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Rogdi loss of function mouse model mimics Kohlschütter–Tönz syndrome phenotype

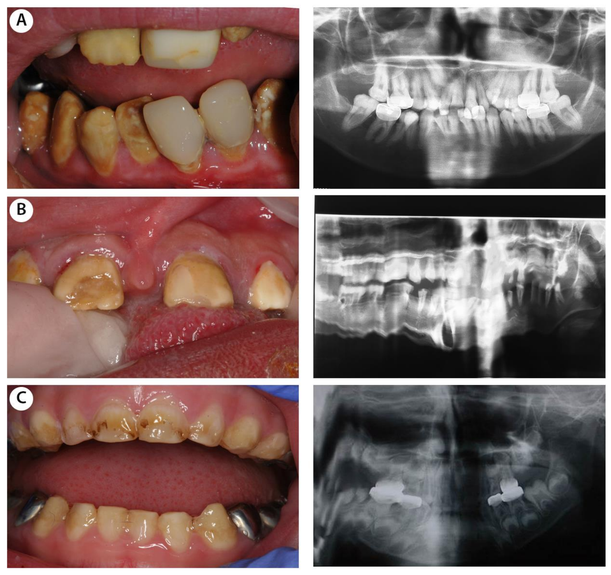


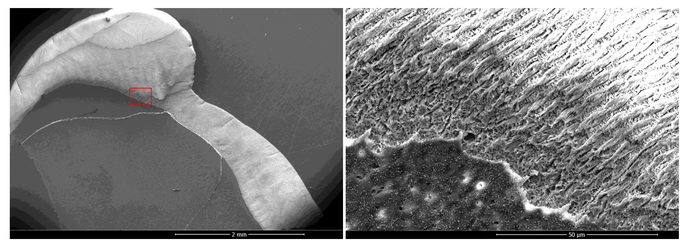
Figure 1. *ROGDI*-associated KTS patients.
We identified, in the CRMR O-Rares, 3 patients with KTS and described the causative *ROGDI* variants. Patients present a yellow-brownish discoloration with a smooth enamel appearance affecting both primary and permanent dentition displaying a Hypomature amelogenesis imperfecta type. 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) [Kohlschutter *et al*, 1974; Schossig *et al,* 2012]. 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 Oral and dental 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
- Enamel defects
  Amelogenesis imperfecta
- Nephrocalcinosis

Dental enamel is the hardest and most mineralized tissue in our body. It is almost fully mineralized and composed of a substituted hydroxyapatite (Hap) of primarily calcium (Ca2) and inorganic phosphate (Pi) [Lacruz et al., 2017].



Element	Mass %	Atomic %
Carbon	4.06	9.17
Phosphorus	17.33	15.18
Calcium	37.02	25.05

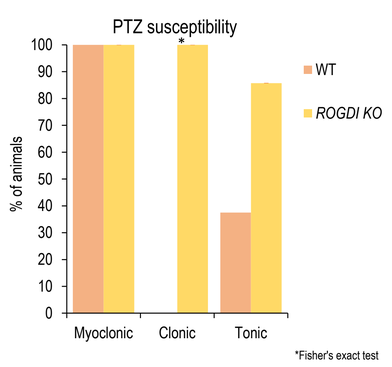
Figure 2. Scanning electron microscopy of *ROGDI*-associated KTS patient (A, figure 1) Premolar (45).
Enamel was present, but this was hypoplastic. Red boxed region show region in which SEM image was obtained. The enamel presents a clear decussating prism pattern. Table of energy dispersive X-ray spectrometry 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

Material and Methods / 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 Oral and dental Diseases in Strasbourg. Knock-out (KO) mice show a higher susceptibility to develop epilepsy (Figure 3).

Epilepsy

Seizure types such as generalized tonic, tonic–clonic, myoclonic, atonic, focal clonic with or without awareness and with secondary bilateral synchronization have been reported in KTS patients [Schossig et al., 2012]. Pentylenetetrazole (PTZ), a GABA receptor antagonist, is used to create a common chemically- induced seizure model. Seizures were induced by a single fixed dose of i.p.PTZ (40 mg/kg) and the onset latency/severity/duration of seizure response was monitored.



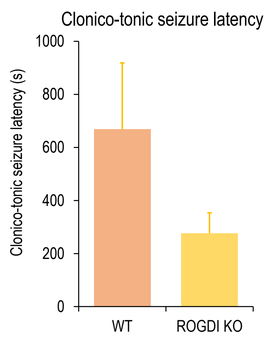


Figure 3. Epilepsy susceptibility in Rogdi KO mice.
All mice manifested myoclonic seizures, but only Rogdi KO mice had clonic seizures. At the end of the convulsion, less than 40% of the WT presented tonic seizure while more than 90% of the Rogdi KO mice developed one. These results show a higher susceptibility of the *Rogdi* KO mice to develop epilepsy.

Intellectual disability and psychomotor regression

People with KTS have obvious motor and cognitive impairments, affecting both men and women, with no known sex difference [Huckert et al., 2014; Lee et al., 2017]. Circadian activity and Novel object recognition tests showed an increased locomotor activity in *Rogdi* -/- both females and males. Additionally, we observed *Rogdi* -/- mice exhibit memory impairment during the novel object recognition test. Grip strength was significantly decreased in *Rogdi* -/- female mice.

Enamel defects

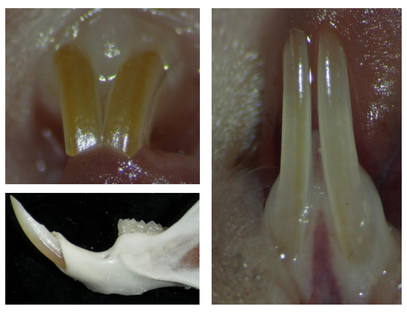


Figure 4 *Rogdi* mouse tooth phenotype.
(A,B,C) Compare incisors of *WT* 8-week-old mice, with normal darker yellow/orange pigmentation in the upper incisor to (D) upper incisors of 8-week-old *Rogdi* -/- mutant mice which have a chalky white color. (E,F) *Rogdi* -/- mutant lower incisors show chalky lightening with white patches in the cervical tooth zone.

Rogdi mutants display severe enamel defects (chalky white color enamel) showing an "amelogenesis imperfecta-like" phenotype (Figure 4). Ultrastructural analysis showed a less mineralized enamel producing a tooth phenotype similar to the one observed in affected individuals (Figure 5).

Enamel defects

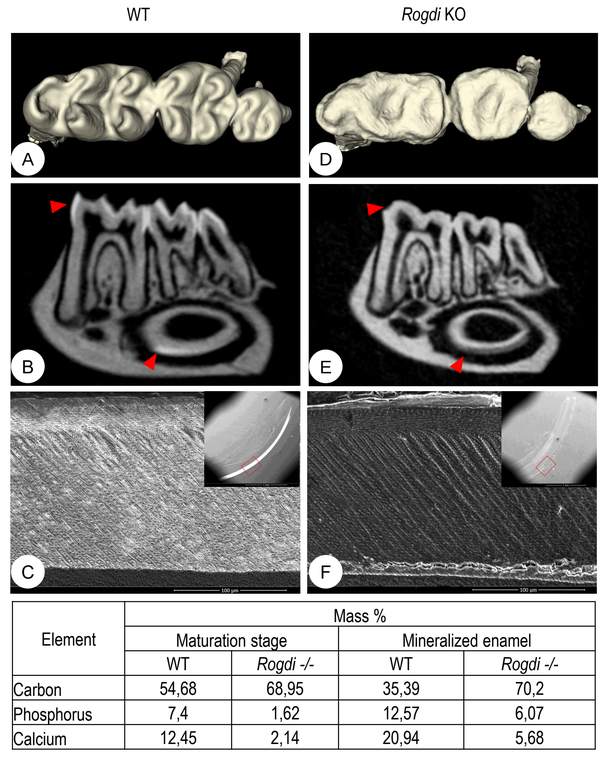
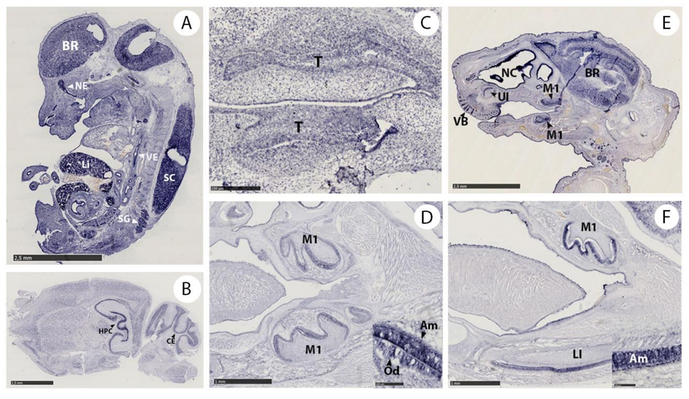


Figure 5. Micro-CT (µ-CT) and scanning electron microscopy (SEM) imaging of 8 weeks-old WT (A,B,C) and *Rogdi* -/- (D,E,F) teeth.
To assay overall structural changes *Rogdi* -/- samples were analyzed using X-ray micro-computed tomography imaging and SEM imaging.
The *Rogdi* KO mouse presented abraded 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 enamel mineral density in lower molars and incisor (arrowheads in B,E) what was also confirmed with SEM imaging (C,F). 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 show calcium and phosphate concentration in the enamel layer 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.

Element	Mass %			
	Maturation stage		Mineralized enamel	
	WT	*Rogdi* -/-	WT	*Rogdi* -/-
Carbon	54,68	68,95	35,39	70,2
Phosphorus	7,4	1,62	12,57	6,07
Calcium	12,45	2,14	20,94	5,68



ROGDI, is an essential basic zipper protein, highly conserved across metazoans, however its function remains unknown. *Rogdi* is expressed in **kidney** and liver since early embryonic stages, 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

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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