

## ORIGINAL PAPER



## Research algorithm for the detection of genetic patterns and phenotypic variety of non-syndromic dental agenesis

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### Abstract

**Introduction:** Dental agenesis (DA), brings together the anodontia, oligodontia, hypodontia, characterized by a deficit in the development of a variable number of teeth. The objectives of the study were to illustrate the phenotypic variability of non-syndromic DA, to identify cases of DA with hereditary genetic transmission, and establish the mode of DA genetic pattern in these cases, together with the determination of DA prevalence in the population group study. **Patients, Materials and Methods:** The cross-sectional observational study was performed on a mixed population group, consisting of 861 Caucasian patients, between January 2018–December 2019. The clinical evaluation protocol of patients with DA, used to illustrate their phenotype, included the following stages: oral examination, photographic examination, and radiological examination. The evaluation protocol specific to the family genetic study of patients with DA, involved the following three stages: family survey, construction of the family tree and analysis of the pedigree structure. **Results:** The prevalence of DA in the population group was 2.78%. Regarding the phenotype, DA mainly affected the upper arch (50% of cases); bilateral DA had a significantly increased incidence (83.33% of cases) compared to unilateral form; in most cases (75%), a patient lacked one to two teeth, the lack of two teeth being the most common form (83.33% of cases); the upper lateral incisors were the teeth most frequently involved in DA (31.11% of the total missing teeth). Regarding the family genetic study, hereditary DA with autosomal dominant inheritance was present in 37.50% of cases. In the other cases (62.50%), isolated, sporadic forms of DA were registered, suggesting a spontaneous *de novo* mutation or a disorder of odontogenesis of a non-genetic nature. **Conclusions:** We consider that this study is of interest for current scientific research with applicability in dental medicine, by bringing actual information on the prevalence of non-syndromic DA in South-East Romania, the variety of phenotypic spectrum of DA for this geographic area, and the role of heredity in the DA genetic determinism in the studied population.

**Keywords:** dental agenesis, genetic pattern, phenotypic variability, family tree, hereditary transmission.

### Introduction

Dental anomalies of number, known as dental agenesis (DA), bring together the triad of anodontia, oligodontia, hypodontia, characterized by a deficit in the development of a variable number of teeth [1–5].

Generally, DA refers directly to the developmental failure of a tooth, anodontia describes a complete failure of development of all teeth, oligodontia refers typically to the absence of more than six teeth, excluding third molars, and hypodontia represents the developmental failure of one to six teeth, excluding third molars [6, 7].

Hypodontia is a rare genetic condition, characterized by the clinical and radiological absence of a small number of teeth, temporary or permanent, at an age that would have been expected to be present on the arch, in the absence of a history of affected tooth extraction or exfoliation [8–10].

The actual prevalence of DA seems to vary in different countries, between 2.6% and 11.3%, registering a higher value among Caucasians in Europe (women 6.3%, men 4.6%) and Australia (6% women, 5.5% men), compared to those in North America (4.6% women, 3.2% men) [11–14]. Regarding hypodontia, the least severe form of DA, its reported prevalence in the general population varies between 2% and 8%, except for the third molar [15–17].

The position, number, shape and size of teeth are under genetic control, the process of tooth development being a complex process involving a number of genes, whose genetic expression explains the great phenotypic variability of dental abnormalities [18–21].

The cause of dental buds' development lack is unknown, but it should be noted that hereditary factors are frequently involved in the etiopathogenesis of DA [22–24].

Family aggregation studies have proved the importance of genetic factors in the occurrence of DA [1]. DA may occur as an isolated condition (non-syndromic DA), or as part of a genetic syndrome (syndromic DA). Non-syndromic DA can be sporadic or may have family aggregation, transmitted as an autosomal or X-linked genetic pattern [25, 26].

Non-syndromic hypodontia may occur sporadically or may have family aggregation, it may be isolated or associated with other syndromic or non-syndromic dental abnormalities, its clinical phenotype being very varied [27, 28].

New horizons have now opened up for understanding the genetic control of syndromic and non-syndromic DA. Thus, a series of genes [axis inhibition protein 2 (*AXIN2*), ectodysplasin A (*EDA*), EDA receptor (*EDAR*), EDAR-associated death domain (*EDARADD*), gremlin 2 (*GREM2*), latent transforming growth factor-beta (TGF- $\beta$ ) binding protein 3 (*LTBP3*), low-density lipoprotein (LDL) receptor-related protein 6 (*LRP6*), paired-box 9 (*PAX9*), Msh homeobox 1 (*MSX1*), nuclear factor-kappa B (NF- $\kappa$ B) essential modulator (*NEMO*), paired-like homeodomain 2 (*PITX2*), secreted-protein acidic and cysteine rich (SPARC)-related modular calcium binding 2 (*SMOC2*), and Wnt family member 10A (*WNT10A*)] have been identified; their mutations are responsible for the appearance of the triad anodontics, oligodontics, hypodontics, the major gene involved in the etiology of hypodontics, being the *WNT10A* gene, consisting of four exons and located on chromosome 2q35, near the *WNT* gene [14, 29–31].

### Aim

The general purpose of the scientific study was to optimize the protocol for preclinical examination of patients with DA, through specialized genetic investigations. Regarding the specific scientific objectives of this research, through the genetic study of patients with DA, we aimed to identify cases of DA with hereditary genetic transmission and establish the mode of DA genetic pattern in these cases, to illustrate the phenotypic variability of DA in the study group, together with the determination of DA prevalence in the population group and the characterization of the distribution by age groups and by genders of the patients with DA; in addition, we compared the results obtained with the data recorded in the specialized literature.

## ☒ Patients, Materials and Methods

### Patients

The scientific research, materialized through the cross-sectional observational study, was performed on a mixed population group, consisting of 861 Caucasian patients, living in Bucharest and neighboring rural areas, who volunteered between January 2018 and December 2019 for consultation and specialized dental treatment, in private dental practices, Lucky Dental SRL Company, Bucharest, Romania.

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Lucky Dental SRL Company, Bucharest, Romania.

All patients and legal representatives were informed

about the purpose, objectives, and methods of scientific research, as well as on the confidentiality of the medical and research act, after which they filled-in and signed the informed consent form for inclusion in the study.

We compiled the study group based on the selective criterion.

### **Inclusion criteria in the study group**

In this group, we included cooperative patients with non-syndromic DA in temporary and/or permanent dentition, belonging to both genders, aged between seven and 50 years, who agreed to participate in this study and who did not abandon it during its process.

### **Exclusion criteria in the study group**

All patients with syndromic DA and associated systemic diseases with DA, patients with a medical history of dental extractions or tooth loss of various causes, uncooperative patients, who refused to be included in the study, patients with comorbidities that could affect the capacity to participate in the study, and all patients who dropped out of the study during its development were excluded from the study group.

Following the selective analysis of the 861 patients belonging to the population group, we concluded that 24 patients meet the criteria for inclusion in the study group.

## Methods

### **Clinical evaluation protocol**

The clinical evaluation protocol of patients with DA, used to diagnose their phenotype, included the following successive stages of examination: oral clinical examination, photographic examination, radiological examination or cone-beam computed tomography (CBCT), performed when necessary to provide supplementary clinical data that cannot be obtained using other imaging techniques.

Diagnostic, extraoral and intraoral medical photographs, which provide a series of details absolutely necessary to highlight the phenotypic heterogeneity of DA, were performed only with the consent of patients or legal relatives, in the case of children, after being informed about the protocol. The photos were taken with the help of a digital single-lens reflex (DSLR) camera, equipped with ring or twin flash and special accessories belonging to the mandatory kit used in intraoral photography, represented by a set of spacers, contrastors and mirrors.

The photographs were taken from different norms: frontal norm in occlusion and disocclusion, right and left lateral norm, in occlusion and disocclusion, maxillary occlusal norm and mandibular occlusal norm. Often, for intraoral photos, we used special soft-part spacers to allow all teeth to be seen. Sometimes, to reduce the anxiety induced in patients, we took these photos without using the spacer.

Extraoral and intraoral diagnostic medical photographs were printed on the highest quality medical diagnostic photographic paper, stored, and transmitted electronically on compact disk (CD) or memory stick.

### **Genetic study protocol**

The evaluation protocol specific to the family genetic study of patients with DA, involved the successive

completion of the following three stages: family survey, construction of the family tree and analysis of the pedigree structure.

The family investigation always started from an initial case, called proband. The proband, in this study, was the patient diagnosed with DA, who belongs to the study group. In the family survey, for each patient with DA (proband) we filled-in, after the anamnesis and the complete medical examination, a standard form called "sheet for congenital malformations and hereditary diseases", in which we recorded on devices and systems the patient's identity, anamnestic data, the general clinical examination results, the results of the local extraoral and intraoral clinical examination, the results of specific laboratory, paraclinical and genetic examinations. Based on all these data, we established the positive diagnosis of certainty, for the patient (proband). Next, we extended the family survey to other family members, looking for whether any of them have or have had the same dental abnormality as the proband or similar dental abnormalities.

Using the data contained in the "sheet for congenital malformations and hereditary diseases", we proceeded to the next stage, represented by the construction of the family tree, with the help of standard international symbols (Figure 1).

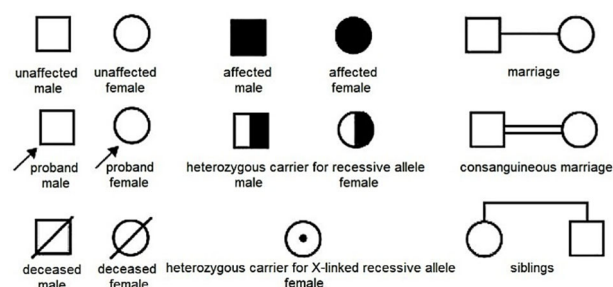


Figure 1 – Common standard pedigree symbols.

According to international standards of pedigree data management system, the graphic representation of the pedigree structure was started with the positioning of the proband, after which, we represented graphically the members of the ascending direct parentage (parents, grandparents, great-grandparents), descendants (children, grandchildren, great-grandchildren) and collaterals (brothers, sisters).

Next, we performed the analysis of the genealogical tree diagram, which materialized both in the diagnosis of non-hereditary and hereditary forms of DA, and of their genetic pattern in the studied family.

After completing all the established work stages, we prepared a personal medical file for each patient, consisting of: the patient's consultation form, his/her informed consent (signed by the patient or relative, in the case of minor patients), the consent regarding to the protection of personal data, radiographs, medical tests reports and results of common laboratory tests, photographs (exobuccal and endobuccal), stage epicrisis, file for congenital malformations and hereditary diseases, the family tree and the result of its analysis.

## Results

### General observations

From the 861 patients belonging to the population

group, 24 patients meet the criteria for inclusion in the study group. The frequency of DA in the population group was 2.78%.

Regarding the distribution by age groups of patients with DA, the majority are children (45.83% of cases), aged between seven and 17 years (11 cases, of which eight boys and three girls).

The study of patients' distribution by gender indicated that 58.33% are male patients (14 men) and 41.67% are female patients (10 women), the prevalence of DA being 1.39 times higher in males compared to females.

### Phenotypic spectrum of dental agenesis

In DA patients, we recorded a significant difference in the location of the disease, at the maxillary or mandibular level.

#### Phenotypic heterogeneity according to the location in the dental arches

Thus, in most cases (50%), DA was located only at the level of the upper arch, the lower arch being affected only in 25% of cases (Figure 2). Severe forms of maxillo-mandibular DA have been found in another 25% of cases (Figure 3).

#### Phenotypic heterogeneity according to bilateral versus unilateral location in the dental arches

Bilateral DA had a significantly increased incidence, being diagnosed in 83.33% of cases, compared to unilateral DA, which was highlighted only in 16.67% of cases (Figure 4).

#### Phenotypic heterogeneity according to the number of congenitally missing teeth

Regarding the number of missing teeth, in 75% of cases, a patient lacked one to two teeth, the lack of two teeth being the most common form (Figure 5).

As a result, hypodontia, characterized by the lack of a small number of teeth, was found in most patients, respectively in 83.33% of cases (Figure 6).

We found oligodontics, characterized by the lack of a larger number of teeth, in four cases of great complexity, in which seven, 10 and 16 teeth were missing (Figure 7). Three of them were cases of genetic DA with hereditary transmission (Figure 8).

In total, in the study group, patients with DA lacked 90 teeth.

#### Phenotypic heterogeneity according to the groups of congenitally missing teeth

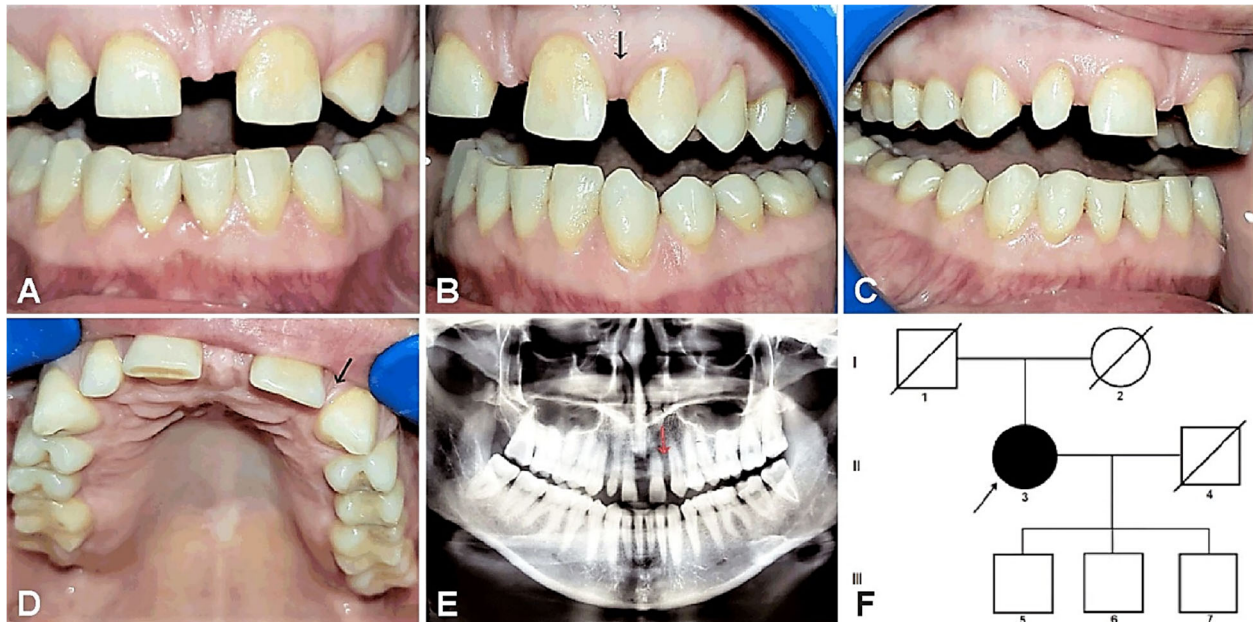
The groups of teeth most frequently involved in DA were the maxillary teeth, namely the upper frontal group (33 teeth, representing 36.67% of the total absent teeth) and the upper lateral group (27 teeth, representing 30% of the total absent teeth), followed in descending order of their frequency by the teeth of the lower frontal group (18 teeth, representing 20% of the total absent teeth) and the teeth of the lower lateral group (12 teeth, representing 13.33% of the total absent teeth) (Figure 9).

#### Phenotypic heterogeneity according to the frequency of congenitally missing teeth

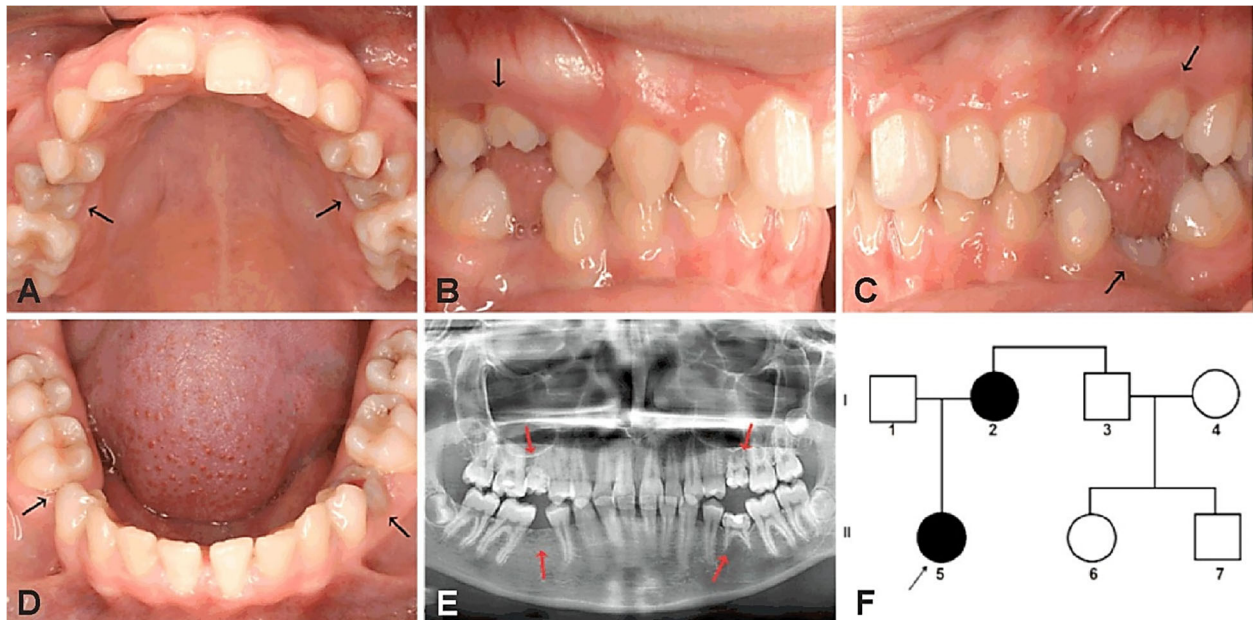
The teeth most frequently involved in DA, in descending

order of their frequency, are the following: upper lateral incisors (28 teeth, representing 31.11% of the total missing teeth), lower central incisors (14 teeth, representing 15.55% of the total absent teeth), the upper second premolars (nine teeth, representing 10% of the total absent teeth), the lower lateral incisors (six teeth, representing 6.66% of the total absent teeth), the upper first premolars (six teeth, representing 6.66% of the of the total absent teeth), the lower second premolars (six teeth, representing 6.66%

of the total absent teeth), the upper second molars (six teeth, representing 6.66% of the total absent teeth), the upper canines (three teeth, representing 3.33% of the total absent teeth), upper first molars (three teeth, representing 3.33% of the total absent teeth), lower second molars (three teeth, representing 3.33% of the total absent teeth), upper third molars (three teeth, representing 3.33% of the total absent teeth) and third lower molars (three teeth, representing 3.33% of the total absent teeth).



**Figure 2 – Unilateral maxillary lateral incisor agenesis in the permanent dentition – isolated, sporadic, non-hereditary, non-syndromic case, suggesting a spontaneous de novo mutation or a disorder of odontogenesis of a non-genetic nature. Intra-oral photographs: frontal view (A), half-lateral views with teeth separated (B and C) and occlusal view of maxillary arch (D) revealing the dental phenotype; panoramic radiograph showing the maxillary lateral incisor agenesis (E); pedigree of the family: isolated, sporadic, non-hereditary case (proband II:3) (F).**



**Figure 3 – Complete, maxillary and mandibular, second premolars agenesis in the permanent dentition, associated with the persistence on the arches of temporary secondary molars (5.5, 6.5 and 7.5) – a very rare condition of non-syndromic familial hypodontia with incomplete penetrance and variable expressivity. Intra-oral photographs: occlusal view of maxillary arch (A), lateral views (B and C), and occlusal view of mandibular arch (D) denoting the dental phenotype; panoramic radiograph showing the agenesis of four second premolars in all the four quadrants (E); pedigree of the family: hereditary familial hypodontia in two successive generations (cases I:2 and II:5) with incomplete penetrance and variable expressivity (F).**

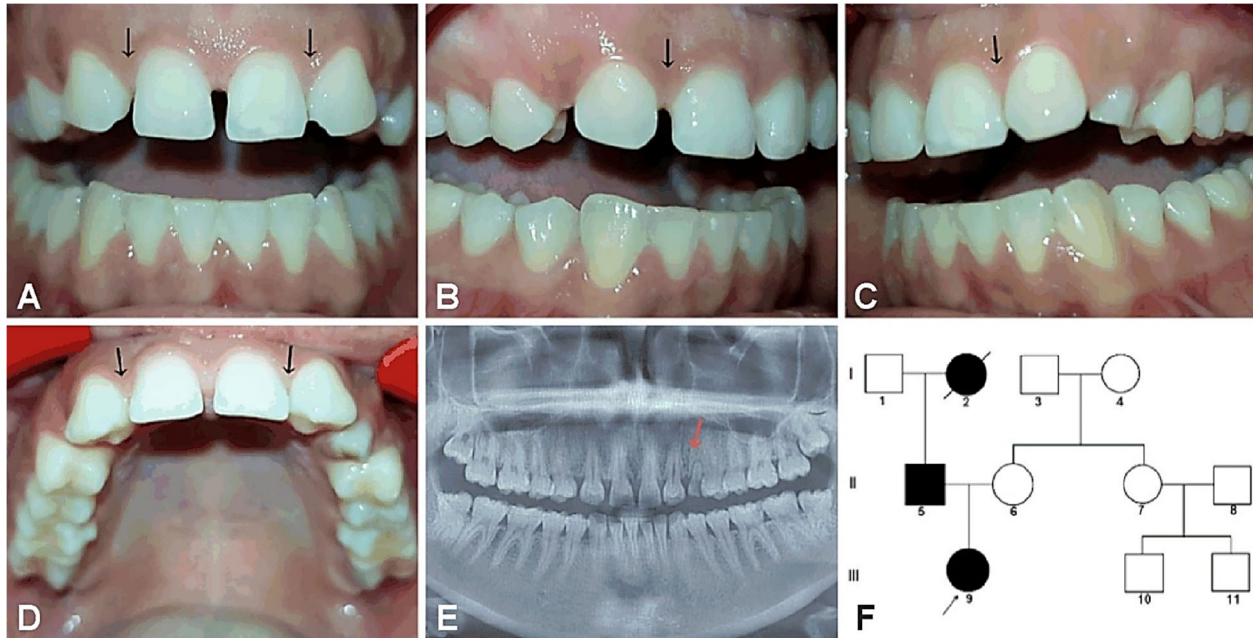
### Genetic patterns of dental agenesis

The study of heredity, family survey, preparation, and analysis of the family tree of patients with DA, included in the study group, indicated that the genetic factor plays a key role in the etiology of DA.

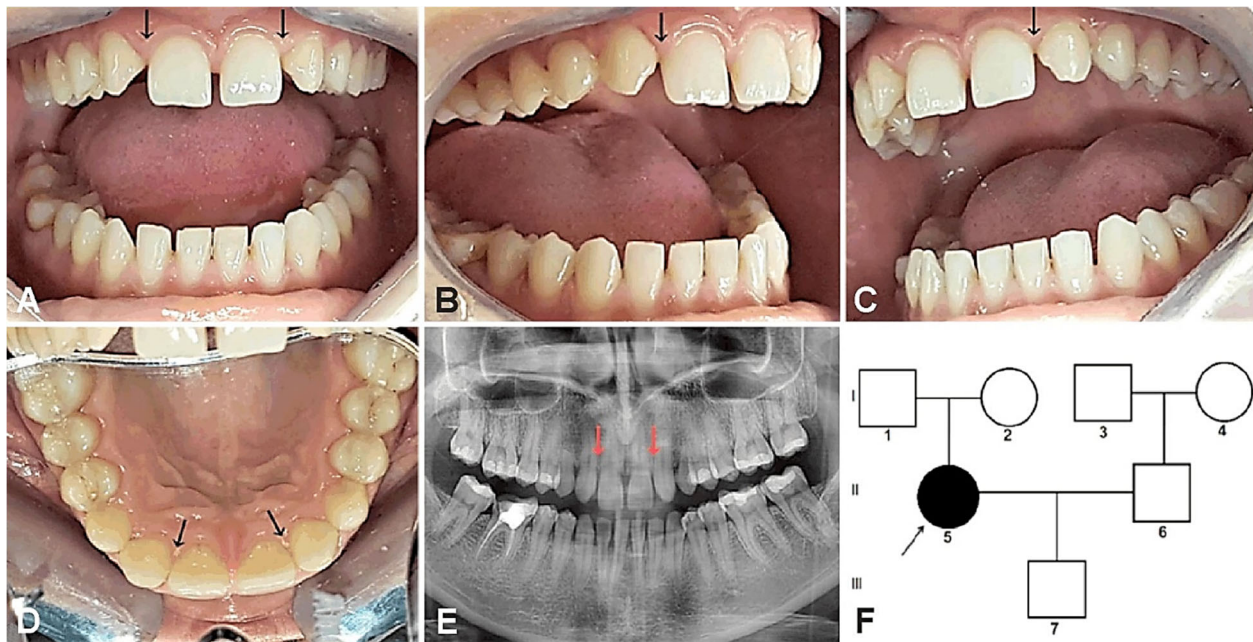
The results of these investigations illustrated that the

hereditary genetic DA with autosomal dominant inheritance was present in 37.50% of cases (nine patients).

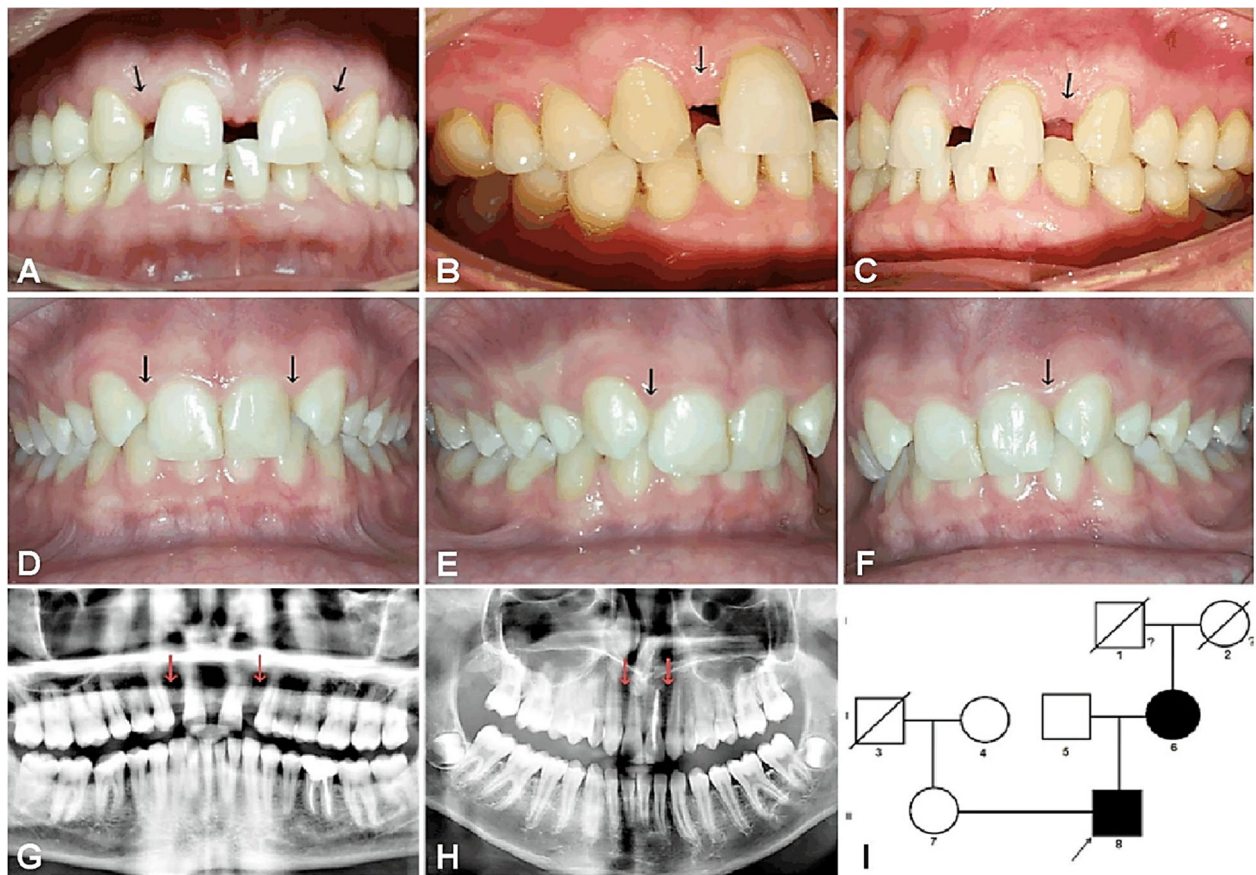
In the other cases (62.50% of cases, representing 15 patients), isolated, sporadic forms of DA were registered, suggesting a spontaneous *de novo* mutation or a disorder of odontogenesis of a non-genetic nature.



**Figure 4** – Bilateral maxillary lateral incisor agenesis in the permanent dentition – hereditary, non-syndromic case, with autosomal dominant inheritance, associated with the persistence on the arch of left temporary upper canine. Intra-oral photographs: frontal view (A), half-lateral views with teeth separated (B and C), and occlusal view of maxillary arch (D) revealing the dental phenotype; panoramic radiograph showing the bilateral absence of maxillary lateral incisors (E); pedigree of the family: hereditary, non-syndromic familial hypodontia in three successive generations (cases I:2, II:5, and III:9) suggest an autosomal dominant inheritance (F).



**Figure 5** – Bilateral maxillary lateral incisor agenesis in the permanent dentition – isolated, sporadic, non-hereditary, non-syndromic case, suggesting a spontaneous *de novo* mutation or a disorder of odontogenesis of a non-genetic nature, associated with the persistence on the arch of both temporary superior canines. Intra-oral photographs: frontal view (A), half-lateral views with teeth separated (B and C) and occlusal view of maxillary arch (D) revealing the dental phenotype; panoramic radiograph showing the bilateral absence of maxillary lateral incisors (E); pedigree of the family: isolated, sporadic, non-hereditary case (proband II:5) (F).



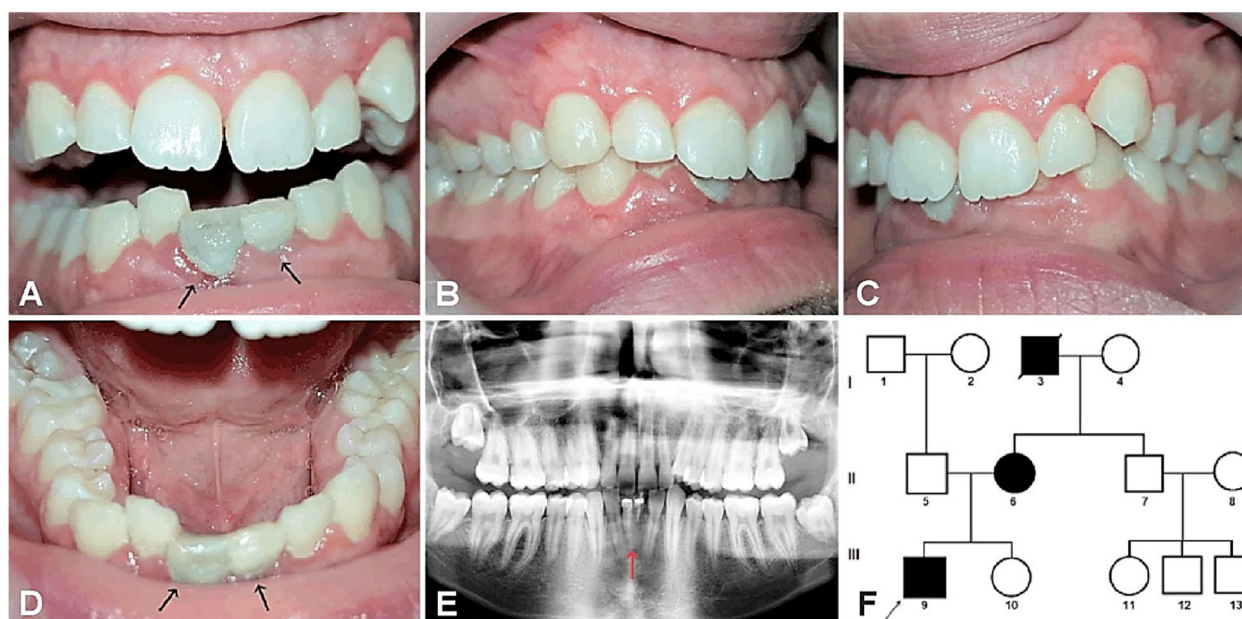
**Figure 6 – Bilateral maxillary lateral incisor agenesis in the permanent dentition – hereditary, non-syndromic case, with autosomal dominant inheritance. Intra-oral photographs of the mother: occlusal frontal view (A) and lateral views (B and C), and intra-oral photographs of the son: occlusal frontal view (D) and lateral views (E and F), revealing the heterogeneity of dental phenotype; panoramic radiograph of the mother (G) and panoramic radiograph of the son: showing the bilateral absence of maxillary lateral incisors (H); pedigree of the family: hereditary, non-syndromic familial hypodontia in two successive generations (case II:6 and III:7) suggest an autosomal dominant inheritance (I).**



**Figure 7 – Oligodontia in the permanent dentition – isolated, sporadic, non-hereditary, non-syndromic case. Intra-oral photographs, occlusal frontal view (A); panoramic radiograph showing a significant number of missing teeth in all the four quadrants (B); pedigree of the family: isolated, sporadic, non-hereditary case (proband II:5) (C).**



**Figure 8 – Oligodontia in the permanent dentition – hereditary, non-syndromic case, with autosomal dominant inheritance. Panoramic radiograph showing a significant number of missing teeth in all the four quadrants (A); intra-oral photographs, occlusal frontal view (B); pedigree of the family: hereditary, non-syndromic familial oligodontia in two successive generations (cases I:3 and II:6) suggest an autosomal dominant inheritance (C).**



**Figure 9** – Bilateral mandibular central incisor agenesis in the permanent dentition – hereditary, non-syndromic case, with autosomal dominant inheritance, associated with the persistence on the arch of both temporary lower central incisors. Intra-oral photographs: frontal view (A), half-lateral views (B and C), and occlusal view of mandibular arch (D) revealing the dental phenotype; panoramic radiograph showing the bilateral absence of mandibular lateral incisors (E); pedigree of the family: hereditary, non-syndromic familial hypodontia in three successive generations (cases I:3, II:6, and III:9) suggest an autosomal dominant inheritance (F).

## Discussions

DA is a dental number anomaly characterized by the total or partial absence of one or more teeth, going to extremely severe forms that consist in the lack of development of all teeth [32–34].

In general, to name the numerical dental deficit, the authors prefer the term DA, because this term describes most accurately the determinant developmental disorder [11, 35, 36].

Worldwide, although the prevalence of DA is uncertain, however, according to the data mentioned in the literature, it varies in relation to the geographical area, between 0.1% and 16.2%, the value range in which the 2.78% corresponds to the frequency of DA in the population group studied by us [5, 12, 22, 37].

In Romania, the previous studies record values of DA prevalence between 3.018% in the south of the country, 6.75% in the west of the country and 8.03% in the central-western part of the country, with an average value of 5.5%, which corresponds to the European average frequency of DA [2, 38–40].

Regarding the gender distribution of patients with DA, in our study group the prevalence of DA was higher in males (58.33%), compared to females (41.67%), contrary to data published in the literature [1, 5, 11, 13]. This result is explained both by the fact that the ratio between the two genders in the study group was 1.39 times higher, in favor of males, and by the fact that almost half of the patients diagnosed with DA are children aged between seven and 17 years old, 72.72% being boys and only 27.27% girls.

Anodontics, oligodontics and hypodontics are dental anomalies of multifactorial cause, in their etiology being involved mainly genetic factors, but also environmental factors, which affect the odontogenetic process in different

evolutionary stages, with variable consequences on the process of tooth development [8, 41, 42].

Family aggregation studies have shown that both hypodontia and oligodontia are inherited, most often, autosomal dominant with incomplete penetrance and variable expressiveness [43].

New horizons have now opened up for understanding the genetic control of dental morphogenesis [44–46]. Thus, the genes whose mutations cause hypodontia were identified: the *MSX1* gene, located on chromosome 4, 4p16.3-p16.1, which is responsible for the autosomal dominant form, and the *PAX9* gene, located on chromosome 14, 14q13.3, which is associated with oligodontia [47, 48].

Mutations in the *MSX1* gene produce specific oligodontia, which are of particular interest to second premolars and third molars [49, 50]. Thus, the *Arg31Pro* mutation of the *MSX1* gene was associated with second premolars agenesis, and the *Ser105Stop* mutation of the *MSX1* gene was associated with premolar hypodontia and orofacial cleft [51–54].

Mutations in the *PAX9* gene have been associated with selective DA, which mainly affects the posterior teeth (agenesis of the permanent molars), being preceded or not by the impairment of the primary dentition [55]. Thus, *219insG. Exon 2*, *A340T. Exon 2*, and *793insC. Exon 4* mutations were especially associated with molar agenesis [56, 57].

Another important gene involved in the process of odontogenesis, which is related to the autosomal dominant form of DA, is the *AXIN2* gene, located on chromosome 17, 17q23-q24, whose mutations are mainly associated with permanent tooth DA and colorectal cancer [1, 58].

Recent genetic studies have indicated that hypodontia is genetically transmitted, not only autosomally, but also gonosomally, the *Thr338Met* mutation of the *EDA* gene

being responsible for non-syndromic familial hypodontia, the X-linked form [59].

We still do not know all the causes of the DA, but the identification of the affected genes of the examined patients, the type and location of the gene mutations, the prevalence and distribution of the mutation types in the study group may help to explain better the patterns and the phenotypic heterogeneity of non-syndromic DA.

The phenotype of DA is very varied, there is a great diversity of clinical forms, in relation to the number of absent teeth and their location on the arches [60]. Thus, the most common DAs, in descending order of their frequency, mainly affect the third molar, followed by the agenesis of the second premolars, upper lateral incisors and lower central incisors [1, 61]. In relation to the type of affected dentition, agenesis of the upper lateral incisor is the most common DA met in temporary dentition, and bimaxillary agenesis of the last teeth of each dental group is the most common DA met in permanent dentition [1, 62–64].

Regarding the unilateral or bilateral dentition damage, in our study group, bilateral DA was five times more frequent than unilateral DA, and the lack of two teeth was the most common form, both results being consistent with the data presented in the literature [5, 11, 65].

DA characterized by a wide phenotypic heterogeneity, can be isolated or associated with other dental abnormalities, such as: delay in tooth development and ectopic eruption, often affected by upper canines, microdontics, enamel hypoplasia and root abnormalities, such as short roots and taurodontism [18, 66, 67].

Regarding syndromic DA, so far hundreds of genetic syndromes have been described, which phenotypically associate cleft lip and/or cleft palate and hypodontia, most commonly affecting the upper lateral incisor. However, gene mutations identified as being involved in the etiology of cleft lip and/or cleft palate could not explain the concomitant occurrence of DA [29].

DA due to its phenotypic heterogeneity and etiological multivalence, is a complex subject, of great relevance in dental medicine. Given that so far, in Romania, there is no similar genetic study of recent data on illustrating the role of the genetic component in DA determinism, in this scientific research we have deepened this topic, given that the genetic study of DA is not only a difficult but also challenging topic, which paves the way for the completion of new specialized studies and future scientific research of a large scale.

## ☐ Conclusions

We found the actual prevalence of non-syndromic DA in South-East Romania, describe the variety of phenotypic spectrum of DA for this geographic area, and identify the role of heredity in the DA genetic determinism in the studied population. We consider that the study is of interest for current scientific research, with applicability in dental medicine, by bringing actual information regarding the research of DA. Further molecular genetic study will be needed to identify novel causal mechanisms of how gene mutations disrupt normal tooth development, helping to explain better the patterns and the phenotypic heterogeneity of non-syndromic DA.

## Conflict of interests

The authors declare that they have no conflict of interests.

## Author contribution

Marina Imre has equal contribution to this paper as the first author.

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