EFTUD2 gene deficiency disrupts osteoblast maturation and inhibits chondrocyte differentiation via activation of the p53 signaling pathway

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INTRODUCTION

Mandibulofacial dysostosis with microcephaly (MFDM, MIM# 610536) is a rare syndrome with a wide spectrum of congenital anomalies , which is characteristic of multiple skeletal anomalies comprising craniofacial anomalies/dysplasia, microcephaly, dysplastic ears, choanal atresia and short stature [1-2]. Heterozygous loss of function variants of the elongation factor Tu GTP-binding domain-containing 2 gene (*EFTUD2*, MIM# 603892) were previously reported in MFDM, and considered to be the cause of MFDM [3-4]. However, the mechanism underlying EFTUD2-associated skeletal dysplasia remains unclear [1, 5].

RESULTS

Clinical and genetic identification of the patient

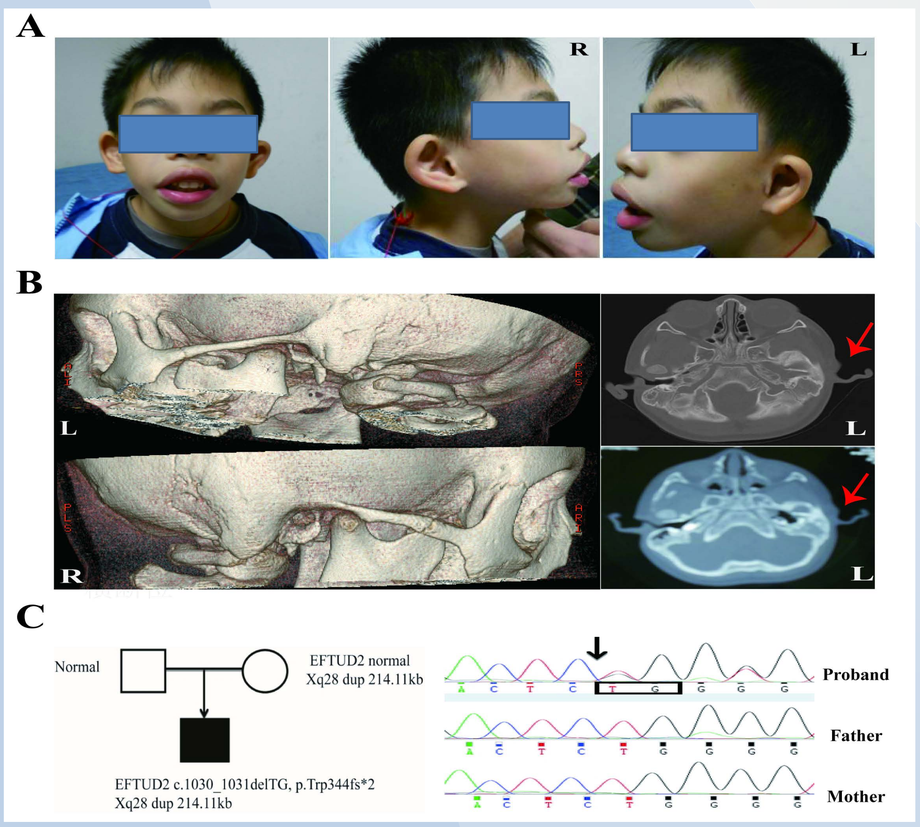

Figure 1. A) The proband presented with microcephaly (head circumference of 45 cm, <P3), severe micrognathia, arched eyebrows, everted lips, broad nasal bridge, abnormal ear structures with hearing loss. B) Malformed structures of the external and middle ear on the left head and temporal bones via CT scan at 37 months of age. R: right, L: left. C) Pedigrees and EFTUD2 variant identified by family trio whole-exome sequencing which showed a *de novo* heterozygous mutation c.1030_1031delTG (p.Trp344fs*2) in EFTUD2 (NM_001258353.1) in the proband.

eftud2 knockout disrupted bone and cartilage development in zebrafish

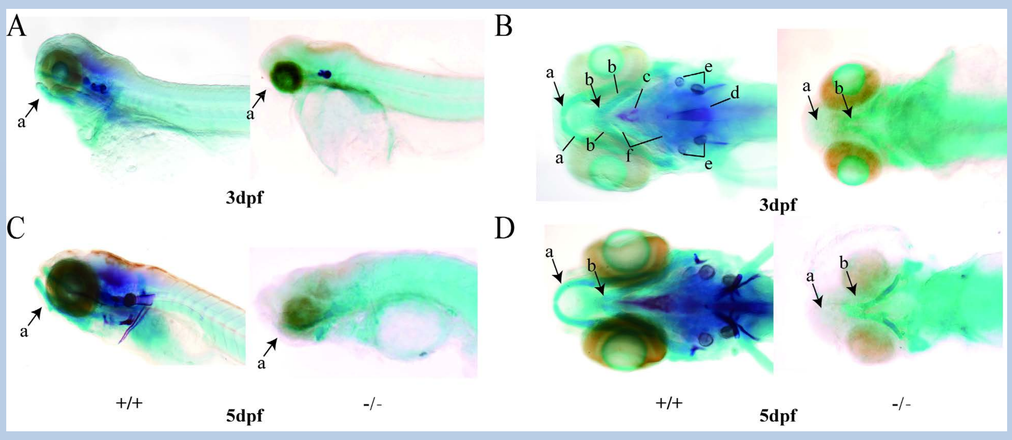

Figure 2. A, C) Lateral view of larvae treated with eftud2 TALEN mRNA (-/-) at 3 dpf and 5 dpf, exhibiting disruption of cartilage and bone formation compared with WT (+/+). B, D) Ventral view of larvae treated with eftud2 TALEN mRNA (-/-) at 3 dpf and 5 dpf, showing dysplasia formation in Meckel's cartilage, ceratohyals, ethmoid bones and otolith loss.

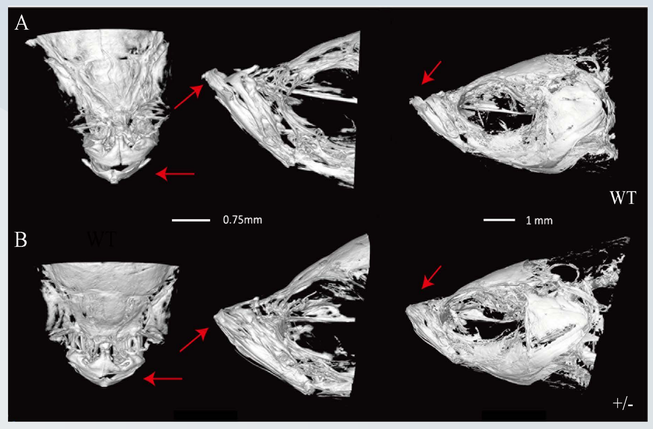

Figure 3 A) Mandible bone of wild-type adults (WT) scanned by synchrotron radiation X-ray microtomography. B) The heterozygous F2 generation (+/-) exhibited a shortened mandibular bone (arrows).

EFTUD2 knockdown and knockout resulted in TP53 pathway activation in vitro and in vivo

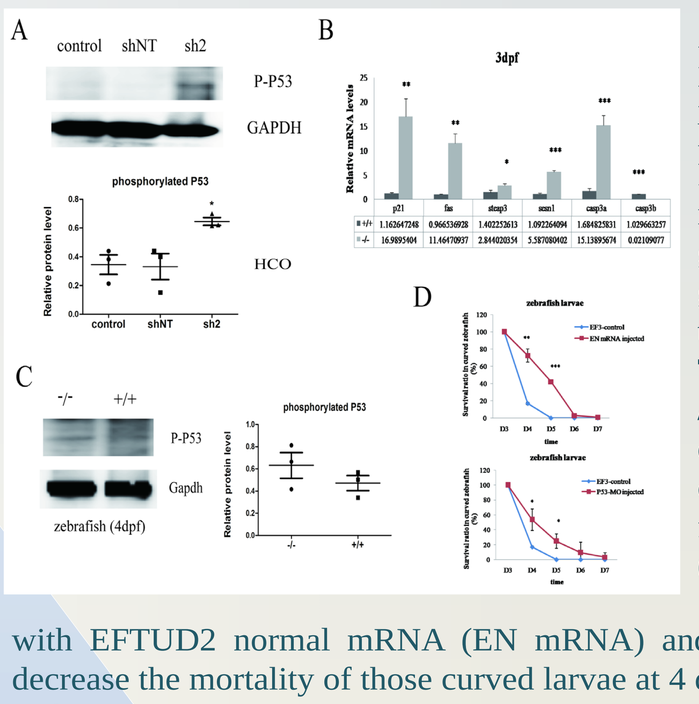

Figure 4 A) HCO cells with EFTUD2 knock-down (sh2) had a higher expression of phosphorylated P53 (P-P53) protein than the nontransfected (control) and shNT groups. B) Expression of relevant genes in P53 pathway were higher in the eftud2 (-/-) than WT (+/+). C) The expression of P-P53 in eftud2 (-/-) larvae was slightly elevated at 4 dpf. D) The survival rate among the curved F3 generation hybridizing from eftud2 heterozygous mutants (EF3 control). EF3 controls injected with EFTUD2 normal mRNA (EN mRNA) and p53 morpholino (P53-MO) could decrease the mortality of those curved larvae at 4 dpf and 5 dpf (*: P<0.05, **: P<0.01, and ***: P<0.001).

CONCLUSIONS

We identify a novel *de novo* frameshift *EFTUD2* gene variant (c.1030_1031delTG, p.Trp344fs*2) in a Chinese MFDM patient, and established an *EFTUD2* deficiency model *in vitro* and *in vivo*. Evidence of cell lines and zebrafish model suggested *TP53* signaling pathway was activated due to *EFTUD2* disruption. Our findings showed that the *EFTUD2* gene could impact the proliferation and differentiation of osteoblasts and chondrocytes, suggesting that premature osteoblasts and chondrocytes differentiation could be responsible for the pathogenesis of MFDM. Further studies on the specific mechanisms involved are necessary in the future.

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