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SHORT COMMUNICATION

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Candidate gene screening for non-syndromic incisive tooth agenesis: preliminary findings

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Abstract

Dental agenesis is the congenital absence of one or more teeth resulting from disturbances during early dental development and is most often associated with genetic variants in odontogenesis-related genes. However, the specific variants associated with permanent incisor agenesis remain unknown. This study was designed as an exploratory pilot case–control genetic association study aimed at identifying the number of polymorphic variants in selected candidate genes potentially related to maxillary and mandibular lateral incisor agenesis. Salivary DNA was collected from eight individuals (both sexes) with non-syndromic agenesis of permanent lateral incisors and matched to eight controls without agenesis. Exome sequencing targeted genes previously implicated in dental agenesis, including MSH homeobox 1 (*MSX1*), Wnt family member 10A (*WNT10A*), Paired box 9 (*PAX9*), axis inhibition protein 2 (*AXIN2*), ectodysplasin A (*EDA*), sprouty RTK signaling antagonist 2 (*SPRY2*), transforming growth factor alpha (*TGFA*), *SPRY4*, and SPARC-related modular calcium binding 2 (*SMOC2*). Individuals with incisor agenesis exhibited 160.2% more variants than the control group. Significant differences in the number of polymorphic variants were observed in *AXIN2* ($p = 0.010$), *EDA* ($p = 0.007$), *SPRY2* ($p = 0.010$), *TGFA* ($p = 0.028$), *SPRY4* ($p = 0.025$), and *SMOC2* ($p = 0.007$), as well as in *MSX1* ($p = 0.023$), whereas no significant differences were found for *PAX9* ($p = 0.078$) and *WNT10A* ($p = 0.756$). Notably, *EDA*, *SPRY2*, and *SMOC2* showed the greatest increase in the number of variants in the affected individuals. Overall, a higher number of genetic variants in these genes was observed in individuals with incisor agenesis, which may suggest a potential role for these genes in lateral incisor agenesis; however, these findings require confirmation in larger, well-powered studies and functional validation of the identified variants.

Keywords

Agenesis; Genes; Genetic variants; Incisors; Polymorphisms

1. Introduction

Dental development is a highly complex process governed by coordinated interactions among multiple tissues and is tightly regulated by signaling molecules, transcription factors, and growth factors. Disruptions in any of these elements can lead to dental anomalies, affecting tooth shape, position, structure, number, and timing of eruption [1]. Dental agenesis refers to the congenital absence of one or more teeth due to failure of tooth development during the early stages of odontogenesis [2]. Agenesis may occur as part of a syndrome or as an isolated (non-syndromic) condition, with the diagnosis established through clinical and radiographic examination. Although environmental factors may contribute to the etiology of non-syndromic dental agenesis, the condition is primarily attributed to mutations in genes involved in tooth development [1–3].

Incisor agenesis specifically involves the absence of central and/or lateral incisors and can significantly affect facial aesthetics and oral function. In pediatric patients, the absence of lateral incisors is clinically important because it may alter craniofacial development, compromise arch symmetry and space maintenance, and influence orthodontic treatment planning during growth. The prevalence of dental agenesis, excluding third molars, varies widely across populations, likely reflecting racial and ethnic variation [1]. The reported prevalence rates range from 1.4% to 11.3% [4, 5]. The condition tends to be more common in females [6, 7], and the lateral incisors are the second most frequently affected teeth after the second premolars [6, 8, 9]. In Colombia, although the overall prevalence of dental agenesis remains unclear, available studies indicate that lateral incisors are the most commonly affected teeth, with a higher incidence in women [10].

Several genes have been implicated in odontogenesis and are associated with dental agenesis, including *PAX9* [11], *MSX1* [12], *WNT10A* [13, 14], and *EDA* [15]. Among these, *WNT10A*

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and *MSX1* have been extensively studied. *WNT10A*, a member of the Wingless/Integrated (WNT) family, is essential for the development of primary and permanent dentition. It participates in tooth morphogenesis by activating the canonical WNT signaling pathway, is expressed in enamel knots, and is associated with odontoblast differentiation [16]. *MSX1*, an Msh homeobox family gene, encodes a transcription factor critical for odontogenesis and regulates bone morphogenetic protein 4 (BMP4) expression. *BMP4*, a member of the transforming growth factor-β (TGF-β) family, plays a vital role during the cap and bud stages of tooth development and is crucial for epithelial-mesenchymal interactions during embryogenesis [17]. *PAX9* encodes a key transcription factor in the dental mesenchyme during morphogenesis, and its loss-of-function mutation arrests tooth development at an early stage [11]. In PAX9-null mouse models, tooth development is arrested at the bud stage, and hypoplastic or absent lower incisors and absence of third molars are observed [18]. Another gene involved in this process is *AXIN2*, which encodes a negative regulatory protein of Wnt/β [19]. Association studies have shown that variants and polymorphisms in *AXIN2* are significantly associated with tooth agenesis in patients with at least one missing incisor [20]. *EDA* encodes a Tumor Necrosis Factor (TNF)-like ligand involved in the EDA/ectodysplasin A receptor (EDAR)/nuclear factor kappa B (NF-κB) pathway, which is essential for the development of ectodermal organs, including teeth [21]. Mutations in *EDA* can cause non-syndromic tooth agenesis, with the most frequently missing teeth being central and lateral incisors (both deciduous and permanent), particularly the mandibular central incisors and maxillary lateral incisors [22]. *SPRY2* and *SPRY4* encode Sprouty proteins that modulate Fibroblast growth factor (FGF)/Receptor tyrosine kinase (RTK) signaling, and risk variants in these genes have been associated with increased susceptibility to maxillary lateral incisor agenesis [13]. Other genes, such as *TGFA* and *SMOC2*, have been proposed as modulators of non-syndromic tooth agenesis. However, current evidence suggests a more limited and less specific role in incisor agenesis than that of major loci, such as *MSX1*, *PAX9*, *EDA*, *AXIN2*, and *WNT10A* [23].

Despite these established associations, the specific mutations and genes directly responsible for incisor agenesis remain poorly defined, and the scientific evidence linking genetic alterations to this condition remains limited. Therefore, the objective of this study was to identify genes with the largest number of genetic variants potentially associated with maxillary and mandibular lateral incisor agenesis in the Colombian population.

2. Materials and methods

2.1 Sample collection and DNA extraction

This exploratory pilot case–control genetic association study aimed to quantify polymorphic variants in candidate genes potentially associated with lateral incisor agenesis, initially focusing on *WNT10A* and *MSX1*, and subsequently extending the analysis to *PAX9*, *AXIN2*, *EDA*, *SPRY2*, *SPRY4*, *TGFA*, and *SMOC2* genes.

Dental agenesis was diagnosed using a standardized protocol that integrated dental history, intraoral clinical examination, and radiographic assessment. Detailed information was obtained through structured interviews with the patients and/or their legal guardians to exclude prior tooth extraction or trauma as potential causes of tooth loss. This evaluation was complemented by the analysis of panoramic radiographs, which confirmed the congenital absence of the affected teeth and ruled out other conditions such as impaction or delayed eruption.

DNA was extracted from saliva samples collected from eight individuals (males and females) with non-syndromic agenesis of the permanent maxillary or mandibular lateral incisors. The control group comprised eight individuals without dental agenesis, frequency-matched for age, sex, and ancestry. Participants were recruited from dental and orthodontic patients at the Dental School of the Universidad Nacional de Colombia. Informed consent and/or assent were obtained from all participants and their legal guardians. The study protocol was approved by the institutional ethics committee (Act B.CIEFO-244-2024).

Given the pilot nature of this investigation, a convenience sampling strategy was used. The inclusion criteria for the agenesis group were individuals older than 7 years with at least one congenitally missing maxillary or mandibular lateral incisor. The exclusion criteria were agenesis of more than six teeth and the presence or suspicion of a syndromic diagnosis.

Saliva samples were collected using Oragene-DNA saliva collection kits (OGD-500-L240315, DNA Genotek, Ottawa, ON, Canada). DNA was extracted from 200 μL aliquots of saliva using the QIAamp DNA Blood Mini Kit (163042987, Qiagen, Germantown, MD, USA) following the manufacturer's protocol. DNA concentration was quantified by measuring the absorbance at 260 nm, and purity was assessed using the 260/280 nm ratio with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

2.2 Exome sequencing and data processing

Whole-exome sequencing, a next-generation sequencing (NGS) approach that targets the coding regions of the genome, was employed to identify genetic variants associated with lateral incisor agenesis. Sequencing libraries were prepared using the Twist Library Preparation EF kit, and paired-end exome sequencing was performed on an Illumina platform (Macrogen, Seoul, South Korea). The quality of the raw sequencing data was assessed using FastQC v0.11.9.

Raw reads were aligned to the human reference genome GRCh38 (GCF_000001405.4) using BWA v0.7.17 [24]. The resulting Binary Alignment Map (BAM) files were sorted using SAMtools v1.13 [25], and duplicate reads were marked and removed using Picard MarkDuplicates (Broad Institute). To improve variant-calling accuracy, base quality score recalibration (BQSR) was performed following the GATK Best Practices using GATK v4.5.0 [26]. Variants were called with GATK HaplotypeCaller to generate Variant Call Format (VCF) files, and functional annotation and effect prediction were performed with SnpEff [27].

Downstream analyses focused on single-nucleotide variants

(SNVs) and small insertions/deletions (indels) located within the coding regions and canonical splice sites of the candidate genes. Variants were filtered using standard quality metrics, retaining only sites with a minimum read depth of $\geq 10\times$, genotype quality ≥ 20, and an allele balance compatible with heterozygous (approximately 0.3–0.7) or homozygous calls, following standard practices for exome variant filtering. To enrich for potentially functional changes, synonymous variants were retained only for descriptive purposes, whereas the primary analysis focused on variants predicted to have moderate or high impact (missense, nonsense, splice-site, or frameshift changes) according to SnpEff functional impact categories.

Population allele frequency information from public databases was used to flag common polymorphisms, and rare variants were highlighted but not excluded a priori, given the exploratory nature and small sample size of this study. Finally, variants within *EDA*, *SMOC2*, and *SPRY2* were extracted from the full variant dataset using AWK scripts based on reference identifiers (rsIDs), chromosomal coordinates, and nucleotide changes for gene-level counting and comparison between groups. Variant counts were calculated at the individual level for each gene by determining the number of variants predicted to have a moderate or high impact on every gene per subject and then averaging these counts across individuals within each group for statistical comparison. Given the relatively small sample size, the statistical power to detect differences between groups was limited, and the risk of type I error (false-positive findings) was increased; therefore, the observed associations should be interpreted with caution.

2.3 Statistical analysis

The primary outcome for statistical comparison was the number of polymorphic variants per gene in the cases versus controls. The normality of the data distribution was assessed using the Shapiro-Wilk test. Given the small sample size and non-normal distribution of variant counts, differences between groups were evaluated using the Wilcoxon rank-sum test.

To provide a measure of effect size beyond *p*-values, we calculated a standardized effect size (r) for the Wilcoxon test, calculated as the z-statistic divided by the square root of the total sample size, as recommended for non-parametric tests. Effect sizes were interpreted as small ($r \approx 0.10$–0.29), moderate ($r \approx 0.30$–0.49), or large ($r \geq 0.50$) to aid in assessing the magnitude and potential biological relevance of the observed differences, particularly in the context of limited statistical power.

Given the multiple comparisons across candidate genes, both unadjusted *p*-values and *p*-values corrected for multiple testing were calculated. For a conservative assessment of the family-wise error rate, Bonferroni-adjusted *p*-values were computed based on the number of tests performed, and, in parallel, the Benjamini–Hochberg false discovery rate (FDR) procedure was applied to control the expected proportion of false positives among significant findings.

Considering the small sample size (eight cases and eight controls), which inherently limits statistical power and may inflate effect size estimates in pilot genetic studies, all results were interpreted cautiously and in the context of prior biological evidence regarding the role of these genes in odontogenesis and dental agenesis. All analyses were performed using GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, MA, USA) and IBM SPSS Statistics version 27 (IBM SPSS Statistics, Chicago, IL, USA).

3. Results

3.1 Demographic characteristics

High-quality genomic DNA was successfully extracted from saliva samples collected from eight patients with lateral incisor agenesis included in this study. The cohort comprised five children and three adults, with ages ranging from 9 to 47 years, and included five males and three females. The most common agenesis pattern was bilateral absence of the maxillary lateral incisors (teeth 12 and 22), observed in 50% of the participants. Assessment of the quantity and quality of the extracted salivary DNA revealed concentrations, 260/280 ratios, and spectrophotometric profiles consistent with high-quality nucleic acids suitable for downstream genetic analyses [28] (Table 1).

3.2 Genetic variants found in individuals with incisor agenesis

The initial analysis focused on determining the total number of genetic variants in individuals with lateral incisor agenesis compared with controls without agenesis. As presented in Table 2, individuals with lateral incisor agenesis harbored a mean of 418.652 ± 70.021 variants, whereas the control group had a mean of 160.903 ± 25.293 variants (Table 2). Overall, individuals with lateral incisor agenesis harbored more variants across the candidate genes than did the controls (160.2% increase; $r = 0.60$, $p = 0.007$; pFDR = 0.028), indicating a large effect size, despite the limited sample size.

Because the genes most frequently associated with dental agenesis are *WNT10A* and *MSX1*, the number of genetic variants in these two genes was initially compared between individuals with lateral incisor agenesis and those in the control group. As shown in Fig. 1, a significantly higher number of variants in *MSX1* was observed in individuals with agenesis than in controls ($r = 0.50$, $p = 0.023$, pFDR = 0.070), whereas the difference in the number of variants in *WNT10A* was not significant ($r = 0.05$, $p = 0.756$, pFDR = 0.80).

Given the observed heterogeneity in the number of variants identified in *MSX1* and *WNT10A* among individuals with agenesis, we extended our analysis to include additional genes previously implicated in dental agenesis. The genes evaluated in this extended analysis were *PAX9*, *AXIN2*, *EDA*, *SPRY2*, *TGFA*, *SPRY4*, and *SMOC2* (Fig. 2). At the single-gene level, *AXIN2* ($r = 0.55$, $p = 0.010$; pFDR = 0.050), *EDA* ($r = 0.62$, $p = 0.007$; pFDR = 0.040), *SPRY2* ($r = 0.48$, $p = 0.010$; pFDR = 0.065), TGFA ($r = 0.45$, $p = 0.028$; pFDR = 0.080), *SPRY4* ($r = 0.47$, $p = 0.025$; pFDR = 0.075), *SMOC2* ($r = 0.60$, $p = 0.007$; pFDR = 0.040), and *MSX1* ($r = 0.50$, $p = 0.023$; pFDR = 0.070) showed moderate-to-large effect sizes and nominally significant differences in variant counts between the cases and controls. In contrast, *PAX9* ($r = 0.30$, $p = 0.078$; pFDR = 0.150) and *WNT10A* ($r = 0.05$, $p = 0.756$;

TABLE 1. General characteristics of individuals with incisor agenesis evaluated in this study.

Patient identification number	Age (yr)	Sex	Type of agenesis
AG 1	11	M	Upper right lateral incisor (12) and Upper left lateral incisor (22)
AG 2	16	F	Upper left lateral incisor (22)
AG 3	20	F	Upper right lateral incisor (12) and Upper left lateral incisor (22)
AG 4	47	M	Upper right lateral incisor (12) and Upper left lateral incisor (22)
AG 5	24	M	Upper right lateral incisor (12) and Upper left lateral incisor (22)
AG 6	14	F	Lower right lateral incisor (42)
AG 7	15	M	Lower right lateral incisor (42)
AG 8	9	M	Upper right lateral incisor (12)

AG: Agenesis; M: male; F: female.

TABLE 2. Total genetic variants found in individuals with and without incisor agenesis.

Sample	Number of Readings	Total Variants (SNPs)	Mean of variants (S.D)
AG 1	40,494,790	423,795	418.652 ± 70.021
AG 2	41,720,550	505,935	
AG 3	45,267,690	273,249	
AG 4	46,665,802	368,737	
AG 5	46,099,858	323,602	
AG 6	42,802,318	477,471	
AG 7	56,305,822	447,736	
AG 8	44,924,294	528,688	
Control 1	45,400,000	132,383	160.903 ± 25.293
Control 2	78,055,021	159,056	
Control 3	47,805,660	188,681	
Control 4	49,254,007	197,900	
Control 5	52,776,108	145,343	
Control 6	43,436,780	158,977	
Control 7	50,103,223	176,233	
Control 8	60,834,523	128,654	

AG: Agenesis; SNPs: Single-nucleotide polymorphisms; S.D: Standard deviation.

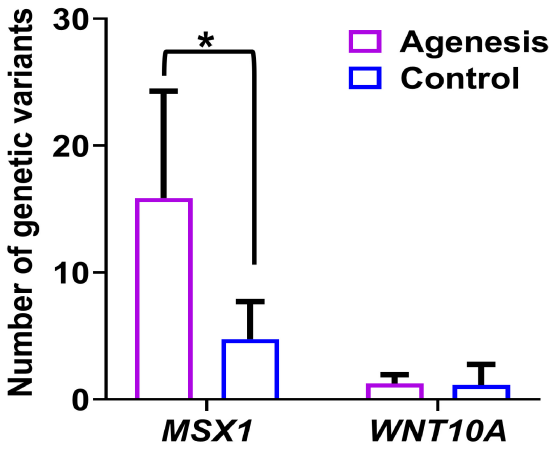


FIGURE 1. Number of genetic variants found in the *MSX1* and *WNT10A* genes of individuals with and without incisor agenesis. The data correspond to the average number of genetic variants in individuals with incisor agenesis compared with individuals without agenesis (Control). *$p < 0.05$. *MSX1*: MSH homeobox 1; *WNT10A*: Wnt family member 10A.

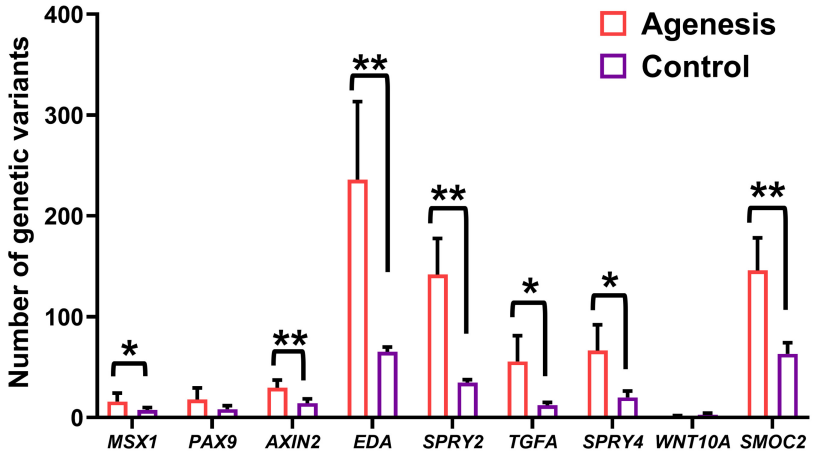


FIGURE 2. Number of genetic variants found in different genes potentially associated with incisor agenesis. The data correspond to the average number of genetic variants in individuals with incisor agenesis compared with individuals without agenesis (Control). $*p < 0.05$, $**p < 0.01$. *MSX1*: MSH homeobox 1; *WNT10A*: Wnt family member 10A; *PAX9*: Paired box 9; *AXIN2*: Axis inhibition protein 2; *EDA*: Ectodysplasin A; *SPRY2*: Sprouty RTK signaling antagonist 2; *SPRY4*: Sprouty RTK signaling antagonist 4; *TGFA*: Transforming growth factor alpha; *SMOC2*: SPARC related modular calcium binding 2.

pFDR = 0.800) displayed small or negligible effects with non-significant *p*-values. Notably, *EDA*, *SPRY2*, and *SMOC2* exhibited the largest differences and the lowest FDR-adjusted *p*-values (pFDR ≈ 0.04) (Fig. 2).

Based on these results, we performed a targeted analysis of specific polymorphisms within *EDA*, *SPRY2*, and *SMOC2*, which have previously been reported to be associated with dental agenesis [13, 17, 29–32]. The polymorphisms selected for this analysis are presented in Table 3.

TABLE 3. Polymorphisms analyzed for the *EDA*, *SMOC2* and *SPRY2* genes.

Gene	Identification number	Location	SNP
EDA			
	rs1160315	chromosome X	C>T
	rs2428151	chromosome X	A>C A>G G>A
	rs2520378	chromosome X	G>C G>T
	rs12853659	chromosome X	C>T
SMOC2	rs150983299	chromosome 6	C>T
SPRY2	rs504122	chromosome 13	G>A

EDA: Ectodysplasin A; SMOC2: SPARC-related modular calcium binding 2; SPRY2: Sprouty RTK signaling antagonist 2; SNP: Single-nucleotide polymorphism.

This targeted analysis revealed that the *EDA* gene had the greatest number of variants, followed by *SMOC2*, with both genes showing significantly more variants in individuals with agenesis than in the control group, whereas *SPRY2* showed fewer variants than *EDA* and *SMOC2* (Fig. 3).

4. Discussion

Dental agenesis affecting the anterior region, particularly the incisors, significantly impacts affected individuals, especially from an aesthetic perspective. Accordingly, clarifying the underlying causes and identifying the factors that increase susceptibility to this condition are of considerable clinical and biological importance. This study aimed to identify genetic variants potentially associated with lateral incisor agenesis. Comparative analysis showed that affected individuals carried a greater number of genetic variants than the controls without incisor agenesis (Table 2).

Previous research has established that *WNT10a* and *MSX1* play crucial roles in tooth development, with numerous studies demonstrating an association between variants in these genes and dental agenesis. For example, Keskin *et al.* [17] (2022) reported that alterations in *MSX1* are primarily linked to agenesis of the second premolars. Alves-Ferreira *et al.* [13] (2014) found WNT10A to be a significant genetic risk factor for maxillary lateral incisor agenesis (MLIA), with certain variants in this gene increasing susceptibility to maxillary lateral incisor agenesis. WNT10A was found to be a significant genetic risk factor for MLIA, with some variants in this gene increasing susceptibility to maxillary lateral incisor agenesis. Similarly, Keskin *et al.* [17] (2022) identified the p.R232W variant in *WNT10A* in a patient with tooth agenesis, suggesting a potential role for *WNT10A* variants in this condition; however, the specific association of these variants with incisor agenesis remains unclear.

Despite the well-established association between *MSX1* and *WNT10a* variants and tooth agenesis, our current study identified only a low frequency of variants in these genes (Fig. 2). This finding may reflect the possibility that variants in these genes are more commonly associated with posterior than anterior tooth agenesis, as previously reported [33, 34].

To capture the broader genetic architecture of lateral incisor agenesis, we expanded our analysis to include additional candidate genes, specifically *PAX9*, *AXIN2*, *EDA*, *SPRY2*, *TGFA*,

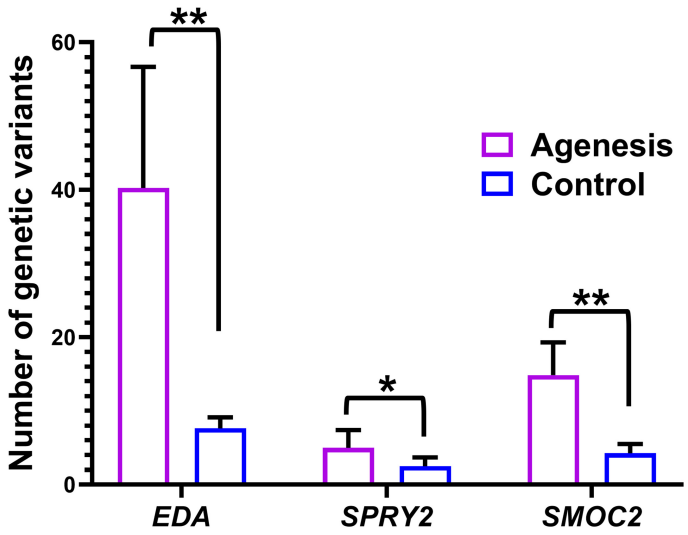


FIGURE 3. Number of variants for *EDA, SPRY2* and *SMOC2* genes in individuals with incisor agenesis and controls. *$p < 0.05$, **$p < 0.01$. *EDA*: Ectodysplasin A; *SMOC2*: SPARC-related modular calcium binding 2; *SPRY2*: Sprouty RTK signaling antagonist 2.

SPRY4, and *SMOC2*, as illustrated in Fig. 2. Among these genes, *EDA*, *SPRY2*, and *SMOC2* exhibited the highest number of variants, with *EDA* harboring the most affected gene in patients with lateral incisor agenesis. Therefore, we focused on specific variants within these genes that have been previously implicated in dental agenesis [13, 17].

The *EDA* gene encodes a type II transmembrane protein with a C-terminal TNF homology domain, which is predicted to form of ten antiparallel β-sheets connected by variable loops. *EDA* is critical for the development of ectodermal derivatives, including teeth, primarily through its interaction with the receptors EDAR and EDA2R [21]. Variants in *EDA* have been associated with X-linked hypohidrotic ectodermal dysplasia and non-syndromic agenesis of incisors [17]. Multiple studies have identified *EDA* variants as significant contributors to non-syndromic oligodontia, particularly during incisor development. Notably, the Thr338Met variant in EDA has been linked to the congenital absence of maxillary and mandibular central and lateral incisors and canines. This mutation affects the TNF homology domain, which is essential for homotrimer formation and receptor interaction at the monomer-monomer interface. Individuals with *EDA* mutations often exhibit distinctive patterns of tooth agenesis, including the absence of central and lateral incisors in both primary and permanent dentitions. Some cases also involve congenitally missing maxillary and mandibular canines, a rare occurrence in isolated oligodontia associated with genetic defects [21]. In a South American population, Alves-Ferreira *et al.* [13] (2014) found that although four *EDA* allelic associations (rs1160315, rs2428151, rs2520378, and rs12853659) were not statistically significant, the gene was nonetheless associated with increased susceptibility to incisor agenesis, consistent with our findings. Other studies, such as Su *et al.* [35] (2024), have linked *EDA* alterations to the agenesis of primary incisors, while our data and other reports suggest a relationship with permanent incisor agenesis.

The sprouty (SPRY) genes encode intracellular inhibitors of receptor tyrosine kinase (RTK) signaling pathways, particularly those activated by fibroblast growth factors (FGFs). *SPRY2* has recently emerged as an important factor in tooth development, and polymorphisms in this gene have been associated with agenesis. *SPRY2* regulates FGF and Sonic Hedgehog (Shh) signaling pathways, both of which are essential for tooth morphogenesis (NCBI). Alves-Ferreira *et al.* [13] (2014) reported that the allele frequencies of *SPRY2* SNPs (rs504122) showed a trend toward an increased risk of incisor agenesis, which is consistent with our results. In one of the largest case-control studies of maxillary lateral incisor agenesis (MLIA), *SPRY2* was identified as a gene contributing to MLIA susceptibility, representing the first evidence implicating sprouty genes in tooth agenesis, particularly involving the lateral incisors [13]. The mechanistic basis for the role of SPRY2's likely involves its antagonism of FGF-induced RTK signaling, which is critical for epithelial-mesenchymal interactions during tooth development. Disruption of this pathway may result in developmental failure and subsequent tooth agenesis [36].

SMOC2 is an extracellular matrix protein that modulates cell-matrix interactions and may influence cell growth and angiogenesis mediated by vascular endothelial growth factor (VEGF) during the formation of dental tissues, including enamel, dentin, and cementum [32]. Mutations in *SMOC2* have been linked to a spectrum of dental anomalies, including dental dysplasia, severe oligodontia, and microdontia [37]. Bloch-Zupan *et al.* [32] (2011) observed higher Smoc2 expression in molar germs compared to incisors during mouse dental development, suggesting a stronger association with molar agenesis. Nevertheless, our findings indicate a significantly higher variant burden in *SMOC2* among individuals with lateral incisor agenesis, with *SMOC2* showing the second highest altered gene after *EDA*, suggesting that *SMOC2* may also contribute to incisor agenesis and merits further functional and genetic investigation. The genes examined in this pilot study were selected based on prior evidence implicating

their involvement in dental agenesis. Our objective was to identify genes with high genetic variability that might show meaningful differences in variant burden between cases and controls, thereby highlighting promising candidates for further investigation.

This exploratory pilot case–control genetic association study has several limitations that should be considered when interpreting the findings. The small sample size substantially limits statistical power, increasing the likelihood of both false-positive and false-negative results, and may result in unstable effect estimates, while precluding robust subgroup or gene–gene interaction analyses. In addition, the study was conducted in a geographically and ethnically specific population, which may restrict the generalizability of the findings to other populations and geographic regions. Finally, no functional assays were performed to assess the biological impact of the identified variants; therefore, any potential causal role in lateral incisor agenesis remains speculative and requires confirmation through functional validation and replication in larger, well-powered cohorts and independent populations.

However, given its exploratory nature, this study provides preliminary evidence that *EDA*, *SPRY2*, and *SMOC2* may play particularly important roles in the etiology of lateral incisor agenesis in this Colombian cohort and offers a foundation for larger population-based studies with more comprehensive statistical modeling and functional validation of the identified variants in these genes.

5. Conclusion

Incisor agenesis is driven by complex genetic interactions involving multiple genes and signaling pathways. The preliminary findings of this exploratory study revealed a higher number of genetic variants in *EDA*, *SPRY2*, and *SMOC2* among Colombian individuals with lateral incisor agenesis, suggesting a potential contribution of these genes to the condition. Nevertheless, these observations must be confirmed in larger, well-powered cohorts and through functional studies to clarify their biological relevance.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

LME—designed the study, performed the research, and wrote the manuscript. LF—performed the research and the data analysis. AMA—performed the research and data analysis. ZB—designed the study and wrote the manuscript. DA—designed the research study. SY—performed the research and wrote the manuscript. JEC—designed the research study and performed the research. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the institutional ethics committee (Act B.CIEFO-244-2024). Written informed consent was obtained from all subjects and/or their parents or legal guardians.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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