Alexandra Jiménez-Armijo[1,2,3], Supawich Morkmued[1,4], Virginie Laugel-Haushalter[1], Naji Kharouf [2,5], Eric Mathieu[5], Joseph Hemmerlé[5], Agnès Bloch-Zupan [1,2,6]

1 Université de Strasbourg, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U1258, CNRS- UMR7104, Illkirch, France.
2 Université de Strasbourg, Faculté de Chirurgie Dentaire, Strasbourg, France.
3 Universidad de Chile, Faculty of Dentistry, Pathology and Oral Medicine Department, Santiago, Chile.
4 Khon Kaen University, Faculty of Dentistry, Pediatric Department, Khon Kaen, Thailand.
5 Laboratoire de Biomatériaux et Bioingénierie, Inserm UMR_S 1121, Strasbourg.
6 Hôpitaux Universitaires de Strasbourg (HUS), Pôle de Médecine et Chirurgie bucco-dentaires, Hôpital Civil, Centre de référence des maladies rares orales et dentaires, CRMR O-Rares, Filière Santé Maladies rares TETE COU, European Reference Network ERN CRANIO, Strasbourg, France.

Impaired enamel maturation in a *Rogdi* loss of function mouse model mimics Kohlschütter–Tönz syndrome phenotype

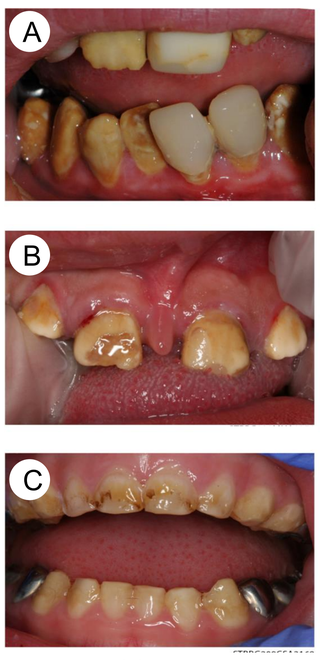
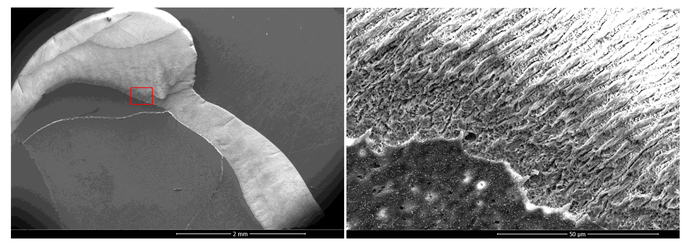


Figure 1. *ROGDI*-associated KTS patients.
We identified, in the CRMR O-Rares, 3 patients with KTS and described the causative *ROGDI* variants. Patients present a yellow-brownish discoloration with a smooth enamel appearance affecting both primary and permanent dentition displaying a Hypomature amelogenesis imperfecta type. Stainless steel are covering the molars in patients A and C. Patient B was published in Huckert et al., 2016.

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]. In patients with KTS, the enamel is soft, rough, and stained with varying shades of brown. This enamel malformation has severe clinical consequences in the form of poor mechanical properties of dental tissues, high susceptibility to caries, high tooth sensitivity, poor aesthetic quality of the dentition, and generalized defects requiring intensive treatment (Figure 1). Teeth from one of the patients with KTZ, from the Reference center for Rare Orodental Diseases, CRMR O-Rares (Strasbourg), were analyzed with scanning electron microscopy imaging. Results showed the lowering of KTS tooth mineral content, which is consistent with the observed phenotype (Figure 2).

KTS clinical features
Epilepsy
Intellectual disability
Psychomotor regression
Amelogenesis imperfecta – Hypomature type
Nephrocalcinosis



Element	Mass %	Atomic %	Error %	Total intensity
Carbon	4.06	9.17	19.56	7.36
Oxygen	28.03	47.51	11.57	77.54
Phosphorus	17.33	15.18	5.41	132.4
Calcium	37.02	25.05	6.31	117.61

Figure 2. Scanning electron microscopy of *ROGDI*-associated KTS patient (A, figure 1) Premolar (45).
Enamel was present, but this was hypoplastic. Red boxed region show region in which SEM image was obtained. The enamel presents a clear decussating prism pattern. Table of energy dispersive X-ray spectrometry data for quantification of element display levels of element content in enamel. Calcium-phosphate ratio (Ca/P) was of 1.65.
Normal values of Ca/P are around 2.17 ± 0.1

Methods and Results

To have a better insight into the understanding of the disease and the function of ROGDI to explore treatment options, a mouse mutant with a loss of function mutation in *Rogdi* was created at the Mouse Clinical Institute (Interreg IV and V RARENET programs). The strategy was to flox exons 6 to 11 to mimic the genotype of some KTS patients treated within the Reference Center for Rare Orodental Diseases in Strasbourg. Knock-out (KO) mice display severe enamel defects (chalky white color enamel) showing an "amelogenesis imperfecta-like" phenotype (Figure 3).

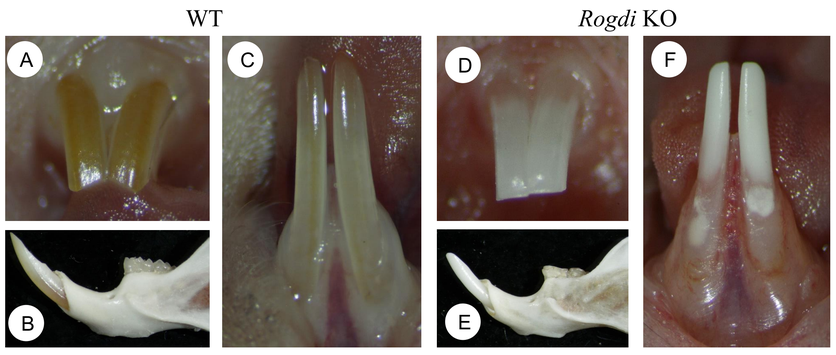


Figure 3 *Rogdi* mouse phenotype.
(A,B,C) Compare incisors of *WT* 8-week-old mice, with normal darker yellow/orange pigmentation in the upper incisor to (D) upper incisors of 8-week-old *Rogdi -/-* mutant mice which have a chalky white color. (E,F) *Rogdi -/-* mutant lower incisors show chalky lightening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

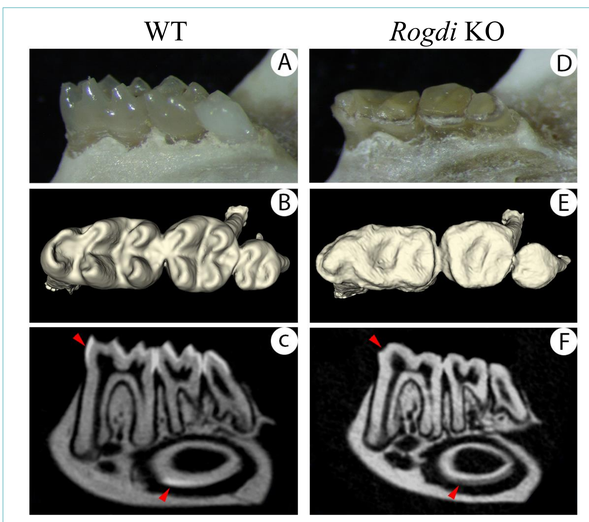
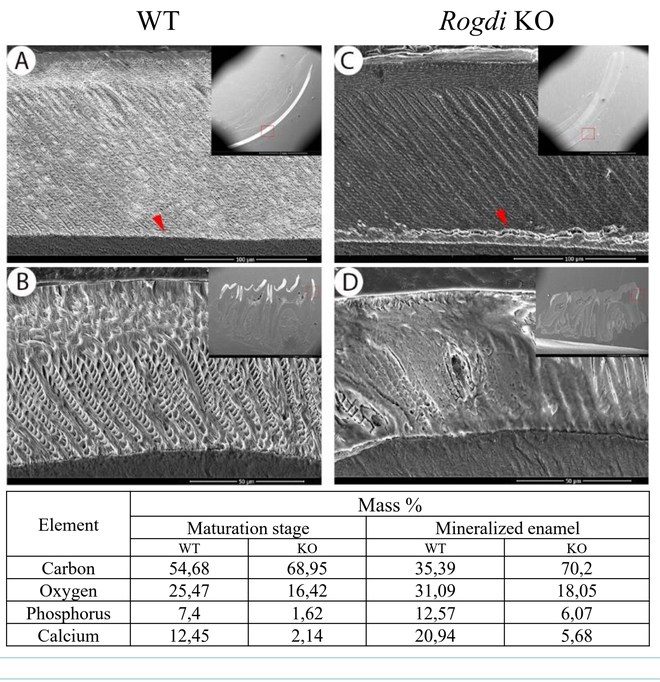


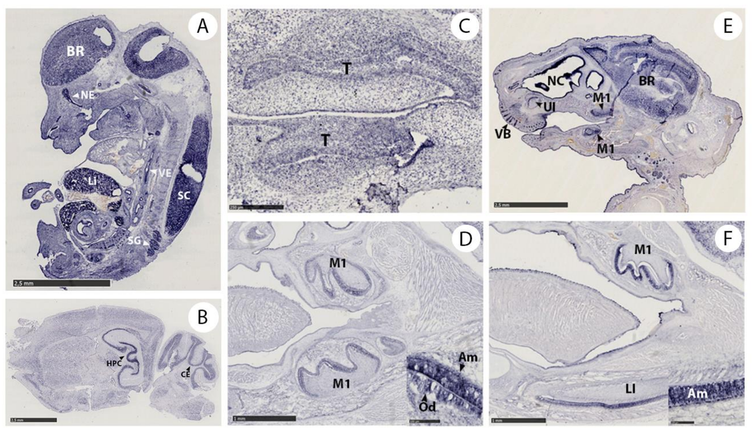
Figure 4. Micro-CT (µ-CT) imaging of 8 weeks-old WT (A,B,C) and Rogdi -/- (D,E,F) teeth.
To assay overall structural changes *Rogdi -/-* samples were analyzed using X-ray micro-computed tomography imaging.
The *Rogdi* KO mouse presented abraded cusps in the molars (D, E) which were severely worn, losing 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) what was also confirmed with SEM imaging (Figure 5).

Rogdi mutants do not form calcified enamel producing a tooth phenotype similar to the one observed in affected individuals (Figure 4,5).



Element	Mass %			
	Maturation stage		Mineralized enamel	
	WT	KO	WT	KO
Carbon	54,68	68,95	35,39	70,2
Oxygen	25,47	16,42	31,09	18,05
Phosphorus	7,4	1,62	12,57	6,07
Calcium	12,45	2,14	20,94	5,68

Figure 5. Scanning electron microscopy (SEM) of 8 weeks-old *WT* (A,B) and *Rogdi -/-* (C,D) teeth.
SEM images of fully-formed lower incisor enamel (A,C) and lower molars (B,D) 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 enamel in maturation stage of amelogenesis and mineralized enamel. The calcium and phosphate concentration in the enamel layer of wild-type mouse is normal but both concentrations are highly diminished in *Rogdi* mutant. Carbon levels are higher in *Rogdi* KO suggesting a lack of enamel matrix degradation during the maturation stage of amelogenesis.



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 6).

Figure 6. *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.

Conclusion

Rogdi mutant mouse model displays **severe enamel defects** in both incisor and molar teeth. *Rogdi* mutants **do not form fully mineralized enamel, producing an "amelogenesis imperfecta" like phenotype** comparable 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.

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jimeneza@igbmc.fr