

A Novel Mutation in the *ROGDI* Gene in a Patient with Kohlschütter-Tönz Syndrome

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Key Words

Amelogenesis imperfecta · Epilepsy · Kohlschütter-Tönz syndrome · Leucine zipper domain · Mutations · *ROGDI*

Abstract

Kohlschütter-Tönz Syndrome (KTZS) is an autosomal recessive disorder caused by mutations in the *ROGDI* gene. This syndrome is characterized by epilepsy, psychomotor regression and amelogenesis imperfecta. In this paper, we report a case of a 13-year-old Malian girl presenting with this rare disease. By genetic analysis, we identified a novel *ROGDI* homozygous mutation NM_024589.1: c.117+1G>T [Chr16 (GRCh37): g.4852382C>A] which confirmed the diagnosis of Kohlschütter-Tönz syndrome. The mutation abolishes the usual splice donor site of intron 2 which leads to the deletion of exon 2 and in-frame assembly of exon 3. Exon 2 encodes a highly conserved leucine-rich region that is essential for *ROGDI* protein function. Hence, this deletion may affect the function of the *ROGDI* protein.

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Kohlschütter-Tönz syndrome (KTZS, OMIM 226750) is a rare autosomal recessive syndrome characterized by epilepsy, psychomotor regression and amelogenesis imperfecta (AI) due to mutations in the *ROGDI* gene in 16p13.3 [Mory et al., 2012; Schossig et al., 2012a].

AI is a heterogeneous group of developmental diseases that affect the formation, structure and clinical appearance of enamel. AI can be classified into different subtypes (hypoplastic, hypomineralized, and hypomature) based on the nature of the enamel defect. The prevalence of AI varies from 1/700 to 1/14,000, and it can be inherited in an autosomal dominant, autosomal recessive or X-linked manner [Aldred et al., 2003; Crawford et al., 2007; Bloch-Zupan et al., 2012; Gadhia et al., 2012]. AI can present as an isolated disease or coexist with other abnormalities and symptoms as part of syndromic disorders such as KTZS.

Since the first case of KTZS reported in 1974 [Kohlschütter et al., 1974], around 20 cases have been published so far delineating a similar phenotype. Several

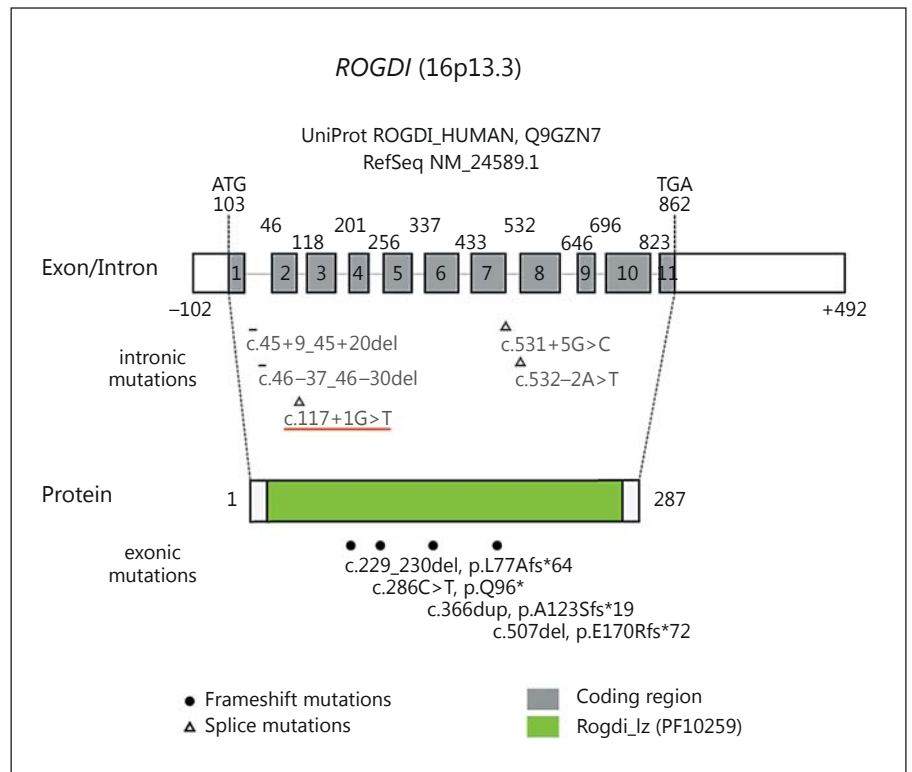


Fig. 1. *ROGDI* exonic map. Locations of the different mutations described in the literature are shown. The new mutation c.117+1G>T is underlined in red.

mutations in the *ROGDI* gene were described since 2012 in European KTZS patients (fig. 1) [Mory et al., 2012, 2014; Schossig et al., 2012a, b; Tucci et al., 2013].

We report the case of a 13-year-old girl, the third child of consanguineous parents from Mali, who presented with hypomineralized/hypomature AI, psychomotor delay and pharmaco-resistant epilepsy. Due to the similarity of this phenotype with KTZS, we investigated the *ROGDI* gene for potential mutations by Sanger sequencing.

Case Report

The girl and her parents were recruited and examined clinically by dentists at the Reference Center for Orofacial Manifestations of Rare Diseases at the Strasbourg University Hospital. The family consented to participate in the French Ministry National Program for Clinical Research, PHRC 2008 HUS No. 4266 'Amelogenesis imperfecta: clinical and molecular study' (ClinicalTrials.gov Identifier: NCT01746121) and in the Interreg/Offensive Sciences A27 projects. Personal and clinical data were entered into D[4]/Phenodent, a dental defects diagnostic database (www.phenodent.org).

The patient was born at term by emergency caesarean section due to pregnancy-induced hypertension. Weight at birth was 3.1 kg at 39 weeks and 3 days of amenorrhea (25th percentile of birth). She required resuscitation at birth due to meconium aspiration

(Apgars 3, 8 and 10), and was transferred to the special care unit where she received oxygen and antibiotics for 24 h before being moved to the transitional care ward.

She presented with seizures starting in her 3rd week of life and manifested by hypotonia, peribuccal cyanosis, and eye rolling. At 1 month she underwent surgery for a strangulated inguinal hernia. Shortly after discharge, she presented to the emergency room with an episode of loss of consciousness, which was attributed to gastroesophageal reflux. Two further events followed at 4 and 5 months; her parents described several additional episodes in which she became hypotonic and unresponsive with signs of central cyanosis. All episodes lasted less than 10 min.

At 1 year of age, after presenting with a clear right-sided partial seizure, she was started on carbamazepine and clonazepam, and the working diagnosis of seizures secondary to suspected hypoxic ischemic encephalopathy was made. The seizures continued despite the use of multiple anti-convulsive agents (topiramate, phenobarbital and vigabatrin) as well as a trial of a ketogenic diet. Physical examination was essentially normal and she continued to grow well along her centile lines. Developmental milestones were delayed: she learned to walk unaided at 19 months and spoke her first words at 3 years.

By 6 years of age, the seizures were occurring on a daily basis, often worse at night and predominantly involving right-sided clonic movements. There was severe developmental delay, with a global functional level of an 18-month-old. There were marked right-sided neglect, ataxic gait and dysmorphic features with frontal bossing, thickened protruding lower lip and genital hypoplasia

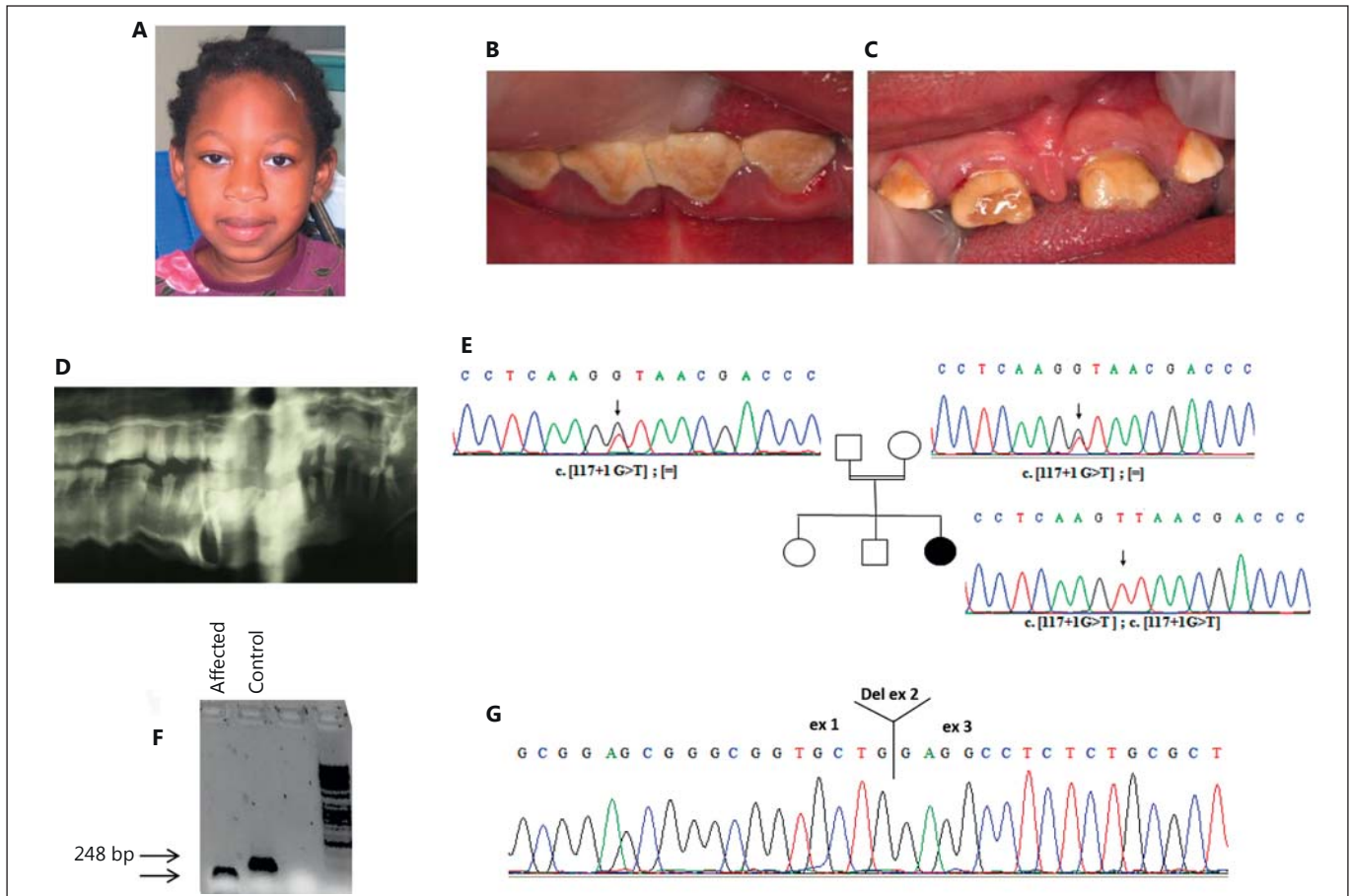


Fig. 2. Phenotype of the affected patient, familial segregation of the mutation and RT-PCR. **A** Facial photograph of the patient. **B** Intraoral view of mandibular incisors. **C** Intraoral view of maxillary incisors. **D** Panoramic radiograph showing hypomineralized/hypomature AI. The enamel is rough, colored, prone to disintegration, and retains oral biofilm. There is no contrast between dentin and enamel on the radiograph. The poor quality of the panoramic radiograph is due to the behavioral difficulties of the patient, which prevent her from remaining still during the radiographic acquisition procedure.

E Identification of a new homozygous mutation c.117+1G>T in the *ROGDI* gene. The affected proband is homozygous with a 100% peak. The parents are heterozygous with two 50% peaks. [=] = normal allele. **F** RT-PCR validation of the biological effect of the mutation. The mutation causes the deletion of exon 2. The amplicon is smaller in the affected patient compared to the control (248 bp). **G** Sequence of cDNA from the patient confirms the absence of exon 2.

(fig. 2A). Head circumference was normal between the 50th and the 75th percentile. Language was limited to less than 5 words and she exhibited aggressive and impulsive behavior.

The orodental phenotype assessed through oral clinical and radiographic (panoramic radiograph) examinations revealed no abnormalities of tooth number or size, but abnormalities of enamel affecting the primary and permanent dentition were noted (fig. 2B–D). The primary teeth presented severe enamel dysplasia with eruption delay. At 5 years of age, a diagnosis of hypomineralized/hypomature AI was proposed. The enamel of the permanent teeth was rough, colored and retained oral biofilm. Because of her medical and behavioral handicap, dental treatments had to be performed under general anesthesia in 2006, 2007, 2009, and 2012.

Materials and Methods

DNA Sequencing and Mutation Screening

Saliva samples were collected using the OG-250 Oragene[®] DNA kit (DNA Genotek Inc., Ottawa, Ont., Canada). Genomic DNA was extracted from saliva according to standard protocols using the prepIT-L2P Oragene[®] DNA kit (DNA Genotek Inc.). Coding exons and flanking intronic regions of *ROGDI* were PCR amplified from 50 ng of genomic DNA (online suppl. table 1, see www.karger.com/doi/10.1159/000366252). Bidirectional sequencing of the purified PCR products was performed by GATC Biotech (Applied Biosystems ABI 3730xl[™], Konstanz, Germany). Sequences were aligned and compared with reference sequences using the (SeqScape) software v.2.6.

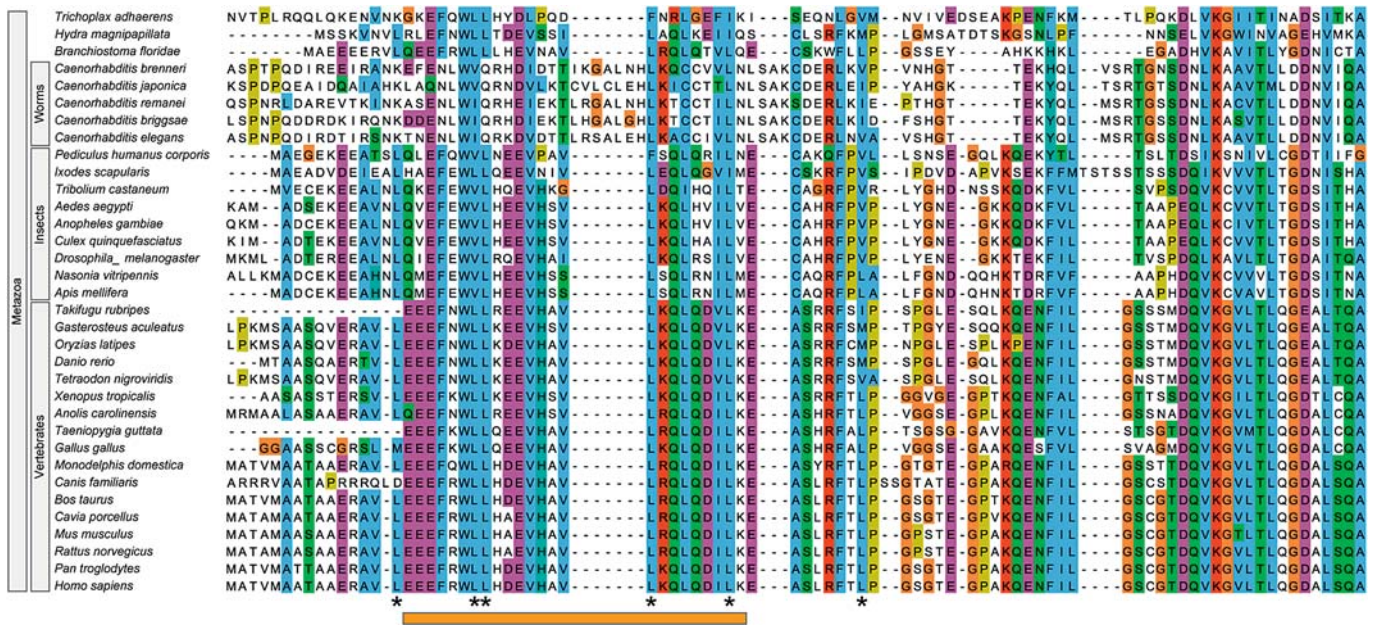


Fig. 3. Multiple sequence alignment. The multiple sequence alignment of the ROGDI protein family amongst metazoans (see Materials and Methods) is shown here. The alignment is displayed using Jalview and colored according to the 'ClustalX' coloring scheme. The proteins are sorted from human to early metazoans

from bottom to top, starting from position 1 to position 85 in the human sequence. The 24-aa deletion from position Glu16 to Arg39 is represented by the orange bar. Possible leucines compatible with the Rogdi_lz domain are marked by asterisks.

Sequencing and Mutation Analysis

The Alamut software v.2.2 (Interactive Biosoftware, Rouen, France) was used to collect the analysis of 3 programs used to assess the effect of potential splice variants (Human Splicing Finder, MaxEntScan, NNSplice) [Reese et al., 1997; Yeo and Burge, 2004; Desmet et al., 2009]. dbSNP (build 137, <http://www.ncbi.nlm.nih.gov/projects/SNP/>) [NCBI Resource Coordinators, 2013] and the Exome Variant Server (NHLBI Exome Sequencing Project (ESP), Seattle, Wash., USA; <http://evs.gs.washington.edu/EVS/>, March 2013) databases were used to test whether the identified variant was a known variant or polymorphism. The PFAM [Punta et al., 2012] database was used to search for protein domain organization and the eggNOG [Powell et al., 2012] database was used to retrieve the sequences of RODGI orthologs in eukaryotic species (KOG3992). The multiple sequence alignment was extracted from the pre-computed alignments available in eggNOG and further manually refined.

Reverse Transcriptase-PCR and Sequencing Analysis of RODGI Transcripts

RNA was extracted from fibroblasts of the affected patient (cutaneous biopsy) and 3 unrelated controls by using a RiboPure™ Kit, followed by DNase treatment with the TURBO DNA-free™ Kit (Life Technologies, Carlsbad, Calif., USA). RNA integrity was assessed by gel electrophoresis and RNA concentration by Eppendorf Biophotometer Plus™ with the Hellma® TrayCell™ (Eppendorf, Hamburg, Germany). Reverse transcription of 1 µg total RNA to cDNA was performed using the iScript™ cDNA Synthesis

Kit (BioRad, Hercules, Calif., USA). RT-PCR was performed to determine the exon content of the cDNA from the patient. PCR primers specific for exons 1–3 were designed to amplify the anticipated altered splice site junction (online suppl. table 1). RODGI cDNA was also partially sequenced to determine the consequence of the genomic mutation on RNA splicing.

Results

Bidirectional sequencing analysis of RODGI identified a homozygous mutation c.117+1G>T in the second intron in the proband. This mutation causes a transversion in the canonical splice donor site of intron 2. Segregation analysis validated that each unaffected parent was heterozygous for this mutation (fig. 2E). The mutation was absent from dbSNP and the Exome Variant Server.

The effect of the mutation on the splice site was assessed using 3 different splice site mutation prediction programs (see Materials and Methods). As expected, all programs predicted the destruction of the donor site. The expected consequences were the skipping of exon 2 and an in-frame assembly of exon 3. The resulting protein is expected to be shorter by 24 amino acids (aa) from posi-

tion Glu16 to Arg39. Thanks to the PFAM database, the protein could be annotated as containing a leucine zipper domain spanning from position 18 to 277 (domain Rogdi_1z: PF10259). Analysis of the multiple sequence alignment of the protein members of the ROGDI family extracted from eggNOG revealed that the 24-aa deletion was localized in one of the regions containing a pattern of leucines organized like in leucine zipper domains, which could then affect the function of the protein (fig. 3).

To validate the biological effect of this mutation, we performed RT-PCR analysis and sequencing of the *ROGDI* cDNA from the proband and unrelated controls. RT-PCR analysis confirmed the absence of exon 2 in the affected patient with a shorter fragment, whereas the control samples yielded a PCR fragment of the expected size (248 bp) (fig. 2F). Sequencing of the RT-PCR products further confirmed the absence of exon 2 in the proband's cDNA and the in-frame assembly of exon 3 (fig. 2G).

Discussion

The *ROGDI* gene located on chromosome 16 is composed of 11 coding exons. The encoded protein is 287 aa long and is annotated as a leucine zipper domain-containing protein (UniProt AC: Q9GZN7). We report a novel mutation c.117+1G>T that abrogates the donor splice site in intron 2 and causes the deletion of exon 2 (72 bp) resulting in a shorter protein (24 aa less). This is the first report of a mutation in *ROGDI* in a sub-Saharan African patient. Our discovery adds to the list of mutations previously identified in this gene in patients with KTZS. Other mutations described in the literature (fig. 1) include deletions, duplications, splicing, and frameshift mutations and are scattered in exons 4–7 and introns 1 and 7 [Schossig et al., 2012a; Mory et al., 2012; Tucci et al., 2013].

The function of ROGDI is yet unknown; however, the protein is present in metazoan species ranging from worms to humans and is highly conserved, suggesting an important functional role (fig. 3).

The loss of 24 aa in the mutant protein likely affects protein function. Indeed, exon 2 contains several highly conserved leucine residues which may contribute to dimerization and DNA binding.

Rare diseases that manifest with neurological symptoms and various dental anomalies have been previously recognized [Bloch-Zupan, 2007]. For example, tuberous sclerosis (OMIM 191100) and Sjögren-Larsson syndrome (OMIM 270200) combine epilepsy and enamel defects

among many other features. The function of the ROGDI protein and the link between the dental and brain phenotypes observed in the presence of *ROGDI* mutations is yet to be understood. Also known as *Lzf*, C76152, AU020118 and 0610011C19Rik, *ROGDI* is transcribed during mouse and human brain development [Jensen et al., 2004]. *Rogdi* is also expressed in the cap stage of odontogenesis in E14.5 mouse embryos [Laugel-Haushalter et al., 2013]. This suggests that ROGDI is present during and may play a role in brain formation and homeostasis as well as in odontogenesis.

Careful examination of the oral cavity may provide invaluable information for the diagnosis of rare diseases. It facilitated the diagnosis of KTZS in this patient and family, who waited 13 years for a confirmed clinical and molecular diagnosis. Collaborative efforts between reference centers for rare diseases and multidisciplinary health care centers are indispensable for providing an integrative approach to the diagnosis and treatment of patient pathologies. Further research on the role of *ROGDI* during brain and tooth development as well as in epilepsy and AI is necessary and may allow the discovery of new treatments for KTZS.

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