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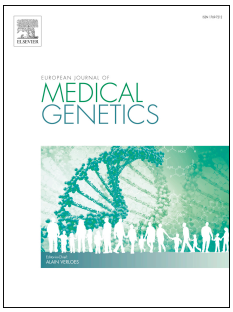

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Expansion of the clinical spectrum of frontometaphyseal dysplasia 2 caused by the recurrent mutation p.Pro485Leu in MAP3K7

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Conflicts of interest

Alice Costantini, Carina Wallgren-Pettersson and Outi Mäkitie declare no conflict of interest.

Supplemental data are included in the submission.

ABSTRACT

Frontometaphyseal dysplasia 2 (FMD2) is a skeletal dysplasia with supraorbital hyperostosis combined with undermodeling of the bones, joint contractures and some extraskeletal features. It is caused by heterozygous mutations in *MAP3K7*, encoding the Mitogen-Activated Protein 3-Kinase 7. MAP3K7 is activated by TGF-β and plays an important role in osteogenesis. Less than 20 patients with FMD2 and *MAP3K7* mutations have been described thus far. The majority of the patients harbor a recurrent missense mutation, NM_003188.3: c.1454C>T [NP_003179.1: p.(Pro485Leu)], which leads to a more severe phenotype than mutations in other domains. Here we describe an additional patient with FMD2 caused by the recurrent c.1454C>T *MAP3K7* mutation, identified as a *de novo* variant by whole-genome sequencing. The 17-year-old boy has the characteristic skeletal and facial features of FMD2. However, some novel features were also observed, including growth retardation and spina bifida occulta. In line with other patients harboring the same mutation he also showed keloid scars and had no intellectual disability. This report expands the clinical spectrum of FMD2 caused by the recurrent c.1454C>T [p.(Pro485Leu)] mutation in *MAP3K7*.

Key words: MAP3K7; TAK1; frontometaphyseal dysplasia 2; skeletal dysplasia; growth retardation

1 INTRODUCTION

Skeletal dysplasias constitute a large and heterogeneous group of disorders characterized by a broad phenotypic and genetic variability (Bonafe et al., 2015). Short stature is the main hallmark but many other features, including deformities and extra-skeletal manifestations, may also be present. Abnormalities in signaling pathways crucial for the growth plate, i.e. Notch, WNT, FGF, Hedgehog, and BMP pathways, are common causes of skeletal dysplasias (Geister and Camper, 2015).

Recently, heterozygous disease-causing mutations in *MAP3K7*, encoding the Mitogen-Activated Protein Kinase 7 (also known as TAK1), were described in Frontometaphyseal dysplasia 2 (FMD2) in less than 20 patients (Wade et al., 2016; Wade et al., 2017). This kinase is stimulated by TGF-β and BMPs and plays an important role in the NF-κB signaling and is consequently a key regulator of osteogenesis (Thouverey and Caverzasio, 2012).

Phenotypically, patients with FMD2 are very similar to patients with frontometaphyseal dysplasia 1 (FMD1), caused instead by mutations in *FLNA* (Robertson et al., 2003). The shared clinical characteristics include sclerosis of the skull and long bones, prominent supraorbital ridges and abnormal fingers. Some patients also present with extra-skeletal manifestations, such as hearing loss, urogenital problems and joint contractures (Wade et al., 2016). In addition, patients with FMD2 also seem to have a predisposition to keloid scars, especially if they are females (Wade et al., 2017).

In some aspects, patients with FMD2 also resemble patients with cardiospondylocarpofacial syndrome, which is also caused by *MAP3K7* mutations (Le Goff et al., 2016). However, this

syndrome is characterized by distinct phenotypic features such as growth retardation, brachydactyly, short limbs and joint laxity (Le Goff et al., 2016).

Here we describe a 17-year-old male with FMD2 caused by the recurrent missense mutation NM_003188.3: c.1454C>T [NP_003179.1: p.(Pro485Leu)] in *MAP3K7* and presenting with several novel phenotypic manifestations.

2 CLINICAL REPORT

2.1 Clinical manifestations

The index patient and his healthy parents were recruited at the Folkhälsan Department of Medical Genetics to a research project exploring genetic causes of skeletal dysplasia. The study protocol was approved by the Research Ethics Board at Helsinki University Hospital and informed consents were obtained from the participants prior to the study.

The patient, a boy born to non-consanguineous Finnish parents, presented at birth with tetralogy of Fallot, a clavicular fracture, ulnar deviation of the wrists, contractures of the elbows, pes metatarsovarus, and hydronephrosis due to ureteral reflux (Table 1). The skull was somewhat dolichocephalic with a sloping forehead, the fontanelle was large, and vertebrae C3 and C4 had reduced height. The fingers and toes were long, with broad distal phalanges of the fingers. His birth weight, length and head circumference (3680g / 51cm / 35.5 cm) were normal, and he received 9 Apgar points.

His slight dysmorphic features included hypertelorism, down-slanting palpebral fissures, broad nasal root, full cheeks, grooved philtrum, small mouth, a H-shaped impression in the chin, and

single palmar creases and camptodactyly in both hands. Hemangiomas were present on the forehead and the occiput

and the neck. He was treated with von Rosen's cast for hip dysplasia and joint contractures and underwent corrective cardiac surgery at the age of 6 months.

On examination at age 1 year, in addition to the above-mentioned dysmorphic features, he was noted to have colobomas of the nostrils. Freeman-Sheldon syndrome was suspected. At the age of 3 years, he was observed to have a submucosal cleft palate and a hearing deficit requiring hearing aids.

At 6 years, the boy was found to have a craniocervical malformation (a sharp angle between the clivus and the dens, and caudal displacement of the conus) in combination with Chiari I-malformation. He underwent occipito-cervical fusion to stabilize his neck (Fig. 1). Decompression of the foramen magnum was performed during the same operation because of the Chiari I malformation. He was found to have spina bifida occulta in several vertebrae and bilateral Sprengel deformities. The Halo bracing, used for stabilization, caused severe keloid formation in his back (Fig. 1D). He also underwent surgery for unilateral cryptorchidism. His orbital ridges were increasingly prominent (Fig. 1A-B) and a corrective surgery was performed at 11 years to flatten the protruding bony parts of the forehead.

The patient had always been sensitive to light but initial examinations revealed no etiology. At the age of 6, he was found to have subepithelial corneal scarring (leucoma corneae), astigmatism, and amblyopia.

At 7 years, he had short stature (-1.5 SD) (Supplemental Fig. S1), scoliosis (Fig. 1C), slightly weak voice, and mild atopy, and was prone to infections. Intellectual development and school performance were normal.

His growth, which decelerated already starting from 3-4 years of age when scoliosis was not present, has progressed slowly despite normal pubertal development (Supplemental Fig. S1). By age 17 years, his scoliosis has significantly progressed (Cobb's angle 42°) and may partly explain the lack of pubertal growth spurt and his severe height deficit (148 cm; -4.3 SDS). He has flexion contractures in the knees, hips and elbows and his feet are deformed and rigid.

2.2 Genetic findings

In order to identify the genetic cause in our index patient, we performed whole-genome sequencing (WGS) on the trio. Pair-end sequencing (2x150 bp) was performed at the SciLifeLab (Stockholm) on the HiSeqX instrument (Illumina), with an average coverage of 30X. Read alignment to the human genome assembly (GRCh37) was performed with the Burrow-Wheeler Aligner (BWA); data processing and variant calling were carried out using Genome Analysis Toolkit (GATK). Variants were annotated using Variant Effect Predictor (VEP) and data analysed using GEMINI. Candidate gene variants were filtered using a MAF < 0.001 in GnomAD and SweGen databases and assuming autosomal recessive/compound heterozygous inheritance pattern or a *de novo* variant (Ameur et al., 2017). Furthermore, we chose the impact severity of the variant to be different than "low" in GEMINI. Findings were validated by Sanger sequencing.

Whole-genome sequencing in our trio led to the identification of two heterozygous candidate genetic variants in the index patient, both identified as *de novo*: 1) a recurrent missense variant NM_003188.3: c.1454C>T [NP_003179.1: p.(Pro485Leu)] in *MAP3K7* (Supplemental Fig. S2A) and

2) a novel missense variant NM_020120.3: c.152C>T [NP_064505.1: p.(Leu51Pro)] in *UGGT1*, encoding the UDP-Glucose Glycoprotein Glucosyltransferase 1. UGGT1 is involved in the quality control of protein folding in the endoplasmic reticulum (Arnold et al., 2000). However, mutations in this gene have not been associated with any disease yet. On the other hand, mutations in *MAP3K7* have been described in two skeletal diseases: FMD2 and cardiospondylocarpofacial syndrome. Based on previous studies and phenotypic correlations, the variant in *MAP3K7* was regarded as the cause of our patient's disorder, confirming a diagnosis of FMD2. This finding, which was also validated with Sanger sequencing (Supplemental Fig. S2B-S2D), allowed us to re-evaluate our patient's clinical features and compare with previously reported patients with the same recurrent p.(Pro485Leu) mutation in *MAP3K7*.

3. DISCUSSION

Our patient had many features in common with the previously reported patients, including prominent supraorbital ridges, hypertelorism, down-slanting palpebral fissures, broad nasal bridge, full cheeks, micrognathia, hydronephrosis, cleft palate, hearing loss, scoliosis, ulnar deviation of the hands, camptodactyly, wrist contractures, long fingers, structural cardiac defect and keloid scars (Table 1, Fig. 1) (Basart et al., 2015; Morava et al., 2003; Wade et al., 2016; Wade et al., 2017). Radiologically, he had a craniocervical malformation, Chiari I-malformation as well as progressive scoliosis requiring surgery, and dislocated and abnormally shaped radial heads.

He did not have valgus but varus deformity of the feet and he had normal intellect. Moreover, he had some features not previously noted in patients with the same mutation: significant growth retardation, only partly explained by scoliosis, resulting in severe short stature (height -4.3 SDS at 17 years) (Supplemental Fig. S1), Chiari I malformation, Sprengel deformity, spina bifida occulta,

single palmar creases, pes cavus, and amblyopia. Amblyopia is common in the general population and it might be a coincidental finding in our patient. Furthermore, Chiari I malformation, Sprengel deformity and single palmar creases are similar to contractures and skeletal deformities described in other individuals with FMD (Basart et al., 2015; Robertson et al., 2003; Wade et al., 2016; Wade et al., 2017). In line with this, spina bifida occulta is part of the cervical spine dysraphism in FMD but it has never been reported hitherto in patients with *MAP3K7* mutations. Finally, leucoma corneae and astigmatism were observed previously in one patient with a *MAP3K7* mutation (Basart et al., 2015), suggesting that this feature may be a part of the phenotypic spectrum of FMD2.

The majority of the patients hitherto reported as having FMD2 harbor the same recurrent mutation as the one identified in our patient, c.1454C>T [p.(Pro485Leu)]. This mutation affects the TAB2 domain of the protein (Table 1; Supplemental Fig.S3) (Wade et al., 2016; Wade et al., 2017). Only 3 patients have been described as having other missense mutations (Wade et al., 2016; Wade et al., 2017). These mutations cluster closer to the N-terminal, in the tyrosine kinase domain, and give rise to milder phenotypes than the ones caused by the recurrent mutation (Table 1; Supplemental Fig.S3). Although the majority of features in our patient overlap with the ones previously described in other FMD2 patients, his phenotype also includes some novel features (Table 1). For example, short stature has not been previously reported in FMD2. However, short stature is one of the hallmarks of patients with cardiospondylocarpofacial syndrome, which is also caused by *MAP3K7* mutations (Le Goff et al., 2016). Furthermore, our patient has additional hand and foot deformities as well as spina bifida occulta, vision problems, undescended testis and hemangiomas that have not previously been described in other patients with FMD2.

In summary, our detailed report corroborates many of the clinical features previously described in patients with FMD2 caused by the recurrent *MAP3K7* mutation c.1454C>T [p.(Pro485Leu)] but also describes some novel or unusual characteristics, in particular growth retardation, which may suggest a partial overlap between FMD2 and cardiospondylocarpofacial syndrome.

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184 **WEB RESOURCES**

185 Burrow-Wheeler Aligner: http://bio-bwa.sourceforge.net

186 GATK: https://software.broadinstitute.org/gatk/

187 VEP: http://www.ensembl.org/info/docs/tools/vep/index.html

188 GEMINI: https://gemini.readthedocs.io/en/latest/

189 GnomAD: http://gnomad.broadinstitute.org

190 SweGen: https://swefreq.nbis.se

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REFERENCES

Ameur, A., Dahlberg, J., Olason, P., Vezzi, F., Karlsson, R., Martin, M., Viklund, J., Kahari, A.K., Lundin, P., Che, H., Thutkawkorapin, J., Eisfeldt, J., Lampa, S., Dahlberg, M., Hagberg, J., Jareborg, N., Liljedahl, U., Jonasson, I., Johansson, A., Feuk, L., Lundeberg, J., Syvanen, A.C., Lundin, S., Nilsson, D., Nystedt, B., Magnusson, P.K., Gyllensten, U., 2017. SweGen: a whole-genome data resource of genetic variability in a cross-section of the Swedish population. European Journal of Human Genetics 25(11), 1253-1260.

Arnold, S.M., Fessler, L.I., Fessler, J.H., Kaufman, R.J., 2000. Two homologues encoding human UDP-glucose:glycoprotein glucosyltransferase differ in mRNA expression and enzymatic activity. Biochemistry 39(9), 2149-2163.

Basart, H., van de Kar, A., Ades, L., Cho, T.J., Carter, E., Maas, S.M., Wilson, L.C., van der Horst, C.M., Wade, E.M., Robertson, S.P., Hennekam, R.C., 2015. Frontometaphyseal dysplasia and keloid formation without FLNA mutations. American journal of medical genetics. Part A 167(6), 1215-1222.

Bonafe, L., Cormier-Daire, V., Hall, C., Lachman, R., Mortier, G., Mundlos, S., Nishimura, G., Sangiorgi, L., Savarirayan, R., Sillence, D., Spranger, J., Superti-Furga, A., Warman, M., Unger, S., 2015. Nosology and classification of genetic skeletal disorders: 2015 revision. American journal of medical genetics. Part A 167A(12), 2869-2892.

Geister, K.A., Camper, S.A., 2015. Advances in Skeletal Dysplasia Genetics. Annu Rev Genomics Hum Genet 16, 199-227.

Le Goff, C., Rogers, C., Le Goff, W., Pinto, G., Bonnet, D., Chrabieh, M., Alibeu, O., Nistchke, P., Munnich, A., Picard, C., Cormier-Daire, V., 2016. Heterozygous Mutations in MAP3K7, Encoding TGF-beta-Activated Kinase 1, Cause Cardiospondylocarpofacial Syndrome. American journal of human genetics 99(2), 407-413.

Morava, E., Illes, T., Weisenbach, J., Karteszi, J., Kosztolanyi, G., 2003. Clinical and genetic heterogeneity in frontometaphyseal dysplasia: severe progressive scoliosis in two families. American journal of medical genetics. Part A 116A(3), 272-277.

Robertson, S.P., Twigg, S.R.F., Sutherland-Smith, A.J., Biancalana, V., Gorlin, R.J., Horn, D., Kenwrick, S.J., Kim, C.A., Morava, E., Newbury-Ecob, R., Orstavik, K.H., Quarrell, O.W.J., Schwartz, C.E., Shears, D.J., Suri, M., Kendrick-Jones, J., Wilkie, A.O.M., Co, O.-S.D.C., 2003. Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. Nature genetics 33(4), 487-491.

Thouverey, C., Caverzasio, J., 2012. The p38alpha MAPK positively regulates osteoblast function and postnatal bone acquisition. Cell Mol Life Sci 69(18), 3115-3125.

Wade, E.M., Daniel, P.B., Jenkins, Z.A., McInerney-Leo, A., Leo, P., Morgan, T., Addor, M.C., Ades, L.C., Bertola, D., Bohring, A., Carter, E., Cho, T.J., Duba, H.C., Fletcher, E., Kim, C.A., Krakow, D., Morava, E., Neuhann, T., Superti-Furga, A., Veenstra-Knol, I., Wieczorek, D., Wilson, L.C., Hennekam, R.C., Sutherland-Smith, A.J., Strom, T.M., Wilkie, A.O., Brown, M.A., Duncan, E.L., Markie, D.M., Robertson, S.P., 2016. Mutations in MAP3K7 that Alter the Activity of the TAK1 Signaling Complex Cause Frontometaphyseal Dysplasia. American journal of human genetics 99(2), 392-406.

Wade, E.M., Jenkins, Z.A., Daniel, P.B., Morgan, T., Addor, M.C., Ades, L.C., Bertola, D., Bohring, A., Carter, E., Cho, T.J., de Geus, C.M., Duba, H.C., Fletcher, E., Hadzsiev, K., Hennekam, R.C.M., Kim, C.A., Krakow, D., Morava, E., Neuhann, T., Sillence, D., Superti-Furga, A., Veenstra-Knol, H.E., Wieczorek, D., Wilson, L.C., Markie, D.M., Robertson, S.P., 2017. Autosomal dominant

238 frontometaphyseal dysplasia: Delineation of the clinical phenotype. American journal of
239 medical genetics. Part A.
240

241

242 **FIGURE LEGENDS**

243 **Figure 1.** At 11 years the patient had significant supraorbital hyperostosis (A), as seen also in the
244 preoperative CT at 11 years (B). He required hearing aids for hearing deficit, had atopic eczema
245 and abnormal scapulae (A). He underwent occipito-cervical fusion to stabilize the neck at 6 years
246 and had by 8 years developed significant scoliosis (C). Halo bracing resulted in keloid formation in
247 the back (D). Radiograph of the pelvis and hips at 3 years (E) showed dysplastic hips and coxa
248 valga. Hand radiograph at 16 years (F) showed undertubulation of the digital bones. Cavovarus
249 deformities were seen in radiographs (G) and clinically (H, I).

250

Table 1. Summary of the clinical features in the 19 thus far reported index patients with FMD2 due to *MAP3K7* mutations. Mutations are given as amino acid changes. The novel or unusual clinical features in our patient are marked in blue text. Colours indicate features that were present (yellow), absent (dark blue) or unknown (grey) in the patients. F= female; M=male; D= deceased.

		This report	Basart et al. 2015; Wade et al. 2016; Wade et al. 2017																
	Individual	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
	Sex	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
	Age at last assessment	17	57	9	52	D	26	29	15	U	U	26	29	7	19	13	8	D	U	34
	Mutation *	P485L	P485L	P485L	P485L	P485L	P485L	P485L	G168R	P485L	P485L	P485L	P485L	P485L	P485L	P485L	P485L	P485L	E70Q	V100E
Facial features	**Facial dysmorphism**																			
	Full cheeks																			
	Micrognathia																			
	Subglottal stenosis																			
	Cleft palate/bifid uvula																			
Limbs	**Long fingers**																			
	Ulnar deviation of the hands																			
	Digital and wrist contractures																			
	Under-modelled phalanges, metacarpals/metatarsals																			
	Elbow contractures/ dislocated radius																			
	Broad thumbs/fingers																			
	Valgus deformity of the feet																			
	Under-modelled metaphyses/diaphyses																			
	Single palmar creases																			
	Pes cavus																			
Cranial and neurological	**Intellectual disability**																			
	Hearing loss																			

features	Skull base sclerosis																			
	Chiari malformation																			
	Cervical vertebral fusion																			
Spine	Scoliosis																			
	Spina bifida occulta																			
	Sprengel deformity																			
Urogenital system	Hydronephrosis																			
	Urethral stenosis																			
Skin	Keloid scars																			
Ocular features	Leucoma corneae																			
	Amblyopia																			
	Astigmatism																			
Other features	Structural heart defect																			
	Growth retardation																			
	Undescended testes