ORIGINAL ARTICLE

SLC13A5 is the second gene associated with Kohlschütter–Tönz syndrome

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ABSTRACT

Background Kohlschütter–Tönz syndrome (KTZS) is a rare autosomal-recessive disease characterised by epileptic encephalopathy, intellectual disability and amelogenesis imperfecta (AI). It is frequently caused by biallelic mutations in *ROGDI*. Here, we report on individuals with *ROGDI*-negative KTZS carrying biallelic *SLC13A5* mutations.

Methods In the present cohort study, nine individuals from four families with the clinical diagnosis of KTZS and absence of *ROGDI* mutations as well as one patient with unexplained epileptic encephalopathy were investigated by clinical and dental evaluation, parametric linkage analysis (one family), and exome and/or Sanger sequencing. Dental histological investigations were performed on teeth from individuals with *SLC13A5*-associated and *ROGDI*-associated KTZS.

Results Biallelic mutations in *SLC13A5* were identified in 10 affected individuals. Epileptic encephalopathy usually presents in the neonatal and (less frequently) early infantile period. Yellowish to orange discolouration of both deciduous and permanent teeth, as well as wide interdental spaces and abnormal crown forms are major clinical signs of individuals with biallelic *SLC13A5* mutations. Histological dental investigations confirmed the clinical diagnosis of hypoplastic AI. In comparison, the histological evaluation of a molar assessed from an individual with *ROGDI*-associated KTZS revealed hypocalcified AI.

Conclusions We conclude that *SLC13A5* is the second major gene associated with the clinical diagnosis of KTZS, characterised by neonatal epileptic encephalopathy and hypoplastic AI. Careful clinical and dental delineation provides clues whether *ROGDI* or *SLC13A5* is the causative gene. Hypersensitivity of teeth as well as high caries risk requires individual dental prophylaxis and attentive dental management.

INTRODUCTION

The combination of epileptic encephalopathy, developmental delay or regression, and yellowish discolouration of the teeth due to amelogenesis imperfecta (AI) was first described by Kohlschütter and colleagues in 1974 in a large Swiss family[1] and is now recognised as Kohlschütter–Tönz syndrome (KTZS, OMIM 226750). To date, 23 KTZS families have been reported in the literature.[1–15] Epilepsy usually starts in the first year of life and is often difficult to treat.[12] The most severely affected individuals have profound intellectual disability, never acquire speech and become bedridden early in life.[16] Clinical and laboratory signs are not specific in the disease,[16] except for dental findings; therefore, the latter are essential for the clinical diagnosis of KTZS. All affected individuals show variable yellow-to-brown discolouration of primary as well as permanent teeth right from eruption.[13]

In 2012, we[12] and others[8] identified biallelic mutations in *ROGDI* (OMIM 614574) as the cause of KTZS in the majority of families, also confirming autosomal-recessive inheritance. However, several KTZS families in the literature and/or investigated by us did not have a pathogenic variant in *ROGDI*, indicating genetic heterogeneity.[3 13] AI in *ROGDI*-associated KTZS is characterised by rough dental surfaces, whereas other KTZS patients showed AI with rather smooth dental surface and sometimes only mild discolouration.[13] Here, we report that *ROGDI*-negative individuals with the clinical diagnosis of KTZS may show biallelic mutations in *SLC13A5*, previously described in individuals with the diagnosis of autosomal-recessive early infantile epileptic encephalopathy (EIEE25, OMIM 615905) who also display variable teeth hypoplasia and/or hypodontia.[17–19] Clinical and histological evaluation identifies distinct dental differences between KTZS caused by *ROGDI* or *SLC13A5* mutations, respectively, allowing clinical determination of the putative candidate gene.

MATERIALS AND METHODS

Ethical considerations

The study was conducted with the understanding and full written parental consent as part of the Biobank for Rare Diseases, approved by the Ethics Committee of the Medical University Innsbruck, Austria (Study No. UN4501) in accordance with the Declaration of Helsinki of 1975 as revised in 2002 or in the context of the D[4/phenodent registry, which is approved by CNIL (French National Commission for Informatics and Liberty, number 908416). This clinical study is registered at https://clinicaltrials.gov: NCT01746121 and NCT02397824 and with the MESR (French Ministry of Higher Education and Research) Bioethics Commission as a biological collection 'Orodental Manifestations of Rare Diseases' DC-2012-1677 within DC-2012-

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1002 and was acknowledged by the CPP (person protection committee) Est IV on 11/12/2012. Family C signed a consent form approved by the Johns Hopkins University School of Medicine Institutional Review Board.

Subjects and families

A cohort of 10 affected individuals from five families under care at the participating centres was included in the study. Data were collected during routine examination in 2015. Nine individuals were clinically diagnosed with KTZS based on the combination of epilepsy, intellectual disability and AI. *ROGDI* analysis by Sanger or massively parallel sequencing[20] on genomic DNA from all affected individuals showed normal results. One additional individual was ascertained through exome sequencing (family E). The families' pedigrees are shown in figure 1. Family D was independently studied and published by Hardies and colleagues (2015, family C).[17] The phenotype of family C has been described before as *ROGDI*-negative KTZS prior to further molecular evaluation.[3]

Histological analyses

Histological examination of the impacted tooth 48 of individual D:II:3 and a partially erupted permanent upper molar (tooth no. 16) assessed from an individual with *ROGDI*-associated KTZS (*ROGDI* NM_024589.1: c.286C>T; p.Gln96Ter in a homozygous state) was performed. The teeth were dehydrated in solutions of increasing alcohol concentrations with added basic fuchsin to achieve block staining. The alcohol was then removed with acetone and the teeth were embedded in methyl methacrylate.[21] The embedded teeth were sectioned perpendicular to the occlusal surface in order to produce slices of the teeth. Sections with a thickness of 25–30 μm were polished and contrast stained with acetic light green.

Genetic analyses

Linkage analysis was performed in family A including genomic DNA samples of the affected siblings, the healthy brother and both parents with a SNP-based mapping-chip (HumanCytoSNP-12v2.1, Illumina, San Diego, California, USA) with bioinformatic tools GenomeStudio 1.6.3, Allegro,[22] ALOHOMORA,[23] GRR,[24] PedCheck[25] and Merlin,[26] using standard settings for a recessive inheritance model.

Exome sequencing was performed on genomic DNA samples of individuals A:II:1 and D:II:2, in the two affected individuals and both unaffected parents of family C, and in individual E:II:2 and her unaffected parents using the SureSelect Human All Exon 50 Mb V5 or 51 Mb V4 enrichment kits (Agilent, Santa Clara, California, USA). Paired-end reads were obtained on the Illumina HiSeq2000 or the HiSeq2500 platforms (Illumina, San Diego, California, USA). Bioinformatic tools Burrows-Wheeler Alignment,[27] SAMtools (V.0.1.18),[28] PINDEL (V. 0.2.4t), ExomeDepth (V.1.0.0), GATK3.1[29 30] and PhenoDB (http://mendeliangenomics.org)[31] were used. Variants were filtered for autosomal-recessive inheritance and a minor allele frequency <0.01 in the 1000 Genomes Project (Build 20130502; The 1000 Genomes Project Consortium, 2012, http://www.1000genomes.org); dbSNP builds 126, 129 and 131 (http://www.ncbi.nlm.nih.gov/SNP); Exome Variant Server (http://evs.gs.washington.edu/EVS; release ESP6500SI-V2) and our in-house control databases.

In individual B:II:1, directed dye-terminator Sanger sequencing of the coding region and adjacent intron sequences of *SLC13A5* (NM_177550.4) was performed. Putative disease-causing mutations

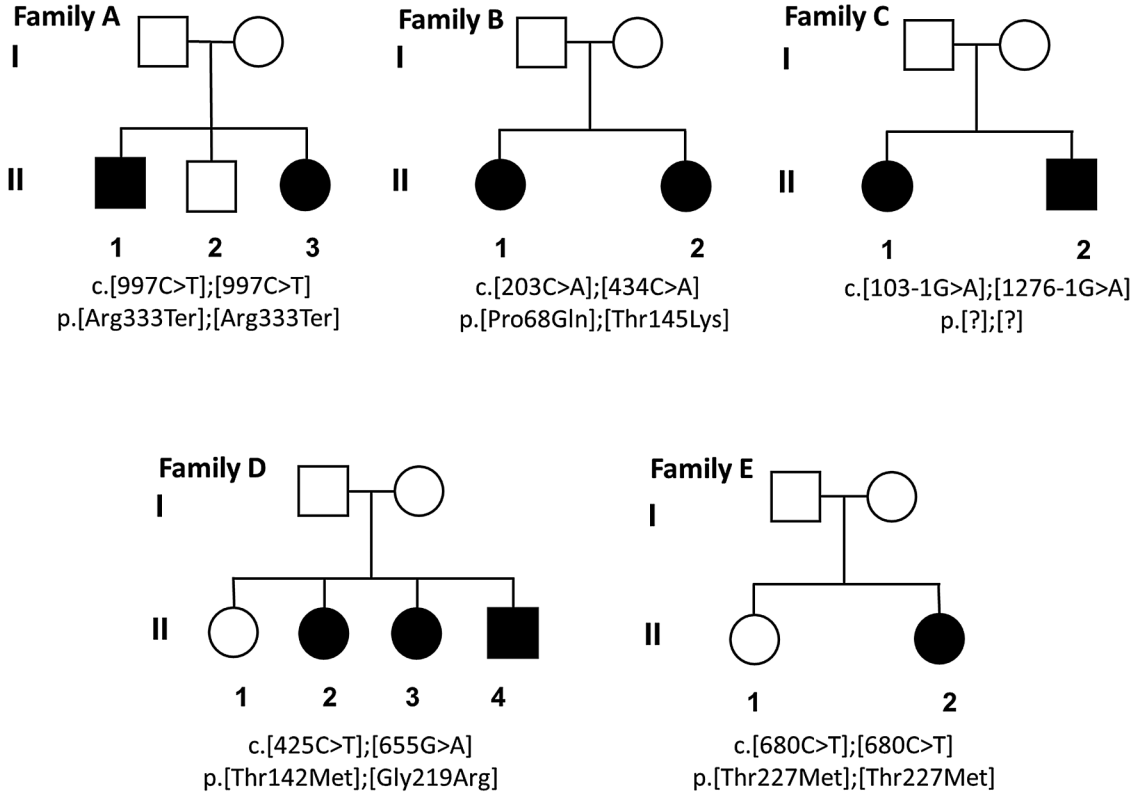

Figure 1 Pedigrees of the families described in this study. Below each pedigree, the *SLC13A5* genotype in the affected individuals is specified on nucleotide and predicted protein levels.

in *SLC13A5* detected by exome sequencing were confirmed by Sanger sequencing in all the affected individuals, and carrier status of the parents was verified in all families using standard methods. The putative pathogenicity of variants was predicted by *in silico* prediction programmes SIFT, MutationTaster and PolyPhen-2,[32–34] and variant frequency was obtained from the Exome Aggregations Consortium ExAC Browser Beta (http://exac.broadinstitute.org, accessed 06/2016) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) (last accessed 10/2015). The *SLC13A5* variants described in this study were submitted into a publicly available database (LOVD 3.0).

RESULTS

Clinical and dental descriptions

Clinical and genetic findings in our patients are summarised in table 1; pedigrees of the families are provided in figure 1; typical dental features in *SLC13A5*-associated KTZS are shown in figure 2.

In *family A* one affected male, one affected female and a healthy male are children of not knowingly related healthy parents originating from southern Italy. The affected male, *A:II:1*, developed neonatal seizures. His seizures were difficult to control and he experienced seizure-free intervals up to 3 years but also regularly episodes with higher seizure frequencies and with various seizure types. EEG abnormalities comprised epileptiform potentials with different foci, sometimes mixed with focal slowing; several EEGs were normal. He showed infantile hypotonia which developed into spasticity at the age of 6 years when he started to walk. At age 33 years, he showed spasticity in all four limbs and an ataxic dystonic gait almost without communication skills. Brain CT at age 15 months was unremarkable.

His younger sister, *A:II:3*, was diagnosed with epilepsy at the age of 7 months. Psychomotor development was delayed: she was able to stand and began to walk at age 26 months, at age 5 she mainly crawled and showed ataxic hypotonia. At 10 years of age, she showed an ataxic dystonic gait, spoke several words and could understand tasks of daily routine. Her verbal skills improved over time, and she was able to write her name at the age of 29 years. Seizures could be controlled by antiepileptic treatment but she showed deterioration of gait at age 11 which ameliorated after change of antiepileptic medication. Brain MRIs at the age of 2 and 10 years, respectively, were unremarkable. EEGs showed different patterns starting with multifocal epileptiform potentials at the age of 7 months, monomorphic theta rhythms at the age of 2½ and later on generalised spike wave activity.

In both individuals, all permanent teeth were erupted, but several teeth had been extracted due to caries. Permanent teeth were small and cylindrical with wide interdental spaces, and marked yellowish to brownish discolorations (figure 2E). Secondary premolars and molars were extremely worn.

Family B consists of two affected sisters, the only offspring of non-consanguineous parents, originating from Martinique and from Cameroon, respectively. Individual *B:II:1* was born at term after an uneventful pregnancy. Six hours after birth, she developed seizures that presented as tonic–clonic, were either right-sided hemiconvulsive or generalised and were resistant to treatment. Development was delayed: ataxic walking without support was achieved at age 22 months, first words were uttered between age 12 and 18 months. There was no regression. At age 11 years, the girl showed intellectual disability, spastic diplegia with pyramidal signs of the legs, cerebellar ataxia and strabismus.

The affected sister, individual *B:II:2*, was delivered by Caesarean section at gestational week 30 because of fetal cardiac arrhythmia. She developed seizures at the age of 2 months, which presented as left-sided hemiconvulsive clonic seizures and were controlled with antiepileptic treatment. Interictal EEGs showed normal results. The girl sat without support at age 18 months ans walked without support at age 5 years. She uttered first words at the age of 3 years and spoke two-word sentences at the age of 6 years. Neurological examination showed pyramidal signs of the legs and cerebellar ataxia. Both sisters' body measurements were in the normal range, and brain MRIs were unremarkable in both.

Both sisters had mixed dentition with delayed eruption of secondary teeth. There was no hypodontia as all permanent teeth were either erupted or radiologically visible as tooth buds. Clinically, the enamel of primary and secondary teeth was hypoplastic with multiple small pits on the surface. There were no contact points between the teeth; lower permanent incisors were sharp and thin. Primary molars are extremely worn, and enamel chipping had occurred (figure 2A, B). In individual B:II:1, the teeth had a yellowish discolouration, whereas in individual B:II:2 the deciduous incisors showed a more opaque enamel. In the dental panoramic radiograph, lack of enamel was obvious on primary and secondary teeth (figure 2C).

Clinical data from *family C*, two siblings with epileptic encephalopathy, have been published elsewhere.[3] Seizures started at the age of 40 days in the affected girl, variable seizure types were documented which could not be completely controlled by antiepileptic treatment. In the affected boy, seizure onset was at the age of 6 hours and seizures were easier to control. Both children had marked intellectual disability and showed hypotonia and ataxia.

Both individuals showed a marked delay in tooth eruption with persistent primary second molars at age 14. Secondary incisors were small with a cylindrical shape, and the incisal edges were sharp and thin with brown discolouration. The tooth surface was rather smooth and hard with small pitting. No enamel was visible on dental panoramic radiographs in primary as well as secondary teeth.

Family D has been reported in Hardies *et al*.[17] The non-consanguineous parents originate from Germany. Briefly, the affected individuals (two girls and one boy) were born at term after uneventful pregnancies. They presented with focal clonic seizures on the first day of life. Transient postnatal hypercalcaemia and hyperphosphataemia requiring treatment were noted in all three affected siblings. All three siblings showed delayed psychomotor development and had intellectual disability.

In all three siblings, permanent teeth were widely spaced with a cylindrical shape and pointed tips. Premolars and molars were extremely worn. Discolouration of secondary teeth varied from dark yellow in individual *D:II:4*, slight yellowish in *D:II:2* and white but opaque in *D:II:3*. In all individuals of family D, the surface of the teeth was hard and rather smooth with multiple small pits. Dental radiographs showed the lack of enamel (figure 2D).

In *family E*, individual *E:II:1* is the child of non-consanguineous parents originating from the Netherlands and has one healthy sister. The girl was born at term after an uneventful pregnancy. Birth measurements were in the low normal range. Epilepsy started 1.5 hours postpartum; the girl showed partial seizures and complex partial seizures with secondary generalisation. Epilepsy was refractory to treatment with high seizure frequency and frequent status epilepticus. Interictal EEG revealed frequent centro-parietal epileptiform activity with sharp waves, isolated spikes as well as spike waves. Epileptic

Table 1 Summary of genetic and clinical findings

Family	Ethnic background	*SLC13A5* mutation (nucleotide level)	Predicted effect (protein level)	Allele frequency (ExAC)	Individual	Sex	Dental findings	Seizure onset	Neurological findings	Cognition	Brain MRI
A	Southern Europe (Italy)	c.997C>T hom	p.Arg333Ter	1.65×10^5	A II 1	M	*Both siblings*: small and cylindrical teeth, wide interdental spaces, marked yellowish to brownish discolouration, worn secondary premolars and molars	1st day	Infantile hypotonia, later spasticity, ataxia, dystonic gait, strabismus	Severe ID	Normal
					A II 3	F		7 months	Infantile hypotonia, later ataxic dystonic gait, Horner syndrome	Severe ID	Normal
B	The Caribbean (Martinique)/ Central Africa (Cameroon)	c.203C>A het c.434C>A het	p.Pro68Gln p.Thr145Lys	0.0 0.0	B II 1	F	Hypoplastic enamel of primary and secondary teeth, wide interdental spaces, yellowish surface, sharp and thin lower permanent incisors, worn primary molars, enamel chipping. Dental radiographs: no enamel visible on primary and secondary teeth	1st day	Spastic diplegia, pyramidal signs of the legs, cerebellar ataxia, strabismus	Severe ID	Normal
					B II 2	F	Primary molars lack enamel and have a yellow surface, opaque enamel of incisors, smooth surface	2 months	Pyramidal signs of the legs, cerebellar ataxia	Severe ID	Normal
C	Europe	c.103-1G>A het c.1276-1G>A het	p.? splicing error p.? splicing error	0.0 0.0	C II 1 C II 2	F M	*Both siblings*: marked delay in tooth eruption, small secondary incisors with cylindrical shape, sharp and thin incisal edges with brown discoloration, smooth and hard tooth surface. Dental radiographs: no enamel visible	1.5 months 1st day	Hypotonia, ataxia Hypotonia, ataxia	Severe ID Severe ID	Normal Normal
D	Central Europe (Germany)	c.425C>T het c.655G>A het	p.Thr142Met p.Gly219Arg	1.71×10^5 2.06×10^4	D II 2 D II 3 D II 4	F F M	*All three siblings*: widely spaced permanent teeth with cylindrical shape, pointed tips, worn premolars and molars; hard surfaces and slight yellow discoloration of teeth. Dental radiographs: no enamel visible	1st day 1st day 1st day	Coordination deficits Coordination deficits Ataxia	Moderate ID Moderate ID Severe ID	Normal Normal Normal
E	Western Europe (The Netherlands)	c.680C>T hom	p.Thr227Met	2.47×10^5	E II 1	F	opaque enamel of incisors, smooth surface, cylindrical shape, wide interdental spaces, worn primary molars, enamel chipping	1st day	Marked hypotonia	Severe global developmental delay	Mild atrophy of left hemisphere

ExAC, Exome Aggregation Consortium Browser (Beta) (accessed 06/2016); F, female; het, heterozygous; hom, homozygous; ID, intellectual disability; M, male.

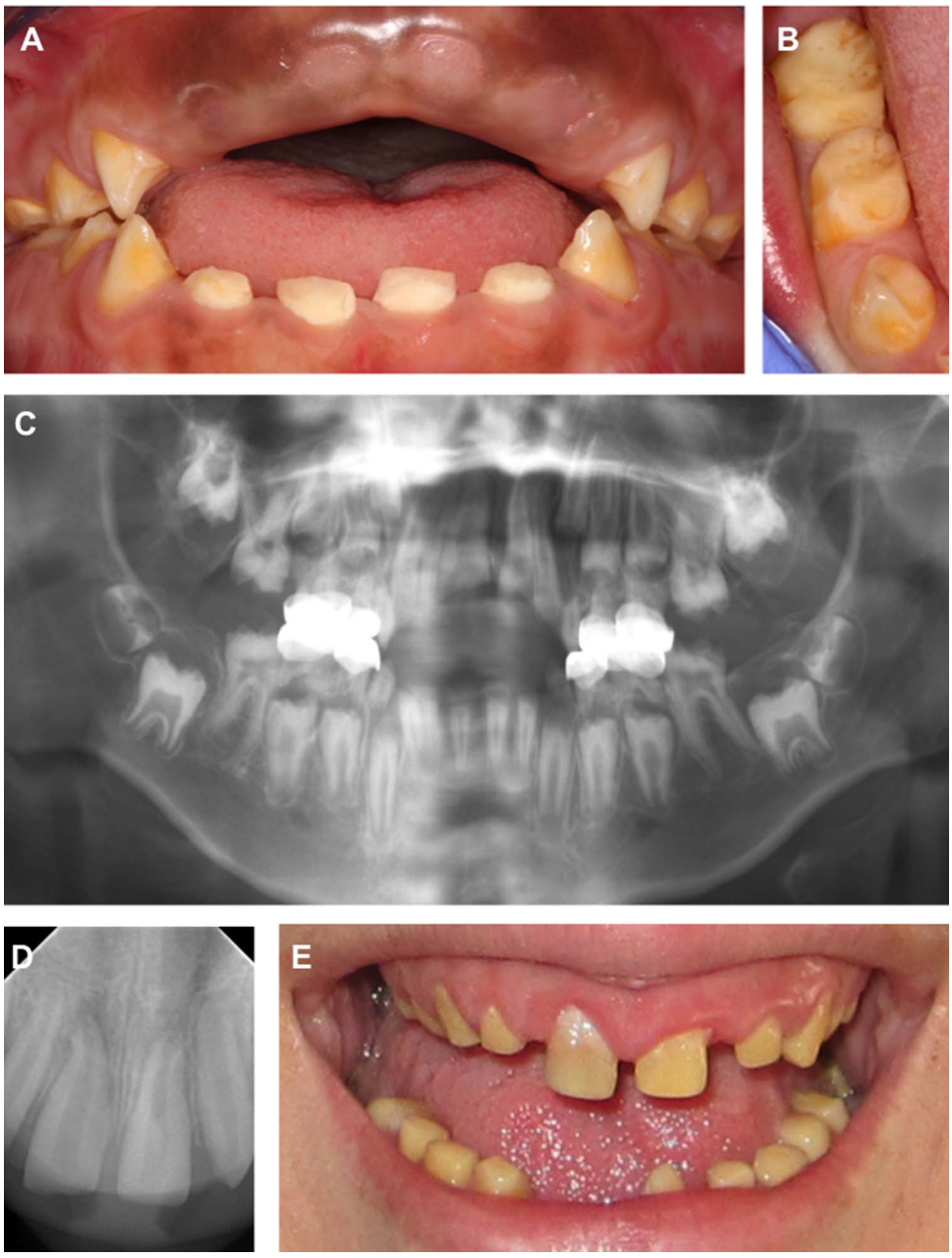

Figure 2 Intraoral photographs and radiographs of ***SLC13A5***-associated Kohlschütter–Tönz syndrome individuals of this study. (A and B) Intraoral photographs of individual B:II:1 with delayed eruption of permanent teeth at the age of 8.3 years. The first right lower permanent molar, 46 (normally erupting around 6 years of age), has not yet erupted. Enamel of primary and secondary teeth is hypoplastic with a yellowish surface. Lower permanent incisors are sharp and thin with opaque colour. (B) Primary molars are extremely worn, and enamel chipping has occurred. (C) Panoramic X-ray of B:II:1 at 9 years of age, and (D) X-ray of secondary upper incisors of D:II:4. No enamel is visible on the dental radiographs. Hyperplastic follicular sacs are present on unerupted second permanent molars. Stainless-steel paediatric crowns have been placed on primary molars to protect them from further wear. They appear very radio-opaque on the panoramic X-ray. (E) Clinical photograph of individual A:II:3 showing the cylindrical shape of permanent teeth due to reduction of the enamel thickness and intense yellow discoloration.

discharges with seizure correlate had different foci of onset and presented with high-voltage polyspike waves. Development was markedly delayed. At age 3, the girl showed satisfactory social contact but no volitional movements, had no head control and exhibited axial hypotonia. A brain MRI at age 11 months showed mild atrophy of the left hemisphere (see online supplementary figure S1).

Individual E:II:1 had a fully erupted primary dentition at the age of 3 years. The incisors had yellowish discolouration and a cylindrical shape. Primary molars were extremely worn, and enamel chipping had occurred.

Histological findings

The magnified photograph of tooth 48 of individual D:II:3 showed multiple pinpoint-sized pits, which were randomly distributed on the tooth surface (figure 3A). Histologically, the enamel layer was very thin (figure 3B). No lines of Retzius or enamel prisms were visible and the surface had small pits. These pits are a typical sign for AI of the hypoplastic type. The magnification of the histological section showed organic debris in the bottom of the pits, which was responsible for additional extrinsic staining (figure 3C). The dentin had a normal structure. The histological findings were compatible with the dental diagnosis hypoplastic AI (figure 3).

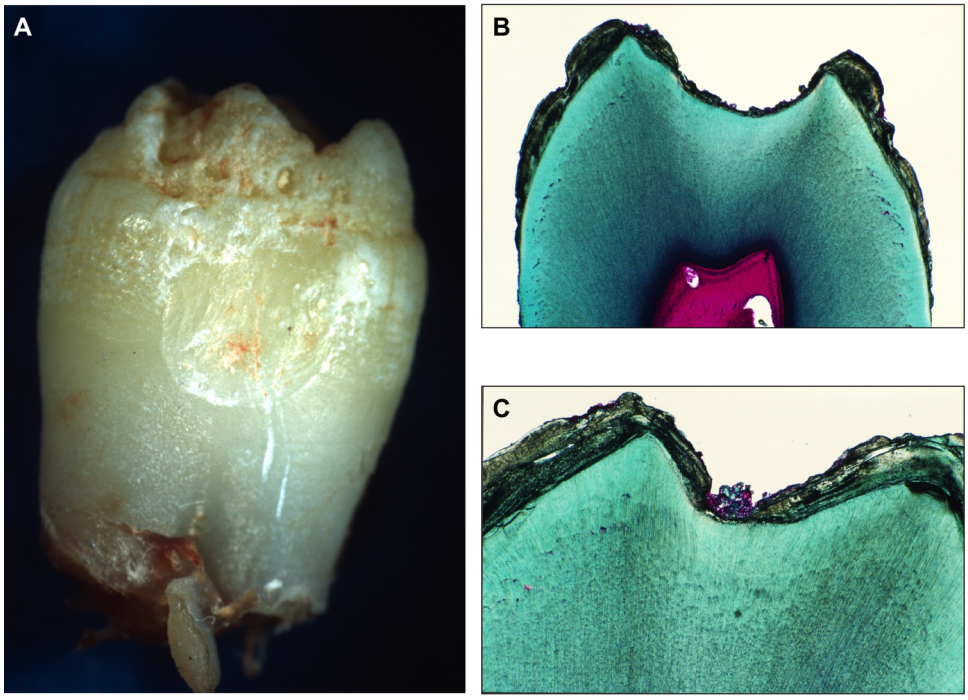

Figure 3 Amelogenesis imperfecta (AI) in *SLC13A5*-associated Kohlschütter–Tönz syndrome is of the hypoplastic type. An impacted wisdom tooth has been assessed from individual D:II:3. (A) The magnified photograph (1.6×) presents multiple pits on the tooth surface. These pits are a typical sign for AI of the hypoplastic type. The reduced thickness of the enamel cap causes an abnormal crown form. (B and C) The tooth was block stained with fuchsin, and after embedding in methyl methacrylate and perpendicular sectioning, the specimens were polished and contrast stained with acetic light green (original magnification 12–60×).[21] The enamel layer is thin, and no lines of Retzius or enamel prisms are visible. The dentin has a normal structure.

The magnified photograph of the partially erupted first molar assessed from an individual with *ROGDI*-associated KTZS showed dark yellow to brown discolouration with severe abfractions where the tooth had been exposed to the oral cavity (figure 4A). Histologically, the enamel presented with a countless number of cracks, prominent enamel tufts and lamellae, which passed from the enamel surface to the dentine–enamel junction (figure 4B, C). Lines of Retzius were missing and enamel prisms were grouped irregularly. Enamel thickness was only reduced in the erupted parts of the tooth where the ill-mineralised enamel could not withstand the abrasive forces during chewing. The dentin had a normal structure. The histological findings point to the diagnosis of hypocalcified AI in *ROGDI*-associated KTZS.

Genetic findings

Linkage analysis in family A showed three candidate regions on chromosomes 9p24.2, 10q21.3 and 17p13.1. A maximum LOD score of ≥2.39 was obtained for the region on chromosome 17, which encompassed more than 4 Mb containing 189 coding genes. Exome sequencing in this family revealed a homozygous non-sense mutation c.997C>T; p.Arg333Ter in *SLC13A5* located on 17p13.1 as the only candidate variant in the linkage regions. Candidate missense and splice site mutations in *SLC13A5* were also identified by exome and/or Sanger sequencing in families B–E (table 1). All mutations were predicted to be damaging by in silico prediction programmes mentioned above; segregation was compatible with autosomal-recessive inheritance in all families. Compound heterozygous mutations c.425C>T, p.Thr142Met and c.655G>A, p.Gly219Arg in family D were as described in Hardies *et al*.[17] The homozygous mutation c.680C>T, p.Thr227Met in family E had also been previously reported in compound heterozygous state with another mutation in unrelated individuals.[17 18] The splice site mutations c.103–1G>A and c.1276–1G>A in family C have been published in another patient during revision of this manuscript.[19] The predicted effect of mutation c.997C>T (in exon 7 out of 12 exons) in family A is a premature stop codon p. Arg333Ter and complete loss of protein function. This variant is listed twice in a heterozygous state in approximately 121 000 alleles of the ExAC database and once in approximately 7000 exomes of our in house database. The *SLC13A5* variants c.203C>A and c.434C>A detected in family B have not been listed in the databases assessed; they are expected to cause amino acid exchanges p.Pro68Gln and p.Thr145Lys, respectively, and are also predicted to cause substantial loss of protein function by in silico assessment.

Over the last 5 years, another 14 individuals with the clinical suspicion of KTZS were referred to our laboratory and showed no mutation in either *ROGDI* or *SLC13A5*. All these individuals were said to display seizures, intellectual disability and discolouration of the teeth; however, in most individuals information on the clinical features was scarce and we were thus unable to confirm the clinical diagnosis of KTZS in them.

DISCUSSION

Here, we report that *SLC13A5* is the second gene associated with the clinical diagnosis of KTZS, the combination of epileptic encephalopathy and AI. Biallelic mutations in *SLC13A5* have been previously reported as a cause of early-onset epileptic encephalopathy, listed as early-onset epileptic encephalopathy-25 (EIEE25) in OMIM (# 615905). Several affected individuals showed tooth hypoplasia and/or hypodontia,[17] but there has been no published systematic evaluation of the dental status in individuals with biallelic *SLC13A5* mutations.

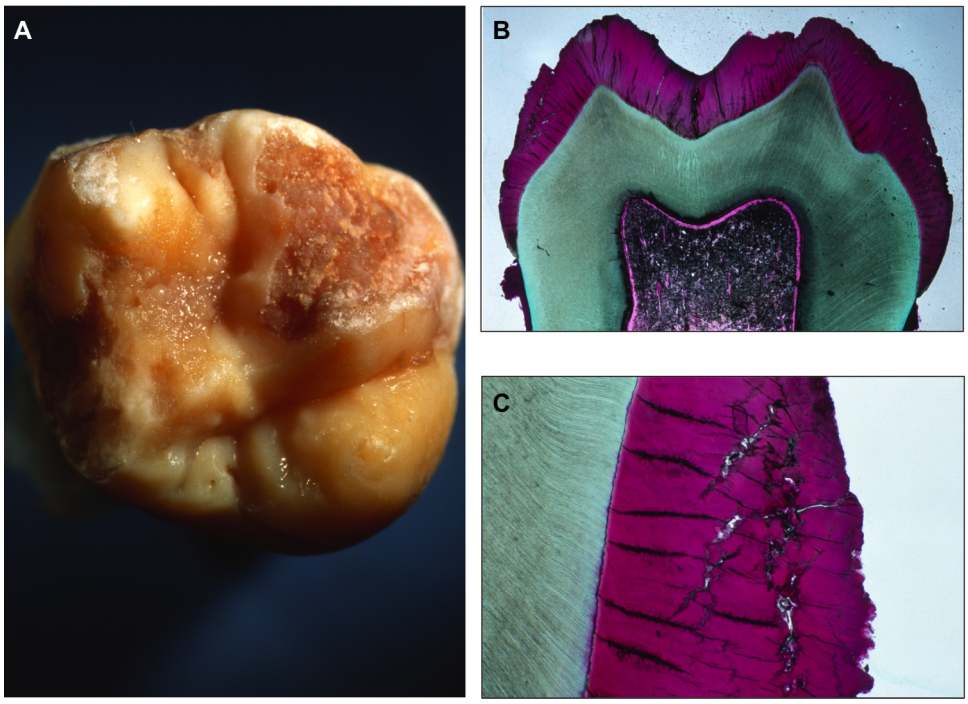

Figure 4 Amelogenesis imperfecta in *ROGDI*-associated Kohlschütter–Tönz syndrome is of the hypocalcified type. A partially erupted lower first molar has been assessed. (A) The magnified photograph (1.6×) presented a normal crown form with intense discolouration and enamel abfractions, where the crown had been exposed to the oral cavity. (B and C) The tooth was block stained with fuchsin, and after embedding in methyl methacrylate and perpendicular sectioning, the specimens were polished and contrast stained with acetic light green (original magnification 12–60×).[21] The enamel has a countless number of cracks, prominent enamel tufts and lamellae, which pass from the enamel surface to the dentin–enamel junction. Lines of Retzius are missing and enamel prisms are irregularly ordered. The dentin has a normal structure.

Both *SLC13A5* and *ROGDI* mutations cause epileptic encephalopathy and AI but show some clinical differences. In *ROGDI*-associated KTZS, the onset of seizures is rarely found in the neonatal period and may be as late as age 3 years. In contrast, 7 out of 10 individuals with *SLC13A5* mutations identified by us and all other previously published individuals presented with seizure onset in the first days of life.[17 18] The remaining three individuals in our cohort were diagnosed with epilepsy at age 6 weeks to 7 months. In *SLC13A5*-associated KTZS, the age of onset is independent from mutation type. Epilepsy is difficult to treat but tends to improve over time. The oldest affected individual in our study is aged 33 years (A:II:1); none of the individuals reported here have died of the disease to-date. Early infantile hypotonia was documented in most of our patients and developed into a mixed movement disorder. Developmental delay and intellectual disability were present in all affected individuals. Mild brain atrophy has been reported in some individuals with *ROGDI*-associated KTZS[16] but has not been shown so far in individuals with *SLC13A5* variants. MRI changes in individual E:II:1 in our study are thought to be postictal. Lack of consistent dysmorphic or specific laboratory signs—apart from dental abnormalities—is a common feature of KTZS caused by mutations in either *ROGDI* or *SLC13A5*.

Yellowish to orange discolouration of both deciduous and permanent teeth is a major clinical sign of KTZS. Enamel may become brown to black after eruption because of stains from food or beverages.[35] No report of a dental histological investigation of an individual with *ROGDI*-associated KTZS has been published before and classification has so far only been based on the clinical presentation. Donnai *et al* described the dental histology in one of two siblings with the clinical diagnosis of KTZS,[4] but *ROGDI* mutation analysis in these two siblings was negative.[13] Our present findings allow the histological differentiation of AI in *ROGDI*-associated KTZS and *SLC13A5*-associated KTZS. For *ROGDI*-associated KTZS, the dental diagnosis has to be revised to hypocalcified AI (figure 4). In the partially erupted first molar assessed from an individual with *ROGDI*-associated KTZS, the ill-mineralised enamel had normal thickness in non-erupted parts of the crown. When exposed to the oral cavity, the hypocalcified enamel was easily worn away or showed abfractions because it could not withstand the abrasive forces during chewing. Because of extreme teeth hypersensitivity, the child had to be fed through a stomach tube. Only after extraction of all erupted and partially erupted teeth, oral feeding was possible.

In contrast, AI in individuals with mutations in *SLC13A5* is of the hypoplastic type (figure 3). Hypoplastic AI is the result of a decreased amount of enamel matrix laid down during tooth formation.[35 36] As mineralisation is normal, the hardness of the remaining enamel layer is normal. However, the reduced thickness of the enamel cap causes an abnormal crown form with small and cylindrical-shaped teeth and wide interdental spaces. Incisors may be sharp and thin, teeth may be extremely worn and enamel chipping may occur. A typical sign of hypoplastic AI is multiple small pits, which are randomly distributed in the dentition and on the tooth surface, and can therefore not be attributed to a certain stage in tooth development.[37] Dental discolouration ranges from discrete to severe, dependent on additional extrinsic staining. Radiologically, no enamel or only a thin radioopaque line of enamel is visible. Delayed eruption has been noticed in two of the families reported here but may have eluded recognition in other individuals.

In both types of AI, exposed dentin may be temperature sensitive, and pits and cracks are predilection sites for caries. Hypersensitivity of teeth as well as high caries risk requires individual dental prophylaxis and attentive dental monitoring and management.

A wide variety of transporters in dental cells regulate processes such as pH regulation and calcium homeostasis, which are important for the biomineralisation and development of sound tooth structures.[38] *SLC13A5* encodes the sodium-coupled citrate transporter NaCT, which is mainly present at the plasma membrane and mediates the cellular uptake of citrate.[39] Previous investigations showed reduced citrate uptake in cell lines with biallelic *SLC13A5* mutations.[17 19] The citrate content of enamel is important during biomineralisation in view of its capacity to chelate calcium ions.[40] *SLC13A5* expression has been detected from

embryonic day 14 in mouse cap stage tooth prior to ameloblasts differentiation and amelogenesis.[41] In two independent gene expression profile studies, *SLC13A5* showed increased expression during amelogenesis; in particular, *SLC13A5* expression was increased in enamel matrix secreting ameloblasts.[42 43] Therefore, hypoplastic AI in *SLC13A5*-associated KTZS is probably a result of the disturbance of citrate homeostasis, which impairs enamel mineralisation and deprives ameloblasts of citrate necessary for energy metabolism. The identification of *SLC13A5* variants in KTZS opens the possibility of a therapy for this subtype of the disease, aimed at increasing intracellular citrate concentration, such as ketogenic diet or triheptanoin treatment.[17 18]

'*SLC13A5*-associated KTZS' as a disease entity is not regarded as different from the previously described "early-onset epileptic encephalopathy-25". Highlighting the dental involvement with the term "KTZS" is important for correct clinical diagnosis and management of affected children: Dental pathologies are sometimes difficult to recognise in individuals with severe intellectual disability but can have a major impact on quality of life due to hypersensitivity of teeth and impaired functionality of chewing. Structural tooth abnormalities with discolourations are often wrongly attributed to poor oral hygiene, with the potential of social stigmatisation. The observation that *SLC13A5* is the second major gene associated with KTZS highlights the need for detailed clinical including dental examination.

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