

A missense variant in LAMB3 underlies variable expressivity in familial tooth agenesis and microdontia



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Abstract

Background Tooth agenesis is a frequent developmental anomaly with both syndromic and non-syndromic presentation. The LAMB3 gene, typically associated to junctional epidermolysis bullosa, has additionally been implicated in isolated dental phenotypes.

Methods We report a three-generation family with non-syndromic tooth anomalies, including oligodontia, microdontia, and delayed eruption. Clinical-exome sequencing was performed on the proband and their parents (trio). Variants were filtered based on frequency, inheritance, and predicted impact. Segregation was confirmed by Sanger sequencing.

Results A heterozygous missense variant in LAMB3, c.547C>T, p.(Arg183Cys), was identified in the affected family members and was absent in the unaffected mother. The variant is rare and affects a conserved residue. In silico tools support its deleterious effect. Notably, the same variant was previously reported in homozygosity in another family with oligodontia. The phenotypic variability in our family underlines autosomal dominant inheritance with variable expressivity.

Conclusions This report highlights the role of heterozygous LAMB3 c.547C>T variant in non-syndromic tooth anomalies and expands its phenotypic spectrum. LAMB3 should be considered in the genetic evaluation of isolated tooth agenesis, particularly in families with intrafamilial variability.

KEYWORDS tooth agenesis, oligodontia, LAMB3 gene, variable expressivity

INTRODUCTION

Human odontogenesis is a tightly regulated developmental process that begins during embryogenesis and continues after birth through sequential and coordinated stages [Kurosaka et al., 2022]. Disruptions at any phase may result in congenital dental anomalies, among which tooth agenesis (TA) is the most common [Howe et al., 2022]. TA is defined as the congenital absence of one or more teeth, excluding third molars, and is classified as hypodontia, oligodontia, or anodontia according to the number of missing teeth [Vastardis, 2000]. Depending on severity, TA may require orthodontic and prosthetic management to preserve oral function, craniofacial growth, and aesthetics [Schroeder et al., 2022; Celli et al., 2018]. In affected children and adolescents, TA may also negatively impact psychological well-being and quality of life [Kotecha et al., 2013].

TA can occur as an isolated trait or as part of a syndromic condition associated with additional dental or systemic abnor-

malities [Fallea, 2025]. Genetic factors play a major role in its pathogenesis, although many isolated cases still lack identifiable pathogenic variants in known TA-associated genes [Costacurta et al., 2020]. Recent evidence also suggests that oligogenic inheritance and variable expressivity may contribute to the broad phenotypic spectrum observed in dental anomalies [Guo et al., 2025; Letra, 2022]. Consequently, broader approaches such as clinical exome sequencing may help identify novel candidate genes involved in tooth development [Modafferi et al., 2025].

Tooth morphogenesis relies on highly coordinated epithelial-mesenchymal interactions and extracellular matrix signalling. In this context, the epithelial basement membrane (BM) plays a crucial role in maintaining tooth germ organization and promoting ameloblast differentiation. Laminin-332, composed of the $\alpha 3$ (LAMA3), $\beta 3$ (LAMB3), and $\gamma 2$ (LAMC2) chains, is an essential BM component involved in epithelial adhesion and tissue organisation [Aumailley, 2013; McLean et al., 1993]. The LAMB3 gene, located on chromosome 1q32.2, encodes the $\beta 3$ chain of laminin-332 [Pulkkinen et al., 1995]. Biallelic pathogenic variants in LAMB3 are classically associated with junctional epidermolysis bullosa (JEB), which may include enamel defects among its clinical manifestations [Kiriti et al., 2013]. More recently, heterozygous LAMB3 variants have also been identified in patients with isolated amelogenesis imperfecta and other dental anomalies without skin involvement [Prasad et al., 2016], supporting an important role for LAMB3 in enamel formation and tooth development.

Here, we present the clinical and molecular findings from a family in which multiple members exhibit non-syndromic dental anomalies, including tooth agenesis, and delayed eruption. Clinical-exome sequencing identified a heterozygous missense variant in LAMB3, classified as likely pathogenic according to ACMG criteria [Richards et al., 2015]. Intriguingly, the same variant was independently identified in a second, unrelated family referred for genetic evaluation due to similar dental phenotypes [Du et al., 2018]. In both families, the variant co-segregated with the dental anomalies and was absent in unaffected relatives. Our findings contribute to the growing body of evidence linking LAMB3 pathogenic variants with isolated odontogenic defects and support the inclusion of LAMB3 in gene panels for the genetic diagnosis of dental developmental disorders. Furthermore, the identification of the same variant in two unrelated families strengthens the argument for a causative role

of this LAMB3 alteration in human tooth development. This report also raises important questions regarding the dosage sensitivity of LAMB3 in enamel formation and the possibility of incomplete penetrance or variable expressivity. By documenting these cases and reviewing the relevant literature, we aim to expand the phenotypic spectrum associated with LAMB3 variants and highlight the importance of considering epithelial basement membrane genes in the differential diagnosis of isolated dental anomalies. Given the significant impact of tooth agenesis and related dental anomalies on oral health, quality of life, and access to dental care, early identification of genetic causes may have implications for personalized treatment planning, anticipatory guidance, and genetic counseling.

MATERIALS AND METHODS

After signed informed consents, genomic DNA was extracted from peripheral blood samples of the four members of the family using standard protocols (Figure 1A). In order to identify potential pathogenic variants, Clinical-exome sequencing (CES) was performed on the proband and her parents (trio) using the capture-based NGS Clinical Exome Solution® v3 kit (SOPHiA Genetics, Saint-Sulpice, Switzerland), while sequencing was achieved using a paired-end read mode with a FastQ-only analysis workflow on the NextSeq550DX® NGS platform

(Illumina, San Diego, CA, USA). FastQ sequencing data were analyzed by Sophia DDM® platform v 7.3.0 (Sophia Genetics) to detect SNVs, indels, and Copy Number Variations (CNVs). Variants were filtered based on quality metrics, allele frequency (excluding those with a minor allele frequency >1% in public databases such as gnomAD) and predicted functional impact. Candidate variants were prioritized based on their relevance to dental development and inheritance patterns. The pathogenicity of the identified variants was evaluated using multiple in sil-

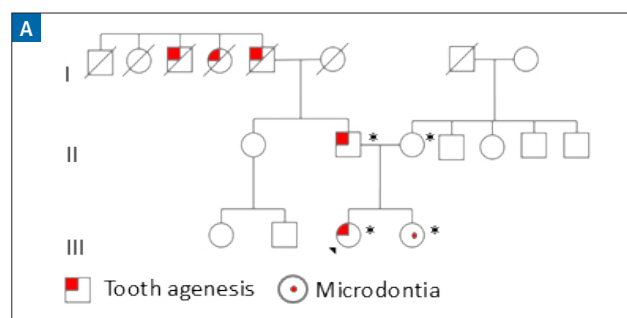
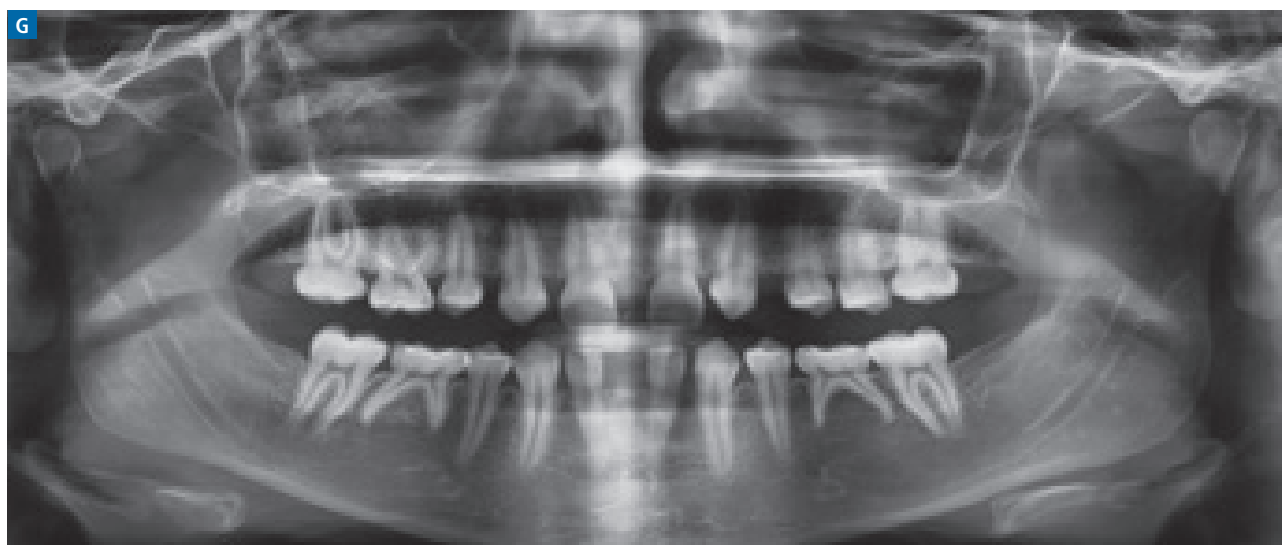
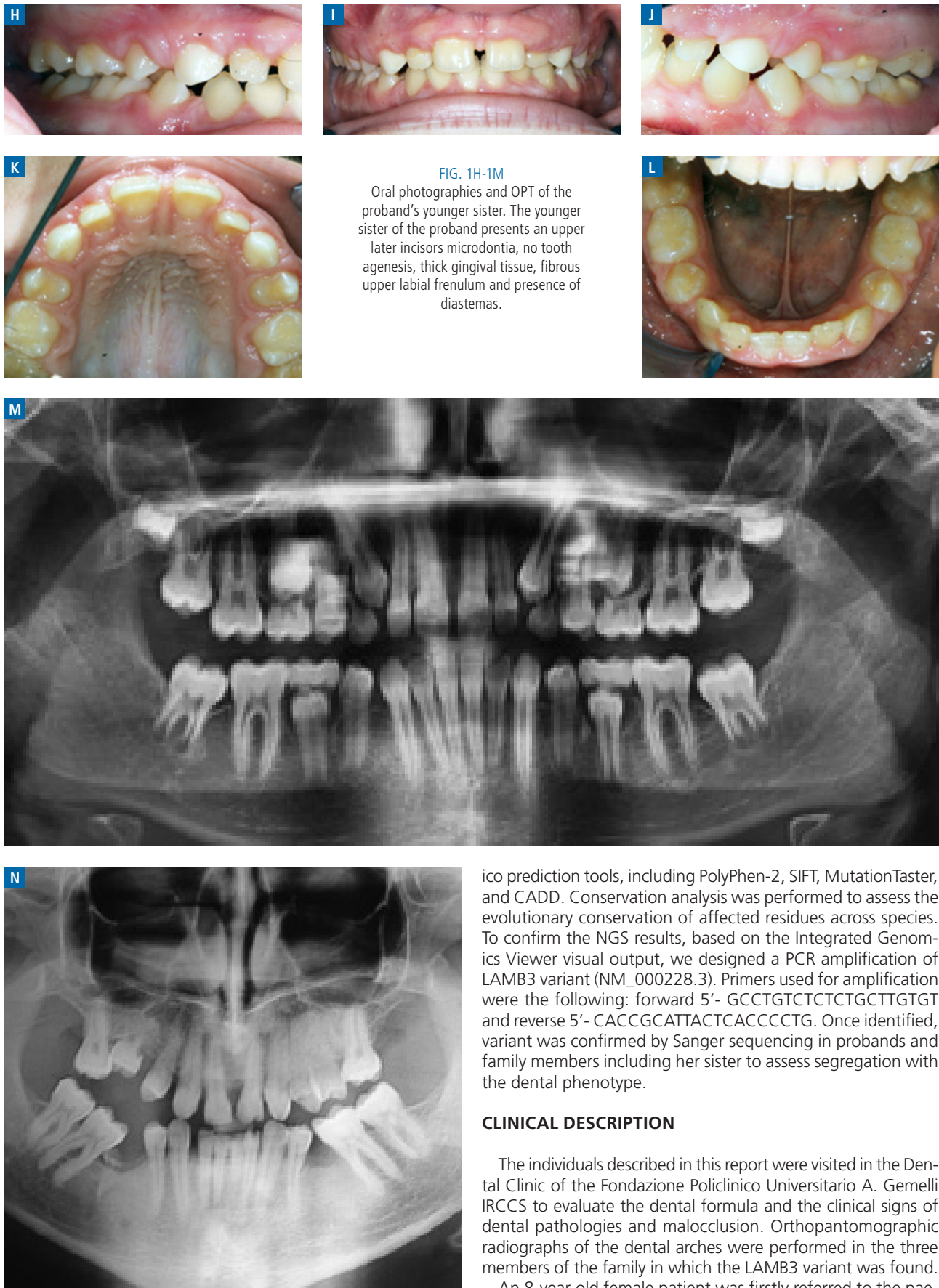


FIG. 1A Family tree. * indicates individuals who underwent molecular genetic testing



FIG. 1B-1G
Oral photographs and OPT of the proband. Note the agenesis of 12, 15, 17, 22, 27, 31, 35, 37, 41, 45, and 47 teeth, while teeth 16 and 26 are visible on the OPT.





ico prediction tools, including PolyPhen-2, SIFT, MutationTaster, and CADD. Conservation analysis was performed to assess the evolutionary conservation of affected residues across species. To confirm the NGS results, based on the Integrated Genomics Viewer visual output, we designed a PCR amplification of LAMB3 variant (NM_000228.3). Primers used for amplification were the following: forward 5'- GCCTGTCTCTCTGCTTGTGT and reverse 5'- CACCGCATTACTCACCCCTG. Once identified, variant was confirmed by Sanger sequencing in probands and family members including her sister to assess segregation with the dental phenotype.

CLINICAL DESCRIPTION

The individuals described in this report were visited in the Dental Clinic of the Fondazione Policlinico Universitario A. Gemelli IRCCS to evaluate the dental formula and the clinical signs of dental pathologies and malocclusion. Orthopantomographic radiographs of the dental arches were performed in the three members of the family in which the LAMB3 variant was found.

An 8-year-old female patient was firstly referred to the paediatrician for an apparent short stature that revealed familial.

FIG. 1N OPT of the proband's father presents multiple missing teeth.

During paediatric evaluation the proband has been brought to the attention of orthodontists due to tooth agenesis. The clinical examination revealed a symmetric face with a harmonic distribution of the facial thirds and an orthognathic profile. Intraoral assessment and orthopantomography confirmed the presence of multiple agenesis, affecting the following permanent teeth: 12, 15, 17, 22, 27, 31, 35, 37, 41, 45, and 47. A delay in the formation and eruption of permanent teeth was observed compared to chronological age, most marked in the upper first molars, whose roots were not yet formed. The change occurred only in the upper central incisors. In the lower arch, all deciduous teeth were present, while in the upper arch, only the deciduous canines and molars were present, while the lateral incisors were missing and there were diastemas. Additional findings included increased overbite, incisors' retro-inclination and enlarged upper labial frenulum with a large diastema between 11 and 21. The patient had an ovoid-shaped dental arch, the deciduous dentition showed a Class I molar relationship bilaterally and a Class II canine malocclusion on the right. The gingival tissues had a thick and fibrous appearance and covered part of the dental crowns (Figure 1B-G). In the proband, the panoramic radiograph and clinical photos were repeated at 8–10 and 15 years of age, to evaluate the evolution of the formation of the existing teeth.

Orthodontic management of the proband was planned in stages, at the beginning with a functional appliance aimed at promoting vertical growth of the maxillary alveolar processes and maintaining spaces during the mixed dentition phase. At 12 years of age, as her canines were erupting, she underwent a frenectomy, immediately followed by diastema closure with fixed orthodontic treatment on upper central incisors, a sectional orthodontic wire and elastic forces. Unfortunately, the outcome was unsatisfactory due to relapse. It was decided to wait until adulthood to replace the missing incisors with dentures.

The patient's younger sister, visited at 12 years of age, presented

dental abnormalities, notably upper lateral incisors microdontia, in the absence of agenesis, thick gingival tissue, fibrous upper labial frenulum and presence of diastemas (Figure 1H-M). Both siblings were otherwise healthy, with no systemic features or dermatological signs suggestive of syndromic conditions. Their father exhibited non-syndromic tooth agenesis, with multiple missing teeth and no mucocutaneous manifestations (Figure 1N). The paternal grandfather and one of his sister and his brother have been referred to have multiple dental agenesis. Unfortunately, it was not possible to include them in the genetic segregation study as they were deceased. This three-generation pedigree highlighted familial segregation of dental anomalies along the paternal lineage, supporting an autosomal dominant inheritance pattern with variable expressivity (Figure 1A). To identify the genetic basis of this phenotype, clinical-exome sequencing (CES) was performed on the trio. Bioinformatic filtering prioritized rare heterozygous coding variants shared among the affected individuals (proband and her father) and absent in the mother (Figure 2A). This approach led to the identification of a heterozygous missense variant in LAMB3: c.547C>T, resulting in a p.(Arg183Cys) substitution in the affected members of the trio (proband and her father) and absent in the unaffected mother. Segregation of the variant within the family was confirmed through Sanger sequencing, including proband's sister. This analysis demonstrated the presence of the variant also in the milder affected sister (Figure 2B).

This variant lies within exon 6 of the LAMB3 gene, which encodes the $\beta 3$ chain of laminin-332, essential component of the epithelial basement membrane. The arginine at position 183 is highly conserved across species, and its substitution with cysteine introduces a potentially disruptive sulfhydryl group, which could alter local protein structure or disulfide bonding (Figure 2C). The variant is rare (535/1614034 alleles; allele frequency of 0,03315%) and never reported in homozygous in gnomAD database V4.1.0 (but only from [Du et al., 2018]), and

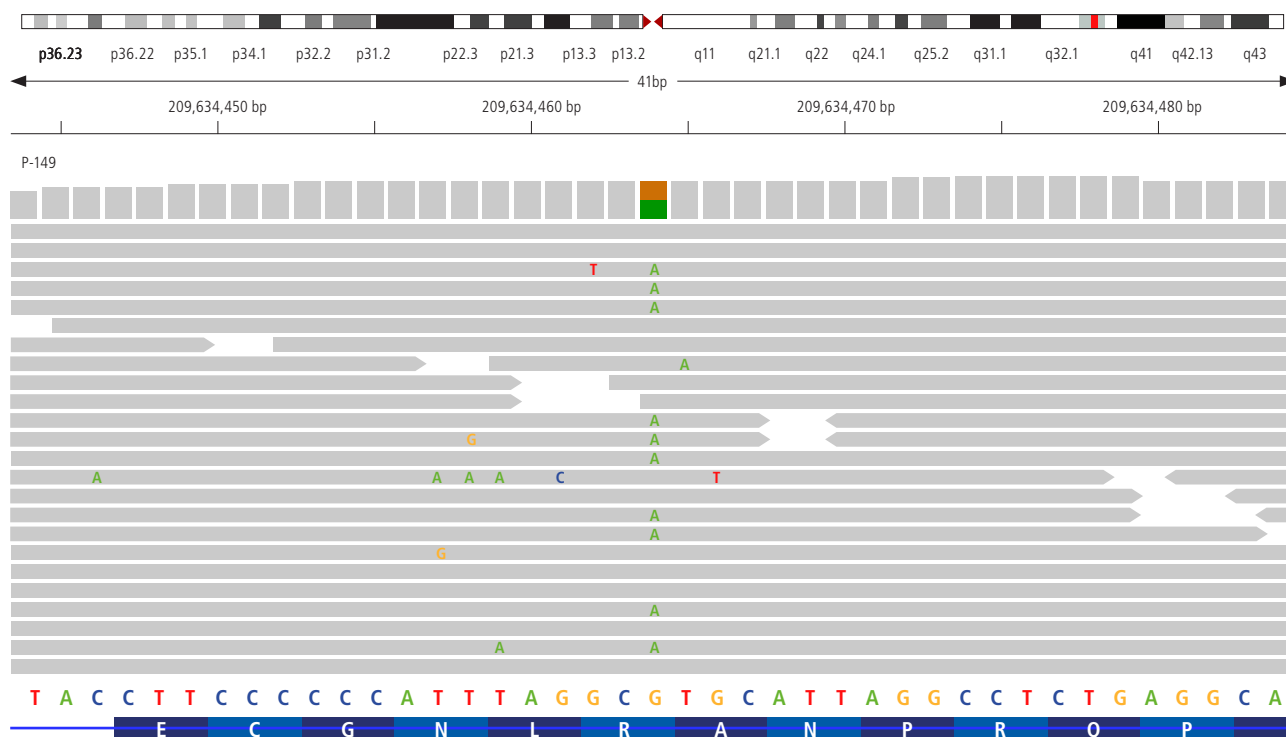


FIG. 2A IGV analysis of the proband variant found in CES. The variant is G>A in the template strand.

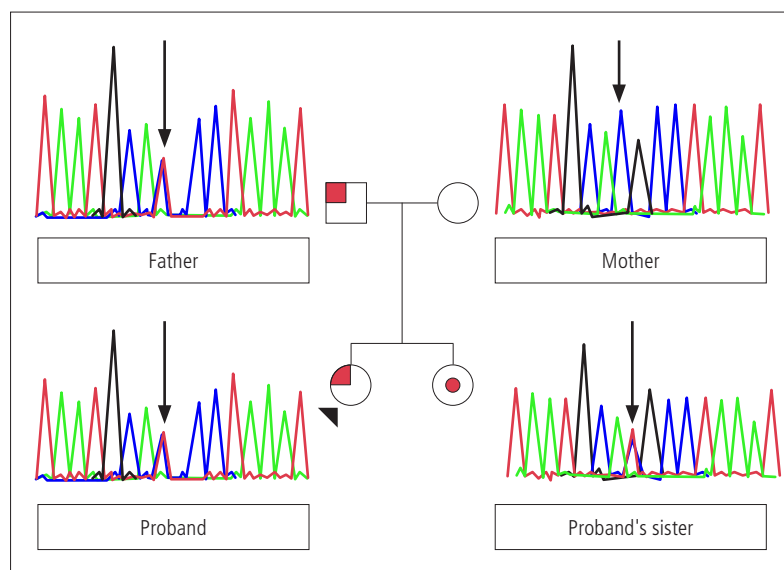


FIG. 2B Electropherogram of tract of exon 6 in which the C>T transition was analyzed in the four members of the proband's family (black arrow).

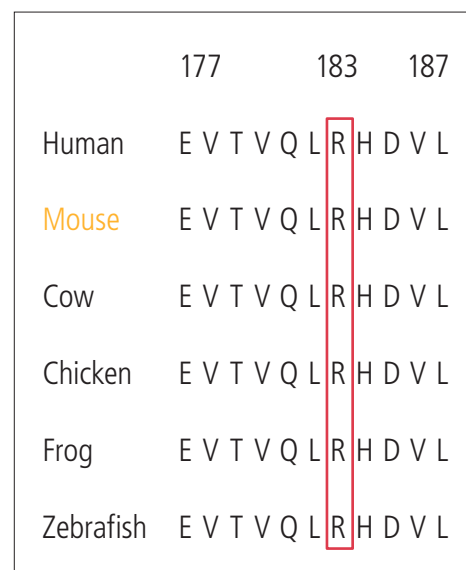


FIG. 2C Alignment of the residue 183 of the LAMB3 protein in different species. Note the conservation of this residue.

has not been previously reported in association with junctional epidermolysis bullosa or other syndromic phenotypes.

Multiple *in silico* prediction tools—including PolyPhen-2 (probably damaging), SIFT (deleterious), CADD (score 26.4), and GERP (score 4.57)—suggest its pathogenic effect. Its classification in ClinVar was conflicting. According to ACMG/AMP criteria, the variant was classified as likely pathogenic based on the following evidence: PS4 (variant frequency enriched in affected individuals), PM2 (absent in population databases), PP1 (segregation with disease), and PP3 (multiple lines of computational evidence).

DISCUSSION

Tooth agenesis is one of the most common congenital craniofacial anomalies, with a heterogeneous genetic basis encompassing both syndromic and non-syndromic forms. Variants in LAMB3 have historically been associated with autosomal recessive junctional epidermolysis bullosa (JEB) and autosomal dominant amelogenesis imperfecta (AI). More recently, heterozygous LAMB3 mutations have been implicated in isolated or familial forms of dental anomalies, including non-syndromic tooth agenesis and enamel hypoplasia [Nakano et al., 2002; Kim et al., 2013; Du et al., 2018].

The variant identified in our family, LAMB3 c.547C>T p.(Arg183Cys), lies within exon 6 and affects a conserved residue in the N-terminal domain of the laminin β 3 chain. This domain plays a key role in the structural assembly of laminin-332 and its interactions with epithelial cells during morphogenesis. *In silico* tools predict this missense change to be damaging (CADD score >20, GERP score 4.57), and the variant is rare from population databases such as gnomAD.

This same variant was previously reported by [Du et al., 2018] in a Turkish family, where it was identified in homozygosity in a child with oligodontia and mild maxillary hypoplasia. In our family, the variant is present in heterozygosity in three affected members: the proband with oligodontia, her sister with microdontia, and their father with multiple missing teeth. This observation supports a model of complex disease with autosomal dominant inheritance with variable expressivity, which has

already been observed in other families with LAMB3-related enamel anomalies [Kim et al., 2013].

The identification of LAMB3 c.547C>T (p.Arg183Cys) in three affected individuals from the same family - each exhibiting a distinct but related dental phenotype (oligodontia in the proband, microdontia in her sister, and agenesis in their father) - supports the hypothesis that this variant contributes to a spectrum of isolated dental anomalies. These findings emphasize the pleiotropic role of LAMB3 in human tooth development, even in the absence of cutaneous involvement. This case reinforces the need to include LAMB3 among the candidate genes considered in the diagnostic evaluation of non-syndromic dental agenesis and microdontia, especially when there is familial recurrence with variable phenotypic expressivity.

Laminin-332, composed of α 3, β 3, and γ 2 subunits, is crucial for epithelial basement membrane integrity. The β 3 chain encoded by LAMB3 is particularly important in hemidesmosome assembly and epithelial attachment during tooth morphogenesis. Defects in laminin-332 can impair ameloblast polarization and function, resulting in hypoplastic or hypomineralized enamel [Dong et al., 2023].

Mouse models have provided significant insight into the role of Lamb3 in tooth development. Lamb3 hypomorphic mice exhibit enamel defects, delayed eruption, and defective adhesion of ameloblasts to the basement membrane [Bailleul-Forrestier et al., 2008]. These phenotypes mirror the spectrum of dental anomalies observed in human patients, ranging from amelogenesis imperfecta to tooth agenesis and microdontia, often within the same pedigree [Dong et al., 2023].

Our findings align with other recent reports that expand the phenotypic spectrum of LAMB3-related conditions. In particular, the case described by Du and colleagues [Du et al., 2018], which shares the exact c.547C>T variant, highlights the possibility of a shared mutational mechanism underlying isolated oligodontia. The overlapping clinical features and co-segregation in both families strengthen the pathogenic link.

The intrafamilial variability observed in our case—oligodontia in the proband, microdontia in the sister, and agenesis in the father—suggests that additional factors such as modifier genes, environmental influences, or epigenetic changes may

modulate the phenotypic expression of LAMB3 variants [Dong et al., 2023]. This is consistent with the concept of mutational burden proposed in complex traits such as non-syndromic tooth agenesis [Modafferi et al., 2025].

From a diagnostic perspective, this case underscores the relevance of including LAMB3 in targeted gene panels for non-syndromic tooth anomalies. As demonstrated by Prasad and colleagues [Prasad et al., 2016], the diagnostic yield of targeted next-generation sequencing for orodental disorders can be significantly improved by expanding the included genes in the panel like LAMB3, even when cutaneous involvement is absent.

In conclusion, our report reinforces the clinical significance of heterozygous LAMB3 c.547C>T, p.(Arg183Cys) in non-syndromic dental anomalies. It highlights the importance of careful phenotyping, familial segregation, and molecular analysis in elucidating the variable expressivity and penetrance of variants in basement membrane genes traditionally associated with syndromic phenotypes. Further functional studies are warranted to clarify the molecular mechanisms underlying the pleiotropic dental manifestations observed in LAMB3-related conditions [Dong et al., 2023].

Author Contributions

GL and BN performed segregation analysis; PCo performed clinical exome sequencing; FMB, PC, CM and DP performed genetic clinical evaluation; ANR and CG performed oral clinical evaluation; CM, CG and ET wrote the paper; ANR, CM, PC and CG reviewed the paper.

Funding

Università Cattolica del Sacro Cuore contributed to the funding of this research project and its publication

Consent to Publish declaration

Parents provided their consent to publish information about themselves and their children.

Acknowledgements

We would thank the family for participating in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

- › Aumailley M. The laminin family. *Cell Adhesion & Migration*. 2013; 7(1), 48–55.
- › Bailleul-Forestier I, Molla M, Verloes A, Bernal A. The genetic basis of inherited anomalies of the teeth: Part 1: Clinical and molecular aspects of non-syndromic dental disorders. *European journal of medical genetics*. 2008; 51(4), 273–291.
- › Celli D, Manente A, Grippaudo C, Cordaro M. Interceptive treatment in ectodermal dysplasia using an innovative orthodontic/prosthetic modular appliance. A case report with 10- year follow-up. *Eur J Paediatr Dent*. 2018; 19(4):307–312.
- › Costacurta M, Di Renzo L, Pujia A, Tomietto M, Gualtieri P, Docimo R. Dental agenesis and evaluation of body composition through bioelectrical impedance analysis. Observational study on a sample of paediatric subjects. *Eur J Paediatr Dent*. 2020;21(2):143–147.
- › Dong J, Ruan W, Duan X. Molecular-based phenotype variations in amelogenesis imperfecta. *Oral Dis*. 2023;29(6):2334–2365.
- › Du R, Dinckan N, Song X, Coban-Akdemir Z, Jhangiani SN, Guven Y, Uygur ZO. Identification of likely pathogenic and known variants in TSPEAR, LAMB3, BCOR, and WNT10A in four Turkish families with tooth agenesis. *Human genetics*. 2018; 137(9), 689–703.
- › Fallea A, Vinci M, L'Episcopo S, Bartolone M, Musumeci A, Ragalmuto A, Cali F. Dissecting the Genetic Contribution of Tooth Agenesis. *International Journal of Molecular Sciences*. 2025; 26(21), 10485.
- › Guo, X, Xu T, Duan X. Genetic-environmental Risk Factors for Tooth Agenesis: A Familial Case-control Study in China. *International Dental Journal*. 2025; 75(5), 100956.
- › Howe BJ, Pendleton C, Withanage MHH, Childs CA, Zeng E, van Wijk A, Xie XJ. Tooth agenesis patterns in orofacial clefting using tooth agenesis code: a meta-analysis. *Dentistry journal*. 2022; 10(7): 128.
- › Kim JW, Seymen F, Lee KE, et al. Enamel defects and mutations in LAMB3. *J Dent Res*. 2013;92(10):995–1000. <https://doi.org/10.1177/0022034513504211>
- › Kiritsi D, Has C, Bruckner-Tuderman L. Laminin 332 in junctional epidermolysis bullosa. *Cell adhesion & migration*. 2013; 7(1), 135–141.
- › Kotecha S, Turner PJ, Dietrich T, Dhoptkar A. The impact of tooth agenesis on oral health-related quality of life in children. *Journal of Orthodontics*. 2013; 40(2), 122–129.
- › Kurosaka, H, Itoh S, Morita C, Tsujimoto T, Murata Y, Inubushi T, Yamashiro T. Development of dentition: From initiation to occlusion and related diseases. *Journal of Oral Biosciences*. 2022; 64(2):159–164.
- › Letra, A. Rethinking the genetic etiology of nonsyndromic tooth agenesis. *Current osteoporosis reports*. 2022; 20(6), 389–397.
- › McLean WHI, Pulkkinen L, Smith FJD, Rugg EL, Lane EB, Bullrich F, Uitto J. Loss of integrin alpha 6 beta 4 in junctional epidermolysis bullosa. *Nature Genetics*. 1993;16(3), 301–305.
- › Modafferi C, Tabolacci E, Grippaudo C, Chiurazzi P. Syndromic and Non-Syndromic Primary Failure of Tooth Eruption: A Genetic Overview. *Genes*. 2025;16(2), 147.
- › Nakano A, Lestringant GG, Paperna T, et al. Junctional epidermolysis bullosa in the Middle East: clinical and genetic studies in a series of consanguineous families. *J Am Acad Dermatol*. 2002; 46(4):510–516.
- › Prasad MK, Laouina S, El Alloussi M, Dollfus H, Bloch-Zupan A, "Amelogenesis imperfecta: 1 gene, several clinical entities?". *Archives of Oral Biology*. 2016; 64: 56–63.
- › Pulkkinen L, Gerecke DR, Christiano AM, Wagman DW, Burgeson RE, Uitto J. Cloning of the $\beta 3$ chain gene (LAMB3) of human laminin 5, a candidate gene in junctional epidermolysis bullosa. *Genomics*. 1995; 25(1), 192–198.
- › Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, ... & Rehm, HL. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine*. 2015; 17(5), 405–423.
- › Schroeder DK, Schroeder MA, Vasconcelos V. Agenesis of maxillary lateral incisors: diagnosis and treatment options. *Dent. Press J. Orthod*. 2022; 27(1), e22spe1.
- › Vastardis H. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. *Am J Orthod Dentofacial Orthop*. 2000; 117(6):650–6.