## P.42 KIAA0753 mutations in skeletal ciliopathies: unveiling disease mechanisms

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#### Conclusions

- Modelling the loss of KIAA0753 in zebrafish recapitulates the phenotypes found in patients, supporting the use of this model to investigate disease mechanisms.

- kiaa0753 skeletal ciliopathy is a result of the complete lack of cilia, a severe phenotype that is incompatible with life in larvae beyond the age of 7 days.

- Larvae survive the first days of embryonic development by using maternally deposited wt kiaa0753 mRNA, which is translated and enables cilia formation.

- We suggest that loss of Kiaa0753 in may affect both centromeres and cilia formation, resulting in abnormal tissue growth and function.

## Introduction

Skeletal ciliopathies are a heterogeneous group of disorders caused by mutations in more than 25 genes, such as KIAA0753. Mutations in KIAA0753 have been associated with Joubert syndrome and orofaciodigital syndrome. Recently, we found that pathogenic variants in KIAA0753 also cause a new type of short rib skeletal dysplasia presenting with skeletal abnormalities in combination with CNS malformations, that can be foetal lethal<sup>1</sup>.

To confirm the role of KIAA0753 in skeletal ciliopathies and how loss of KIAA0753 results in this type of disorders, we used the zebrafish model. The mutant zebrafish line carries a nonsense mutation in the same conserved domain as the mutations found in our patients (kiaa0753p.Arg360\*), and homozygote loss of Kiaa0753 results in comparable presentations<sup>1</sup>. Detailed understanding of such phenotypes will allow further understanding of KIAA0753-related skeletal ciliopathies and the test of candidate compounds that may ameliorate the patients' presentations.

#### Clinical presentations



(A) Patients carrying KIAA0753 mutations present with short stature and dysmorphic facial features (not shown), narrow thorax and prominent abdomen, and delayed motor development. (B,C) X-ray investigations showed metaphyseal dysplasia and cone-shaped epiphyses. (D) Exome analysis revealed patients carried the  $p.Arg324^{\star}$  mutation in homozygosity or the p.GIn315\* and p.Pro424Hisfs\*9 in compound heterozygosity.

### Zebrafish models KIAA0753 skeletal ciliopathy



While heterozygous (A) carriers (kiaa0753+/-) (kiaa0753+/+) wild-type embryos have a straight zebrafish body, mutant present ciliopathy-like а phenotype characterised by a curved body, visible from 2 days post fertilisation (dpf). (B) By 5 dpf, it is visible that mutant larvae have significantly shorter head size, as well as shorter cranial bones (arrows, jaws), visible using cartilage staining (C). The findings correlate with the shorter bones seen in patients.



## Cell disorganisation may affect bone growth



H: hypertrophic zone

(A) Histology of the growth plate of affected foetus showed an enlargement of the resting zone and disruption of the chondrocyte column architecture in the proliferative zone when compared to normal controls. (B) Investigation of the cellular architecture in the jaw of zebrafish larva showed cellular disorganisation, namely cell clustering in kiaa0753<sup>-/-</sup> larvae. Cell quantification showed an increase in the number of cells for the same area in kiaa0753-/when compared with kiaa0753<sup>+/+,+/-</sup> larvae (not shown). This may be due to an increase in cell proliferation or defects in the orientation of cell division, which needs to be further investigated.



Loss of Kiaa0753 results in complete loss of cilia

KIAA0753 has been reported to be important for cilia biogenesis<sup>2-4</sup>. (A) kiaa0753-4 zebrafish larvae present with a complete absence of cilia (shown in 5 dpf larvae), never seen in non-phenotypic siblings. Images shown are confocal projections of A' (coronal view). Despite the severe loss of cilia, mutant larvae survive the first week of life. We therefore suspected that embryogenesis occurs normally due to the presence of maternally deposited kiaa0753 mRNA in the egg. (B) RT-PCR on unfertilised eggs showed the presence of wt kiaa0753 mRNA in eggs extracted from wt and kiaa0753+/females, confirmed by Sanger sequencing. The maternal mRNA may rescue the lack of wt Kiaa0753 during the first hours of development in kiaa0753-/- larvae. In fact, when younger embryos were analysed (12 hpf), they all presented with cilia and and were indistinguishable between (C). These findings strongly suggest maternally deposited mRNA is translated and induce cilia formation, recuing endogenous lack of Kiaa0753..

References <sup>1</sup>Hammarsjö, A. et al.. Sci. Rep. 7, (2017). <sup>2</sup>Firat-Karalar, E. N. et al. Curr. Biol. 24, 664–670 (2014). <sup>3</sup>Chevrier, V. et al. Hum. Mol. Genet. 25, 497–513 (2016) <sup>4</sup>Stephen, J. et al. Hum. Genet. 136, 399–408 (2017).

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