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Rogdi mutation arrests enamel maturation creating a mouse model to investigate Kohlschütter–Tönz syndrome

Introduction

Kohlschütter–Tönz syndrome (KTS) is a rare autosomal recessive disorder caused by mutations in *ROGDI* gene, affecting neurological (early onset epilepsy, psychomotor regression, autism) and tooth development (amelogenesis imperfecta, AI) [Huckert M. *et al,* 2014]. ROGDI, is an essential basic zipper protein, highly conserved across metazoans, however its function remains unknown. *Rogdi* is expressed in fetal-stage tooth, developing nervous system, and adult brain (Figure 1).

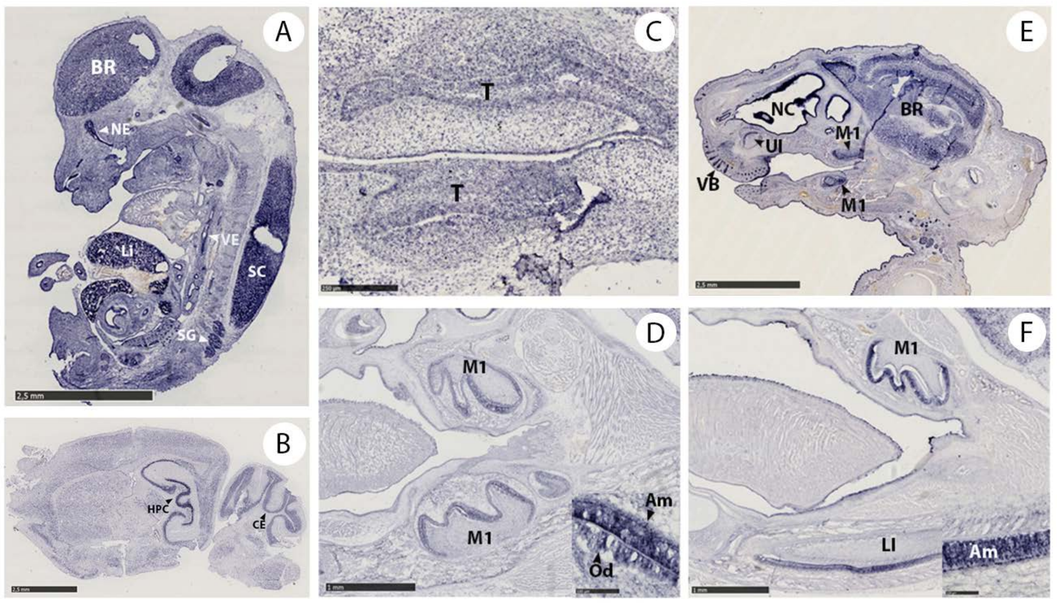


Figure 1 *Rogdi* expression (A) *Rogdi* mRNA is expressed in the brain (BR), nasal epithelium (NE), spinal cord (SC), spinal ganglion (SG), and liver (Li) at E12.5. (B) Adult mouse brain. Pronounced expression of *Rogdi* in the hippocampus (HPC) and in the cerebellum (CE) is observed. (C) *Rogdi* molar odontogenic expression at E14.5 cap stage (T, tooth). (D) At post-natal day P1, *Rogdi* mRNA is present in molar ameloblasts and odontoblasts. (E) Localization of *Rogdi* transcripts is seen at E16.5 in brain (BR), nasal cavity (NC), vibrissae (VB), upper incisor (UI) and first molar (M1) at the bell stage. (F) At P5, enriched expression is visible in ameloblasts.

Methods and Results

We identified, in the CRMR O-Rares, 3 patients with KTS and described the causative *ROGDI* variants. A *Rogdi* mouse mutant with a matching genetic deficit, was created in the Mouse Clinical Institute (ICS - IGBMC) to investigate *Rogdi* function (Figure 2). Knock-out (KO) mice display severe enamel defects (chalky white color enamel) showing an “amelogenesis imperfecta-like” phenotype (Figure 3). *Rogdi* mutants do not form calcified enamel producing a tooth phenotype similar to the one observed in affected individuals (Figure 4,5,6).

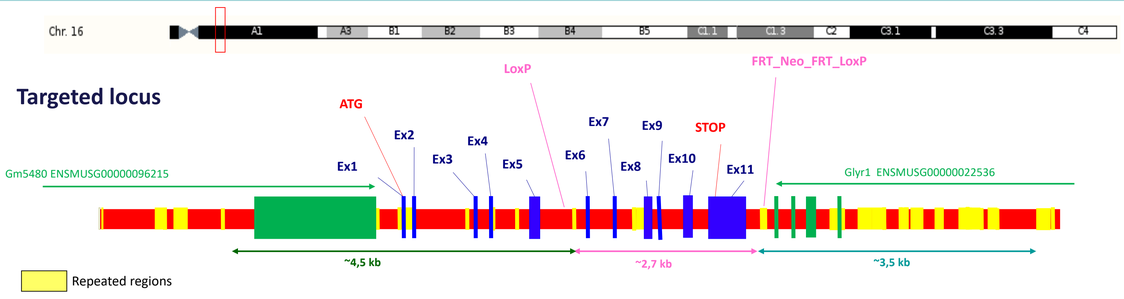


Figure 2 *Rogdi* knockout construct.
Illustrates the mutation strategy. A *Rogdi* mouse mutant was created targeting exons 6 to 11 by homologous recombination.

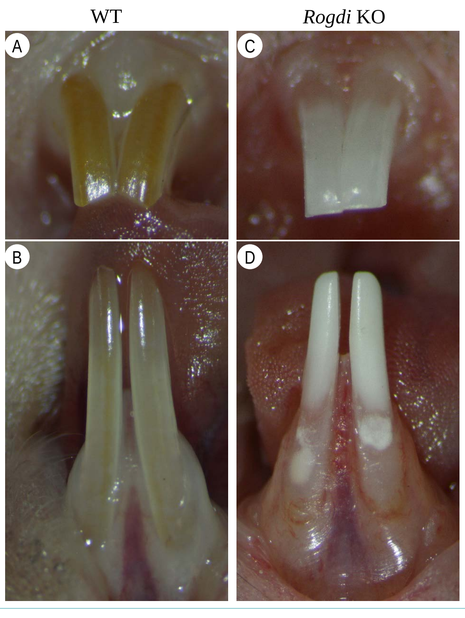


Figure 3 *Rogdi* mouse phenotype.
(A,B) Compare incisors of *WT* 8-week-old mice, with normal darker yellow/orange pigmentation in the upper incisor to (C) upper incisors of 8-week-old *Rogdi* -/- mutant mice which have a chalky white color. (D) *Rogdi* -/- mutant lower incisors show chalky lightening with white patches in the cervical tooth zone.

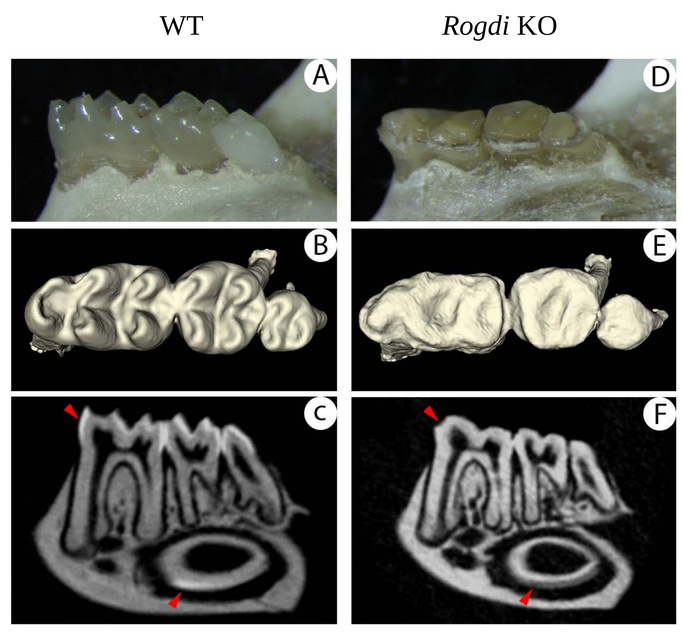
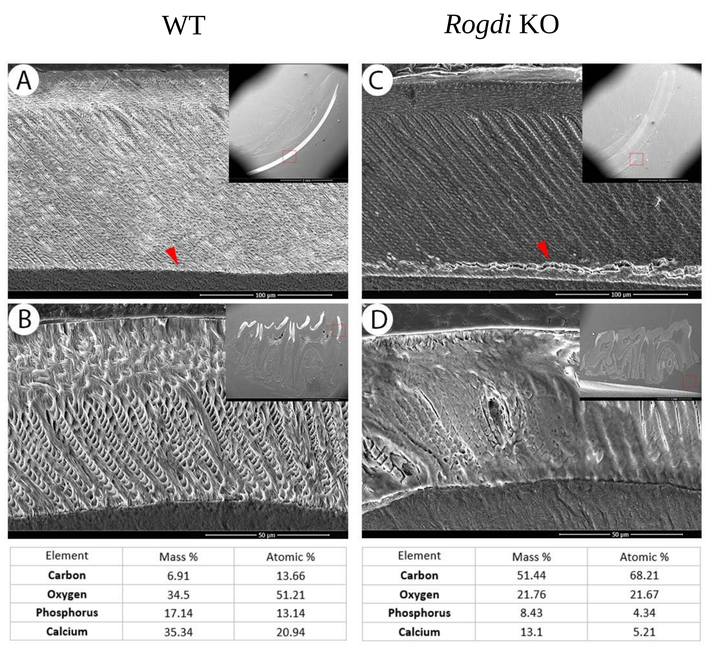


Figure 4. Micro-CT (uCT) imaging of 8 weeks-old WT (A,B,C) and Rogdi -/- (D,E,F) teeth.
The *Rogdi* KO mouse presented abraded cusps in the molars (D, E) which were severely worn, losing enamel at occlusal surfaces, exposing the dentin that remained relatively intact, confirming the phenotype. Optical sections in a sagittal plane show reduced density of enamel in lower molars and incisor (arrowheads in C,F).



Element	Mass %	Atomic %
Carbon	6.91	13.66
Oxygen	34.5	51.21
Phosphorus	17.14	13.14
Calcium	35.34	20.94

Element	Mass %	Atomic %
Carbon	51.44	68.21
Oxygen	21.76	21.67
Phosphorus	8.43	4.34
Calcium	13.1	5.21

Figure 5 Scanning electron microscopy (SEM) of 8 weeks-old *WT* (A,B,C) and *Rogdi* -/- (D,E,F) teeth.
SEM images of fully-formed lower incisor enamel (A,D) and lower molars (B,E) from control and *Rogdi* -/- samples show a reduced enamel mineral density in both incisors and molars in *Rogdi* KO. Red boxed region in insert panels show region in which SEM image was obtained. The enamel of *WT* presents a constant thickness and a clear decussating prism pattern, while *Rogdi* -/- variant produces a near complete absence of opaque mineralized enamel matrix. Table of energy dispersive X-ray spectrometry data for quantification of element composition of tissues displayed in C,D. Left side columns are from *WT*, right side columns are from *Rogdi* -/-. The calcium and phosphate concentration in the enamel layer of wild-type mouse is normal but both concentrations are highly diminished in *Rogdi* mutant.

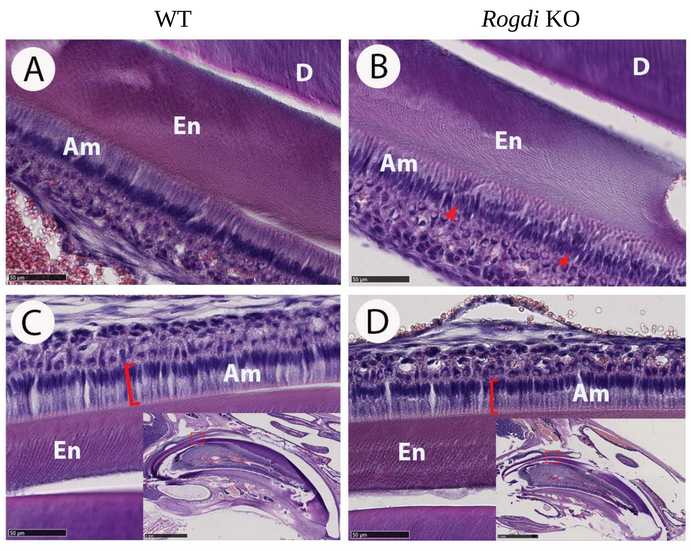


Figure 6 Histological analysis of 4-month-old hemimaxilla.
(A,B) Secretory stage in the ameloblast layer of lower incisors, the red arrowheads point towards a region of disorganization in the ameloblast layer in *Rogdi* -/-. In the upper incisor the ameloblast layer is slightly thinner in the -/- (D) compared to the WT control (C) - compare length of red bracketed regions in C vs. D, enamel thickness.

Abbreviations: Am, ameloblasts; D, dentin; En, enamel.

Conclusion

Rogdi mutant mouse model displays **severe enamel defects** in both incisor and molar teeth. *Rogdi* mutants **do not form calcified enamel, producing an “amelogenesis imperfecta” like phenotype** similar to KTS affected individuals. The generation of this *Rogdi* mutant creates a novel model to investigate the origins of KTS and the unknown function of this protein. Further research on the role of ROGDI during brain and tooth development, as well as in epilepsy and AI is necessary as a possible avenue to continue exploring the mouse model for the discovery of therapeutic treatments.

https://clinicaltrials.gov: NCT01746121 et NCT02397824; MESR, Commission Bioéthique Collection Biologique ”Manifestations bucco-dentaires des maladies rares '' DC-2012-1677 et DC-2012-1002. www.phenodent.org www.rarenet.eu INTERREG V RARENET

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