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Clinical report

A novel single nucleotide splice site mutation in *FHL1* confirms an Emery-Dreifuss plus phenotype with pulmonary artery hypoplasia and facial dysmorphology

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ABSTRACT

We describe a Danish family with an, until recently, unknown X-linked disease with muscular dystrophy (MD), facial dysmorphology and pulmonary artery hypoplasia. One patient died suddenly before age 20 and another was resuscitated from cardiac arrest at the age of 28. Linkage analysis pointed to a region of 25 Mb from 123.6 Mb to 148.4 Mb on chromosome X containing over 100 genes. Exome sequencing identified a single nucleotide splice site mutation c.502-2A > T, which is located 5' to exon 6 in the gene encoding four and a half LIM domain 1 (FHL1) protein. *FHL1* expresses three main splice variants, known as FHL1A, FHL1B and FHL1C. In healthy individuals, FHL1A is the predominant splice variant and is mainly found in skeletal and cardiac muscle. The *FHL1* transcript profiles from two affected individuals were investigated in skin fibroblasts with quantitative real-time PCR. This demonstrated loss of isoform A and B, and an almost 200-fold overexpression of isoform C confirming that lack of FHL1A and overexpression of FHL1C results in an extended phenotype of EDMD as recently shown by Tiffin et al. [2013].
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1. Introduction

Emery–Dreifuss muscular dystrophy (EDMD) is known for its heterogeneity and affects patients from a young age. It is characterized by early-onset contractures of the Achilles tendons, elbows and post-cervical muscles in the childhood, followed by limitations in the neck flexion and eventually the entire spine. This occurs before slowly progressive muscle wasting and weakness sets in, mostly affecting the limbs but rarely becoming profound. Finally, patients will show a variety of cardiac conduction defects, ranging from sinus bradycardia to a complete heart block [Emery, 2000].

Abbreviations: *FHL1*, four and a half LIM domain 1; EDMD, Emery–Dreifuss muscular dystrophy.

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The most common type of EDMD is caused by X-linked recessive mutations in *EMD* (OMIM 310300). However, also autosomal dominant forms with mutations in *LMNA* (OMIM 181350), *SYNE1* (OMIM 612998) and *SYNE2* (OMIN 612999) have been described. These genes code for proteins that contribute to the assembly and function of the nuclear membrane. However, EDMD can also be caused by mutations in the *FHL1* gene, which codes for a protein that is not involved in the nuclear membrane (OMIM 300696).

Splice site variation and nonsense mutations in *FHL1* do not only cause EDMD, but are known to give rise to strongly varying symptoms [Sarkozy et al., 2011]. For example, *FHL1* mutations are seen in isolated hypertrophic cardiomyopathy [Binder et al., 2012] and several distinct skeletal myopathies [Windpassinger et al., 2008] [Schessl et al., 2011]. Patients with the same mutation also show variable expression.

The FHL1 protein is predominantly expressed in skeletal muscle and exerts multifunctional effects on signaling, development of muscle tissue as well as structural maintenance [Cowling et al., 2011]. The three main splice variants of this gene are FHL1A, FHL1B and FHL1C. The dominant splice form, FHL1A, contains the original four and a half LIM domains and is highly expressed in skeletal muscle. The expression in the heart is at intermediate levels and at a lower level in other tissues. Isoform B is made up of only three and a half LIM domains, as well as three nuclear localization signals, one nuclear export sequence and a C-terminal binding site for RBP-Jκ, which is the transcriptional co-activator recombination signal-binding protein for immunoglobulin kappa J. FHL1B is shuttled from the cytoplasm to the nucleus, predominantly in skeletal muscle, in the brain and to a lower extent in cardiac muscle. FHL1C, contains the same C-terminal RBP-Jκ binding domain as seen in FHL1B, preceded by two and one half N-terminal LIM domains. This isoform is expressed in the skeletal muscle, testis and, again at lower levels, in cardiac muscle [Poparic et al., 2011].

The function of FHL1 is linked to the rat sarcoma – mitogen-activated protein kinase (RAS-MAPK) pathway. Examples of this, are the interaction of the first two LIM domains with RAF1, MEK2 and ERK2, and binding of the transcription factor RBP-Jκ by FHL1B and FHL1C which leads to decreased response of the ERK/JNK/p38 complex after Notch signaling [Cowling et al., 2011]. Several syndromes are causes by dysregulated RAS-MAPK signaling, and this group is therefore called RASopathies. One of the best known is Noonan Syndrome, which can be caused by a number of different gene mutations. These neurodevelopmental disorders cause facial dysmorphology, such as downslanted palpebral fissure, hypertelorism and ptosis, and cardiac abnormalities [Rauen, 2013]. Additional symptoms can consist of webbing of the neck, postnatal reduced body growth, cryptorchidism, eye abnormalities and visual problems, skeletal malformation and a short stature. Depending on the gene involved, patients are often delayed in motor development and have learning problems. The cardiac defects, of which pulmonary stenosis and hypertrophic cardiomyopathy occur most often, show large variation in severity, ranging from trivial to life threatening.

In this report, a family was investigated of which three male members are affected by an X-linked syndrome with a phenotype comparable to EDMD, but also show several additional symptoms, possibly related to dysfunctional RAS-MAPK signaling. Using linkage analysis and exome sequencing we identified a novel single nucleotide splice site mutation that was found to cause aberrant splicing of the *FHL1* gene transcripts in patients, leading to a complex phenotype suggesting that this mutation results in both a gain- and loss-of-function depending on the pathway that is involved. The correlation between the genotype and the resulting phenotype is discussed in detail.

2. Materials and methods

2.1. Patients

All patients were counseled by a medical geneticist (UBJ) before exome sequencing and the guidelines from the Danish National Ethical Committee were used. Written consent was obtained from either the patient or their parents. Blood samples and skin biopsies were taken from patient 2 and 3. Patient 4 and the control family member only provided a blood sample. Written consent for publishing photographs was given as well.

2.2. Samples and cell culture

Leukocyte DNA was obtained with the Chemagic Magnetic Separation Module I (PerkinElmer, Waltham, MA, USA) according to the protocol provided by the manufacturer. Fibroblasts of patients 2 and 3 were obtained from skin biopsies, while control fibroblasts were grown from stock cells of a healthy male. All fibroblasts were grown in RPMI1640 medium (Lonza, Basel, Switzerland) with 10% fetal calf serum, 2 mM L-glutamine (Life Technologies, Carlsbad, CA, USA) and 100 U/ml penicillin-streptomycin (Life Technologies, Carlsbad, CA, USA) added.

2.3. Linkage analysis

Genomic DNA from the three patients and an unaffected family member was analyzed using the Affymetrix SNP6.0 array at AROS Applied Biotechnology (Aarhus, Denmark). Genotypes were called using the Genotyping Console (Affymetrix, Santa Clara, CA, USA) and uploaded to BCSNP data management platform (BC Platforms, Espoo, Finland). PLINK ([Purcell et al., 2007]PMID: 17701901) was used to remove monomorphic markers and perform LD pruning using a sliding window of 50 SNPs and a r2 threshold of 0.5. MERLIN [Abecasis et al., 2002] was used to remove markers with unlike genotypes and markers with Mendelian errors. This resulted in a set of 278 chromosome X markers in approximate linkage equilibrium with each other. A parametric linkage analysis was performed using MINX [Abecasis et al., 2002] using a model of X-linked inheritance, full penetrance, and a population disease-allele frequency of 0.0001. Marker allele frequencies from the Affymetrix GW6 frequency files (CEU) and genetic distances from the Affymetrix GW6 Marshfield cM map were used.

2.4. Exome sequencing

Whole exome sequencing was performed at BGI (Shenzhen, China) on genomic DNA from patient 2 and 3, and the control family member. Agilent SureSelect 51 Mb exome capture kit (Agilent Technologies, Santa Clara, CA, USA) was used, as this provide good coverage of the linked region. SOAPaligner (soap2.21) and SOAPsnp (v.1.03) were used for SNP calling while BWA and GATK (Genome-Analysis-Tool-Kit) were used for indels detection. Reads were aligned to the UCSC reference genome (Hg19) and variants were called with a probabilistic variant detection tool. All variants present in dbSNP137 (MAF $>$ 0.005), 1000 Genome variants database, HapMap exomes and an in house database, which contains WES data of 1000 normal individuals, were then filtered out. For the remaining variants, amino acid changes and splice site effects were predicted. This allowed filtering of all synonymous variants and those without possible splice site effects. Next, variants located in the linked region were selected and these were compared between the patients and the control individual.

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