	Male

Number of sequenced reads - total number of reads sequenced; Total no. aligned reads - the total number of reads aligned to the reference sequence; Total no. unique align- the number of reads that uniquely mapped to the reference sequence; Mapped to target reads +/-150bp (%)- the percentage of reads mapped ±150 base pair to the target; Mapped to target reads (%)- the percentage of reads mapped to the target sequence; Target bases with coverage >1,5,10,20- the percentage of targets with 1, 5, 10 and 20 read depth; Mean coverage - the mean of the depth coverage; % X heterozygosity - calls mapped to X chromosome heterozygous.

Supplementary Table 3 – Nine variants across nine genes prioritized after pan-exomic filtering across the two individuals.

We identified 16,543 variants shared between the cousins, of which 16,505 were removed as these were observed in any zygosity state within the local control cohort of exomes (n=329). Of the remaining 38 variants, 10 synonymous variants were discarded due to their low likelihood to impact protein function and 2 splicing variants with a MaxEnt score < 3 were removed. Of the 26 variants remaining, 17 were removed due to their low conservation across species (PhyloP < 0.99). Nine variants across 9 genes satisfied the filtering criteria. Eight variants were discounted after literature review as functionally less relevant to the phenotype of this family. The novel nonsynonymous variant in *FLNA* represented a likely causal variant.

Chromosome	**Location hg19**	**Gene**	**Variant type**	**Variant info**	Zygosity	phylop	1-sift	polyphen2	mutationtaster	gerp++	**dbSNP137**	**Frequency 1KG**	**Frequency EVS**	**Disease associated**
19	10334726	*S1PR2*	ns	S1PR2:NM_004230:exon2:c.G856A:p.V286I,	Het	0.998378	0	0.002	0.326224	3.64	.	.	0.000233	Pulmonary edema, and abdominal aortic aneurysm.
18	77104320	*ATP9B*	ns	ATP9B:NM_198531:exon21:c.A2438G:p.D813G,	Het	0.996477	0.83	0.024	0.533513	4.29	rs141182661	.	0.000581	prostate cancer, and malaria
12	45168574	*NELL2*	ns	NELL2:NM_001145108:exon9:c.C950T:p.S317L,	Het	0.999332	0.99	0.157	0.87028	4.8	.	.	.	central neurocytoma, and contact dermatitis.
21	16337279	*NRIP1*	ns	NRIP1:NM_003489:exon4:c.G3235T:p.V1079F,	Het	0.999335	0.96	0.588	0.475793	4.41	rs140803495	.	0.000581	Nuclear receptor interacting protein 1 (NRIP1) is a nuclear protein that specifically interacts with the hormone-dependent activation domain AF2 of nuclear receptors
12	57549887	*LRP1*	ns	LRP1:NM_002332:exon9:c.T1238C:p.L413P,	Het	0.996845	1	0.989	0.976967	3.73	.	.	.	Aortic atherosclerosis; vasculitis; colorectal cancer, endotheliitis, prostate cancer, myocardial infarction, rheumatoid arthritis, meningitis

2	27500825	DNAJC5G	ns	DNAJC5G:NM_173650:exon4:c.A317C:p.D106A,	Het	0.997837	0.98	0.995	0.359931	4.75	rs143064063	.	0.000116	No disorders were found for DNAJC5G Gene.
X	153586662	FLNA	ns	FLNA:NM_001110556:exon28:c.G4660A:p.G1554R,	Hemi	0.998857	1	0.999	0.999045	5.23	.	.	.	Gene associated with cardiac malformations and midline skeletal defects
1	12887433	PRAMEF11	ns	PRAMEF11:NM_001146344:exon3:c.T424C:p.C142R,	Het	.	.	.	.	.	.	.	.	melanoma
12	70953191	PTPRB	ns	PTPRB:NM_001206971:exon15:c.A3722G:p.N1241S	Het	.	.	.	.	.	rs201449398	.	0.000362	hyperkalemic periodic paralysis drug addiction gastric ulcer endotheliitis

Het – heterozygous; Hemi – hemizygous; Phylop, 1-sift, polyphen2, mutationtaster, gerp++ are scores for assessing variant deleteriousness; dbSNP137 - rsID in dbSNP 137; Frequency in 1KG - AAF in CEU population in 1000 Genomes Project; Frequency in EVS - AAF in European Americans within the Exome Sequencing Project; Dots denote missing data. Ns: nonsynonynmous,

Supplementary Table 4. Known disease causal *FLNA* mutations (n=107) extracted from HGMD.

Accession Number	Amino acid change	Protein change position	Phenotype	Reference
CD071346	small deletion	22	Chronic idiopathic intestinal pseudo-obstruction	Gargiulo (2007) Am J Hum Genet 80, 751
CM050053	Ala-Gly	39	Heterotopia, periventricular	Sheen (2005) Neurology 64, 254
CM105278	Gly-Arg	74	Heterotopia, periventricular	De Wit (2010) Clin Res Cardiol 100, 45
CM020937	Glu-Val	82	Heterotopia, periventricular	Moro (2002) Neurology 58, 916
CD983414	small deletion	95	Heterotopia, periventricular	Fox (1998) Neuron 21, 1315
CM042050	Met-Val	102	Heterotopia, periventricular	Guerrini (2004) Neurology 63, 51
CM098084	Ile-Asn	119	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM098085	Var-Gly	122	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM098086	Ser-Tyr	123	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM061003	Ala-Val	128	Ehlers-Danlos syndrome & heterotopia, periventricular	Gomez-Garre (2006) J Med Genet 43, 232
CM061003	Ala-Val	128	Ehlers-Danlos syndrome & heterotopia, periventricular	Gomez-Garre (2006) J Med Genet 43, 232
CM061003	Ala-Val	128	Ehlers-Danlos syndrome & heterotopia, periventricular	Gomez-Garre (2006) J Med Genet 43, 232
CM042051	Ser-Phe	149	Heterotopia, periventricular	Guerrini (2004) Neurology 63, 51
CM030660	Gln-Pro	170	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CM030661	Leu-Phe	172	Otopalatodigital syndrome 1	Robertson (2003) Nat Genet 33, 487
CM983403	Gln-Term	182	Heterotopia, periventricular	Fox (1998) Neuron 21, 1315
CM030663	Arg-Trp	196	Otopalatodigital syndrome 1	Robertson (2003) Nat Genet 33, 487
CM030662	Arg-Gly	196	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CM030664	Ala-Ser	200	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CM052233	Asp-Tyr	203	Otopalatodigital syndrome 1	Hidalgo-Bravo (2005) Am J Med Genet 136A, 190
CM030665	Pro-Leu	207	Otopalatodigital syndrome 1	Robertson (2003) Nat Genet 33, 487
CM071770	Cys-Phe	210	Otopalatodigital syndrome 2	Marino-Enriquez (2007) Am J Med Genet A 143A, 1120
CM067671	Arg-Term	226	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CM012745	Gln-Term	230	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CD067865	small deletion	232	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CM062728	Glu-Lys	245	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CM062730	Asp-Glu	253	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CM030666	Glu-Lys	254	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CM030667	Ala-Pro	273	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CM070131	Gly-Arg	288	Cardiac valvular dystrophy	Kyndt (2007) Circulation 115, 40
CM098087	Tyr-Term	319	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM107863	Gly-Trp	352	Melnick-Needles syndrome	Foley (2010) Mol Syndromol 1, 121
CM023918	Val-Met	528	Heterotopia, periventricular	Kakita (2002) Acta Neuropathol 104, 649
CM030668	Thr-Lys	555	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CI045115	frameshift insertion at nucleotide 568	568	Heterotopia, periventricular	Parrini (2004) Neurogenetics 5, 191
CD098092	small deletion	596	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM070130	Pro-Gln	637	Cardiac valvular dystrophy	Kyndt (2007) Circulation 115, 40
CM012746	Leu-Phe	656	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CM067668	Gln-Term	668	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CM070132	Val-Asp	711	Cardiac valvular dystrophy	Kyndt (2007) Circulation 115, 40
CM098088	Tyr-Term	731	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM098089	Arg-Term	863	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CD050140	small deletion	920	Heterotopia, periventricular	Sheen (2005) Neurology 64, 254
CM105277	Ser-Leu	1012	Heterotopia, periventricular	de Wit (2011) Clin Res Cardiol 100, 45
CD092208	small deletion	1014	Heterotopia, periventricular	de Wit (2009) J Neurol Neurosurg Psychiatry 80, 426

CM062726	Pro-Lwu	1149	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CM030669	Asp-Ala	1159	Frontometaphyseal dysplasia	Robertson (2003) Nat Genet 33, 487
CX101192	Gly1176Asp	1176	Melnick-Needles syndrome	Santos (2010) Am J Med Genet A 152A, 726
CM030670	Asp-Glu	1184	Melnick-Needles syndrome	Robertson (2003) Nat Genet 33, 487
CM030671	Ser-Leu	1186	Frontometaphyseal dysplasia	Robertson (2003) Nat Genet 33, 487
CM030672	Ala-Thr	1188	Melnick-Needles syndrome	Robertson (2003) Nat Genet 33, 487
CD105280	small deletion	1193	Heterotopia, periventricular	de Wit (2010) Clin Res Cardiol 100, 45
CM030673	Ser-lEu	1199	Melnick-Needles syndrome	Robertson (2003) Nat Genet 33, 487
CM062727	Pro-LEu	1223	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CM107862	Tyr-Ser	1229	Melnick-Needles syndrome	Foley (2010) Mol Syndromol 1, 121
CI098094	frameshift insertion at nucleotide 3767	1230	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM062731	Var-Ala	1249	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CM073069	PR-Leu	1291	FG syndrome	Unger (2007) Am J Med Genet A 143A, 1876
CD067866	small deletion	1346	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CD050141	small deletion	1382	Heterotopia, periventricular	Sheen (2005) Neurology 64, 254
CD012846	small deletion	1434	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CD098093	small deletion	1472	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM0910544	Gln-Arg	1484	Mental retardation	Hu (2009) Hugo J 3, 41
CI098095	frameshift insertion at nucleotide 4573	1499-1498	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM067669	Arg-Term	1515	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CD030738	small deletion	1619	Frontometaphyseal dysplasia	Robertson (2003) Nat Genet 33, 487
CD050451	small deletion	1634	Otopalatodigital-spectrum disorder	Stefanova (2005) Am J Med Genet 132A, 386
CM030674	Cys-Phe	1645	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CD067868	small deletion	1650	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CM061004	Gly-Cys	1720	Frontometaphyseal dysplasia	Zenker (2006) Am J Med Genet A 140A, 1069
CM012747	Ala-Thr	1756	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CM062729	Leu-Arg	1780	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CD012847	small deletion	2203	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CD105281	small deletion	2203	Heterotopia, periventricular	de Wit (2010) Clin Res Cardiol 100, 45
CM067670	Arg-Term	2234	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CM012748	Tyr-Term	2297	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CM098090	Gln-Term	2341	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CM062732	Phe-Ser	2345	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CD067867	small deletion	2436	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CD056813	small deletion	2474	Frontometaphyseal dysplasia	Robertson (2006) Am J Med Genet A 140A, 1726
CX067919	.	.	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CI067900	frameshift insertion at nucleotide 6287	2057-2056	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CD021016	small deletion	2534	Heterotopia, periventricular	Moro (2002) Neurology 58, 916
CD067869	small deletion	2588	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CI067901	frameshift insertion at nucleotide 7800	2593	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CM100148	Trp-Term	2624	Heterotopia, periventricular nodular	Jefferies (2010) Am J Med Genet A 152A, 165
CG070586	1944 bp incl. ex. 16-19 (described at genomic DNA level)	.	Cardiac valvular dystrophy	Kyndt (2007) Circulation 115, 40
CN0910657	Duplication of incl. ex. 4-11 (described at genomic DNA level)	.	Chronic idiopathic intestinal pseudo-obstruction	Clayton-Smith (2009) Eur J Hum Genet 17, 434
CN106956	Duplication of incl. ex. 1-28 (described at genomic DNA level)	.	Chronic idiopathic intestinal pseudo-obstruction	Kapur (2010) Am J Surg Pathol 34, 1528
CG107864	c.4738_4755+10del28 (described at genomic DNA level)	.	Melnick-Needles syndrome	Foley (2010) Mol Syndromol 1, 121
CN075891	Duplication of 0.3 Mb, entire gene+GDI1	.	Mental retardation,	Froyen (2007) Hum Mutat 28, 1034

	(described at genomic DNA level)			
CP035434	Del 9 bp nt 4838-4846, ins 35 bp	.	Otopalatodigital syndrome 2	Robertson (2003) Nat Genet 33, 487
CS012827	IVS6 as -2 A-G	Intronic splicing	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CS012828	IVS19 as -1 G-A	Intronic splicing	Heterotopia, periventricular	Sheen (2001) Hum Mol Genet 10, 1775
CS041120	IVS44 ds -19 C-A	Intronic splicing	Heterotopia, periventricular	Zenker (2004) Am J Hum Genet 74, 731
CS042166	IVS11 as -2 A-G	Intronic splicing	Heterotopia, periventricular	Guerrini (2004) Neurology 63, 51
CS062053	IVS12 as +95 C-T	Intronic splicing	Heterotopia, periventricular	Hehr (2006) J Med Genet 43, 541
CS067820	IVS31 ds -64 C-T	Intronic splicing	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CS067821	IVS44 as -2 A-G	Intronic splicing	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CS067822	IVS46 ds +8 A-G	Intronic splicing	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CS067823	IVS5 ds +2 T-A	Intronic splicing	Heterotopia, periventricular	Parrini (2006) Brain 129, 1892
CS085940	IVS6 ds -1 G-C	Intronic splicing	Heterotopia, periventricular	Tsuneda (2008) J Mol Neurosci 35, 195
CS098091	IVS12 ds +2 T-G	Intronic splicing	Heterotopia, periventricular	Solé (2009) J Neurol Neurosurg Psychiatry 80, 1394
CS983409	IVS2 ds +1 G-A	Intronic splicing	Heterotopia, periventricular	Fox (1998) Neuron 21, 1315
CS983410	IVS3 as -3 C-G	Intronic splicing	Heterotopia, periventricular	Fox (1998) Neuron 21, 1315
CS983411	IVS4 ds +2 T-C	Intronic splicing	Heterotopia, periventricular	Fox (1998) Neuron 21, 1315
CS104078	IVS31 ds -1 G-A	Intronic splicing	Terminal osseous dysplasia	Sun (2010) Am J Hum Genet 87, 146