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### **► To cite this version:**

Lyse Ruaud, Ricarda Flöttmann, Malte Spielmann, Fabienne Escande, Lionel van Maldergem, et al.. Split hand/foot malformation associated with 20p12.1 deletion: A case report. *European Journal of Medical Genetics*, 2020, 63, pp.103805 -. <10.1016/j.ejmg.2019.103805>. <hal-03489526>

**HAL Id: hal-03489526**

**<https://hal.science/hal-03489526v1>**

Submitted on 22 Aug 2022

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## Split hand/foot malformation associated with 20p12.1 deletion: a case report

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M.S. was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) (SP1532/3-1, SP1532/4-1, SP1532/5-1)

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## Abstract

Split hand/foot malformation (SHFM) or ectrodactyly is a rare congenital disorder affecting limb development characterized by clinical and genetic heterogeneity. SHFM is usually inherited as an autosomal dominant trait with incomplete penetrance. Isolated and syndromic forms are described. The extent of associated malformations is highly variable and multiple syndromes with clinical and genetic overlap have been described. We report here a 28 year-old man presenting with SHFM, sparse hair and widespread freckles. Array-CGH identified a 450 kb *de novo* 20p12.1 microdeletion encompassing three exons (exon 6 to 8) of *MACROD2*. Although *MACROD2* mutations have not been associated with limb malformation until now, it is located next to *KIF16B*, which is involved in fibroblast growth factor receptor (FGFR) signaling. Additionally, the deletion encompassed a histone modification H3K27ac mark, known as a provider of quantitative readout of promoter and enhancer activity during human limb development. Altogether, these findings suggest that the 20p12.1 CNV is causative of SHFM in the present case through disturbance of regulatory elements functioning.

Keywords: Split hand/foot malformation, 20p12.1 deletion, *MACROD2*, *KIF16B*, positional effect

## **Introduction**

Split hand/foot malformation (SHFM) or ectrodactyly is a rare congenital anomaly of limb development defined by a defect of the central rays of hands and/or feet. Its prevalence is estimated at 1/100 000 births (Carter et al., 2017; Sivasankaran et al., 2016). SHFM is usually inherited as an autosomal dominant trait with incomplete penetrance. Clinical and genetic heterogeneity is well documented. Mutations in several genes, alongside with recurrent copy-number variants have been described. These include 10q24 (Vergult et al., 2013) and 17p13.3 microduplications (Armour et al., 2011) and microdeletions at 2q31 (Theisen et al., 2010) and 17q25 (Carter et al., 2017). Isolated and syndromic forms have been reported. The extent of associated malformations is highly variable. Multiple syndromes coined with a series of distinct acronyms have been described, although clinical overlap between them is considerable. Split hand/foot associated with long-bone anomalies (SHFLD) is part of SHFM and has been linked to 17p13.3 duplication/triplications encompassing *BHLHA9* (Nagata et al., 2014; Petit et al., 2014) as well as *TP63* mutations (Enriquez et al., 2016).

We report here the first patient with congenital SHFLD and a *de novo* constitutive 20p12.1 microdeletion.

## **Clinical history**

The patient, a singleton male offspring born to unrelated caucasian parents was born at term after an uneventful pregnancy. SHFM was diagnosed at birth without any other malformation. He had normal growth and intellectual development. His first daughter is unaffected. When evaluated at 28 years of age for a preconceptual advice, clinical examination showed cleft hands with oligodactyly, right 2-3 syndactyly (requiring surgery during infancy) and mesomelic brachymelia. A typical SHFLD associated with sparse hair and multiple freckles were observed. Review of upper limbs X-rays indicates bilateral post axial longitudinal ectromelia with three

carpal bones, bowed radii, ulnar hypoplasia, two complete rays and a supernumerary bone in the first ray of the left hand and three normal rays in the right hand (Figure 1A-B).

## Methods and results

Written, informed consent for DNA analysis and blood samples from the patient and his parents were obtained. Genomic DNA from peripheral blood leukocytes was extracted by standard procedures. *BHLHA9* qPCR testing, *TP63* Sanger sequencing and panel NGS of >400 genes known to be mutated in skeletal disorders yielded normal results. Whole exome sequencing didn't find any pathogenic variant.

Array-CGH was performed using Agilent SurePrint G3 Human CGH 1x1M, according to the manufacturer's recommendations. Analyses were carried out using Agilent Cytogenomics. qPCR was used to confirm identified CNVs and their segregation. Interpretation was based on GRCh37/hg19. Chromosomal microarray identified a 450 kb deletion at 20p12.1 (arr[GRCh37] 20p12.1(15046533-15487218)x1dn (Figure 2A). qPCR analysis confirmed this rearrangement and established its *de novo* occurrence (Figure 2B).

## Discussion

Copy number variants account for *ca* 50 % of SHFM. 17p13.3 and 10q24 duplication are by far the most frequently reported microrearrangements (Klopocki et al., 2012). We report here an undescribed 20p12.1 microdeletion in a patient presenting SHFLD. It encompasses three exons (exons 6 to 8) of *MACROD2* (MIM #611567) a highly conserved gene with desacetylase and hydrolase activities. It plays a role in brain development, cellular response to DNA damage stimulus, protein de-ADP-ribosylation and purine nucleoside metabolism. A heterozygous deletion of exon 5 of *MACROD2* has been suggested to be causative for a neurodevelopmental disorder in a patient with a Kabuki-like facial dysmorphism (Maas et al., 2007). However, this association was not confirmed by other observations.(Kuniba et al., 2008) (Bradley et al., 2010).

Recent genome-wide association studies suggest that *MACROD2* is a potential player implicated in bone modeling (Pei et al., 2018), in accordance with *MACROD2* K-O mice model displaying shorter femur length as compared to controls (Pei et al., 2018). However, these results do not appear sufficient to sustain a role for *MACROD2* haploinsufficiency as the single cause of the malformation observed in our patient. Interestingly, another mechanism could be operating in our case. Recently, Carter et al (Carter et al., 2017) performed SNP-array in 25 SHFM cases and observed five CNV of potential deleterious effect through their interference with regulatory elements. Following the same hypothesis, we consider that our observation of a microdeletion 860 kb upstream of *KIF16B* (MIM #614642) is significant. *KIF16B* encodes a kinesin-like protein that is likely to be involved in intracellular trafficking and in fibroblast growth factor receptor signaling (Ueno et al., 2011), thus being essential to limb development. Furthermore, the microdeletion encompasses a histone modification H3K27ac mark. Peaks in H3K27ac are associated *in vivo* with increases in gene expression and enhancer activity in the human limb and reveal thousands of promoters and enhancers with potential lineage-specific functions (Cotney et al., 2013). Due to the missing H3K27ac mark, an aberrant regulation of *KIF16B* could be speculated. This might cause changes in FGFR signaling and subsequent misexpression of FGFR in the developing limb bud. Taken together, these factors are likely to be causative of the malformation observed in our patient. Further similar observations will be necessary to confirm these results.

## Legends to figures

Figure 1 : A-B : Upper limbs X-rays. Note bilateral post axial longitudinal ectromelia with only three carpal bones. Bowed radii and ulnar hypoplasia are striking.

Figure 2 : A-20p12.1 deletion showing MACROD2 location

B- qPCR results indicating de novo occurrence of the deletion. Blue and orange bars represent probes outside of the deletion and the other ones are inside the deletion. Dark overriding bar represent a probe located on chromosome Y.

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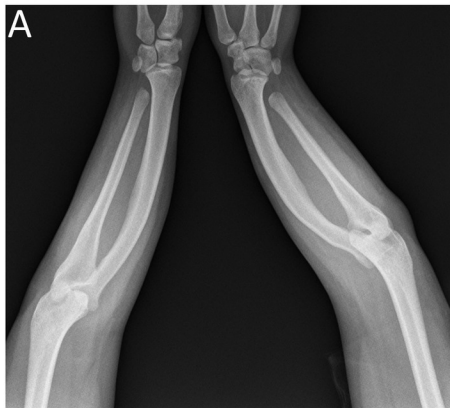


Figure 1



Figure 2

