AMERICAN UNIVERSITY OF BEIRUT

GENETIC BASIS OF FAMILIAL OLIGO-HYPO-HYPERDONTIA IN EASTERN MEDITERRANEAN FAMILIES

by
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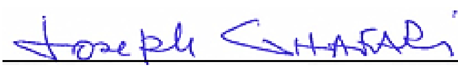

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ABSTRACT
OF THE THESIS OF

Josephine Michel Boueri for Master of Science
Major: Orthodontics

Title: Genetic Basis of Familial Oligo-Hypo-Hyperdontia in Eastern Mediterranean Families

Background:

Tooth agenesis is one of the most common congenital malformations in humans. This may affect either the primary or permanent dentition and can range from 5 or fewer missing teeth (hypodontia), 6 or more (oligodontia), to complete absence of teeth (anodontia). Tooth agenesis may originate from either genetic or environmental factors. Hypodontia as genetically determined can either occur as an isolated condition (non-syndromic hypodontia) involving 4 common genes types: MSX1, PAX9, WINT10A and AXIN2 or can be associated with a syndrome (syndromic hypodontia). Early tooth development can lead also to supernumerary teeth known for "hyperdontia" which refers to the presence of extra teeth compared to a normal dentition. Genetic factors play a role in the occurrence of hyperdontia, but the isolated condition (non-syndromic hyperdontia) is rare compared to the syndromic condition.

Objective:

1. To gain more insight into the prevalence of dental agenesis and dental excess.
2. Clarify the inheritance pattern of dental agenesis and dental excess.
3. Highlight the concomitance of hypodontia/hyperdontia in one family.

Methods:

An initial cohort of 16 patients seeking orthodontic treatment at AUBMC will be recruited for the study. The patients were from different families affected by hypodontia, oligodontia, and hyperdontia. Inclusion criteria were: at least two generations affected in each family; agenesis ranging from 1 to ≥ 6 teeth, hyperdontia ≥ 1 tooth; no genetic syndromes or systemic conditions, no history of facial trauma, previous extractions, or orthodontic treatment. Tooth agenesis and hyperdontia were evaluated on panoramic radiographs and pedigrees were established using the progeny software. Whole exome sequencing will be performed on all family members and variants would be analyzed using the Illumina variant studio based on minor allele frequency of <1% concomitant with a segregation of the genotype with the underlying phenotype.

A second cohort study will be conducted on the affected members (with dental agenesis) of the included families and their relatives to evaluate the dental and arches dimensions compared to control groups. The group are as follow: group 1 (affected patients N=18); group 2 (control of group 1 N=18); group 3 (relatives of group 1: non affected N=10) and group 4 (control of group 3 N=10). The inclusion criteria for the control groups are: no dental extractions or implant restorations; no genetic syndromes or systemic conditions, no history of facial trauma and no orthodontic treatment. Variables assessed were: dental crown width and length; root length; maxillary/ mandibular arch perimeters: arch circumference available, required and deficiency; maxillary/ mandibular inter-molar and inter-canine widths.

Results:

Most of the pedigrees suggest a Mendelian inheritance pattern and segregate in an autosomal-dominant manner. Pedigree analysis indicated an equal number of reported generations per family (n=3-4), number of families with female predominance (n=11), number of families with male predominance (n=4) and families with equal number of reported affected males and females (n=1). Genetic screening did not show any aberration in the previously reported genes linked to hypodontia/hyperdontia, but did point out to 14 potential candidate novel genes (CRACR2A, PER3, NOV/CCN3, EDAR, APCDD1, CDH26, NME8, LAMC2, LIMD1, WNT10A, FGFBP1, DFFA, OR10A6 and DYRK1A) that could be implicated in hypodontia/hyperdontia.

Dental evaluation through panoramic x-ray and intra-oral 3D scans from the 8 families having hypodontia, confirmed a reduced arch dimension, less crowding and smaller teeth with shorter roots in the affected group compared to the control and to their relatives. In addition, dental evaluation confirmed as well that the relative group have smaller arch and dental dimensions compared to the control group and larger compared to the affected group.

Conclusion:

This study gains its strength and novelty from its design as it is the first genetic study on large families having hypodontia alone (n=5), hyperdontia alone (n=1) and combined hypo and hyperdontia (n=3) in the Eastern Mediterranean population using the NGS/WES.
An “accordion pattern” exists between the two phenotypes:
Hypodontia is an evolutionary effect and hyperdontia is a compensatory effect.
A “continuum pattern” exists between all groups for all dental variables:
Teeth crowns tend to get thinner and shorter, and root length tend to get shorter as we move from control to non-affected to agenesis group.

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ABBREVIATIONS

DE	Dental excess
DA	Dental agenesis
NGS	Next generation sequencing
WES	Whole Exome sequencing
WGS	Whole Genome sequencing
ACD	Arch circumference discrepancy
ACA	Arch circumference available
ACR	Arch circumference required
CW	Crown width
CL	Crown length
RL	Root length
Chrom	Chromosome
R side	Right side
L side	Left side
M/m	Maxilla/ Mandible
CL	Cleft lip
CL/P	Cleft lip and palate
VWS	Vander woude syndrome
ED	Ectodermal dysplasia
DTD	Diastrophic dysplasia
CHH	Concomitant hypo/hyperdontia
SNP	Single nucleotide polymorphism
INDEL	Insertion deletion
MAF	Minor allele frequency
IO-scan	Intra-oral scan

CHAPTER I
INTRODUCTION

A. Background

A tooth is defined to be congenitally missing if it has not erupted in the oral cavity and not visible on radiographs. The use of panoramic radiography is recommended, along with clinical examination in detecting or confirming dental agenesis.(Thesleff, 2000)

Hypodontia (dental agenesis) is the most common developmental anomaly in humans (0.3-2.4% and 2.8-8% in primary and permanent dentition respectively), constituting a clinically challenging problem and genetics play a fundamental role in its etiology (Garib et al., 2010). Several studies of odontogenesis at the molecular level, mostly using mouse teeth as models, have indicated that the development of teeth is under strict genetic control, which determines the position, number, size, and shape of teeth.(Arte, 2001)

Three types of dental agenesis are to be mentioned according to the number of teeth missing: Hypodontia is when less than six teeth are missing; Oligodontia when equal or more than six teeth are missing; Anodontia is when all teeth are missing excluding third molars. (Arte, 2001; Symons et al., 1993).

Dental agenesis is frequently associated with various dental anomalies such as:

• Craniofacial development defects (especially maxillary retrognathism and reduced anterior facial height).

• Reduction in jaw size.

• Abnormal occlusion, the severity of which is dependent on the number of missing teeth.

• Reduction in tooth dimensions (microdontia) and morphology or dental malformation (ex: peg shaped lateral incisors), delays of development, root anomalies, abnormal positions (ectopias and transpositions) and enamel hypoplasia.(Arte, 2001; Miletich and Sharpe, 2003)

These different dental anomalies commonly appear together in the same patient; which can be explained by the fact that a certain genetic mutation might cause a series of different phenotypic expressions. (Baccetti, 1998; Garn and Burdi, 1971; Peck et al., 1996)

Teeth agenesis affects mostly the last element within each category of teeth: incisors, canines, premolars, molars (Figure I) (De Coster et al., 2009a). The most common teeth to be missing are the mandibular second premolars (20.3 and 18.1%) followed by the maxillary lateral incisors (17.8 and 17.7%) and the maxillary second premolars (7.4 and 6.3%). (Gracco et al., 2017; Polder et al., 2004; Symons et al., 1993)

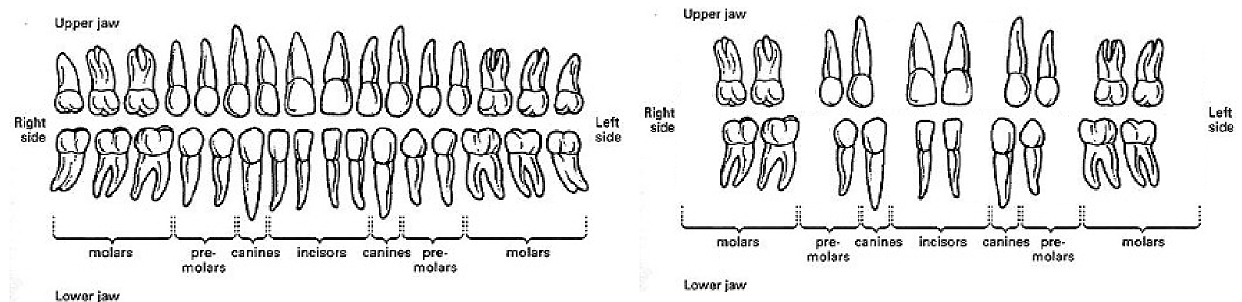


Figure I.1: Teeth categories and dental agenesis

Early defect in tooth development can lead also to supernumerary teeth known for “hyperdontia” which refers to the presence of extra teeth compared to a normal dentition.

(Wang et al., 2018). Hyperdontia may occur in both dentitions, unilaterally or bilaterally and in both jaws. While mesiodens, which is a supernumerary tooth located between the maxillary central incisor, is the most frequent type of supernumerary tooth, multiple supernumerary teeth are rare in individuals with no syndromes associated. (Rajab and Hamdan, 2002).

B. Significance

Identifying the candidate genes responsible for the dental agenesis/supernumerary and its familial transmission in different Mediterranean population is a major advancement in orthodontics because it can help comprehend the molecular mechanisms of dental development and how gene mutation at different levels can affect dental development. Also, better understanding of the specific genes contributing to variation in the risk for dental agenesis/excess in the Mediterranean population can help estimate the genetic susceptibility to this condition in families with affected individuals. By extension, the research emphasizes the need for early diagnosis and treatment.

In addition, by early forecast of the condition following a blood test to assess the patient’s genes, earlier treatment may be instituted (if needed) to try to intercept the development of malocclusion if possible or to minimize its severity. More importantly, present intervention in case of dental agenesis, is in favor to prevent later dental implants or decrease their number and manage spaces in severe cases of hypodontia. Such achievement can improve treatment modalities and outcome and help in the prevention of moderate to severe cases where maxillofacial surgery might be needed or facilitate it.

Recent gene mapping and linkage analysis give hope that the genetic determinants of dental development in general and hypo/hyperdontia in particular will be better understood in the near future.

An emphasis should be placed on studying the heritable pattern of dental agenesis and/or supernumerary. However, only a few genome-wide family-based linkages have been performed in different ethnic populations to identify the gene(s) involved in the trait, and no previous study was conducted in the Mediterranean population, underscoring the fact that the genetic determinants of dental agenesis and/or excess remain unclear.

In this study, the genetic determination is based on the assumption that dental agenesis/excess is genetically determined. Accordingly, the inclusion criteria were set in a stringent way that may lead to proper identification of a candidate gene(s).

C. Research objectives

The aims of this study are to:

1. Clarify the Inheritance pattern of dental agenesis in Mediterranean population especially Arab population.
2. Explore the concomitance between hypo/hyperdontia in one family in Mediterranean population.
3. Compare between the genotype of families having isolated teeth agenesis and families having concomitant hypo/hyperdontia.

D. Hypothesis

Our main hypotheses are:

1- Specific candidate loci and genes have an etiological role in the susceptibility to dental agenesis in the Mediterranean population.

2- Inheritance may be related more to dental agenesis than dental excess.

CHAPTER II

LITERATURE REVIEW

A. Definitions and concepts

1. Dental morphogenesis:

Dental morphogenesis and differentiation are part of the embryonic development and the result of complex interactions between the ectoderm and the mesenchyme at molecular level (Figure II) (Arte, 2001). During dental development the enamel develops from ectoderm of the oral cavity, and all other tissues come from the associated mesenchyme. The first teeth buds to develop are in the anterior mandibular region, later in the anterior maxillary region, then posteriorly in both jaws. (Lowder and Mueller, 1998)

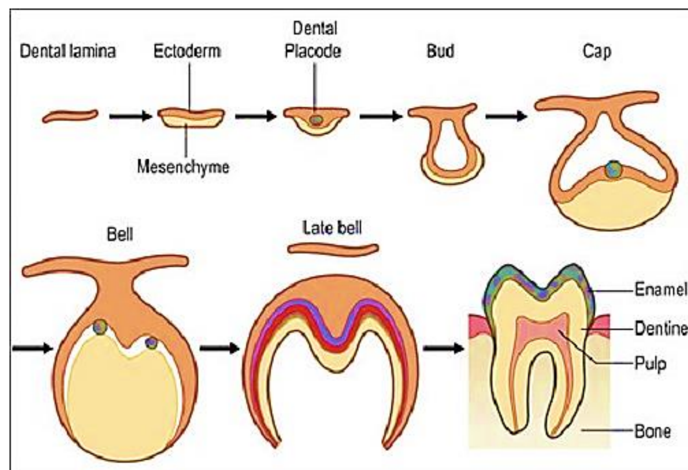


Figure II.1: Stages of dental development

At least 300 genes are involved in the processes of dental morphogenesis through a bind between the transcription factors homeodomains and a growth factor encoding genes acting upstream and downstream of the homeobox genes. (Arte, 2001; Thesleff, 2000)

The growth factors genes involved in dental morphogenesis are: the fibroblast growth factors (FGF), transforming growth factors (TGF, including BMP4 - bone morphogenetic protein 4), the family of Wnt genes (Wingless), and the morphogenesis molecule Shh (Sonic hedgehog). (Miletich and Sharpe, 2003)

The growth factors play an important role in the regulation of the initiation, budding, epithelial morphogenesis and the differentiation of the dental cells. (Thesleff, 2000)

The orchestrated work and fine-tuned expression of these genes are essential for the general scheme of dentition that is determined even before the development of the visible teeth (Miletich and Sharpe 2003). Thus, tooth development requires genetic and molecular regulation to establish accurate tooth number and precise location, size, morphology, and composition of each tooth (Cobourne and Sharpe 2013).

The sequence of eruption can vary from one child to another but follow continuous developmental stages. Dental morphogenesis and differentiation begin in the third month prenatally. The development of all primary teeth and permanent first molars starts before birth. (Garn and Burdi, 1971)

At birth neither the primary nor permanent teeth are well developed. The first primary tooth normally does not erupt before the age of 6 months. The timing and sequence

of eruption of the primary dentition, along with root formation are shown in table II.1. A delay or acceleration of 6 months is considered normal. (Proffit et al., 2018)

Table II.1 Sequence of eruption of the primary dentition

Tooth	Calcification begins	Crown completed	Eruption		Root completed	Exfoliation	
	Maxillary and mandibular	Maxillary and Mandibular	Maxillary	Mandibular	Maxillary and Mandibular	Maxillary	Mandibular
Central	14 wk in utero	1 ½ - 2 ½ mo	10 mo	8 mo	1 ½ yr	7 - 8 yr	6 – 7 yr
Lateral	16 wk in utero	2 ½ -3 mo	11 mo	13 mo	2 yr	8 - 9 yr	7 – 8 yr
Canine	17 wk in utero	9 mo	19 mo	20 mo	3 ¼ yr	11 – 12 yr	9 – 11 y
1st Molar	15 wk in utero	5 ½ - 6 mo	16 mo	16 mo	2 ½ yr	9 – 11 yr	10 -12 yr
2nd Molar	19 wk in utero	10 - 11 mo	29 mo	27 mo	3 yr	9 – 12 yr	11 -13 yr

The failure of exfoliation can be related to several causes: Defect in bone and root resorption due to ectopic position of the underlying permanent tooth. Ankylosis of the primary tooth and absence of the succedaneous permanent tooth are factors causing the failure of exfoliation. (Proffit et al., 2018)

Eruption of permanent teeth follows a sequence of stages as follow:

- *Pre-emergent stage:*

Two processes are necessary for the pre-emergent stage, first is the bone resorption and the primary teeth roots overlying the crown of the erupting tooth. Second is a propulsive mechanism that moves the tooth through the path of eruption. Although the two processes occur in a concert but in some circumstances, they do not.(Proffit et al., 2018)

Failure in the eruption of the permanent teeth is caused by factors related to the surrounding tissue: defect or lack of bone resorption covering the permanent tooth, presence of supernumerary teeth causing obstacle for the eruption. This condition occurs frequently in the cleidocranial dysplasia syndrome. Heavy fibrous gingiva, cystic lesions pushing against the path of eruption are causes for eruption failure. Another factor may relate to the tooth itself such as the anomaly of formation that can be genetic or caused by trauma impeding dental eruption. (Garn and Burdi, 1971)

- *Post-emergent stage:*

Once a tooth emerges into the mouth, it erupts rapidly until it approaches the occlusal level and is subjective to the forces of mastication.

This stage of eruption is divided into two phases:

Post-emergent spurt "relatively rapid eruption": from the time the tooth penetrates the gingiva until it reaches the occlusal level.

Juvenile occlusal equilibrium "slow eruption": As the tooth is subjected to biting forces that oppose eruption, the overall rate of eruption will be slow. During this phase, teeth erupt at a rate parallel to the growth of the mandibular ramus which moves the mandible away from the maxilla creating space into which the teeth will erupt more.(Proffit et al., 2018)

The transition from the primary to the permanent dentition begins at age 6 with the eruption of the first permanent molars followed soon by the permanent incisors. The sequence of eruption of the permanent teeth is shown in (*table II.2)*.

Table II.2 Sequence of eruption of the permanent dentition

Tooth	Calcification begins		Crown completed	Eruption		Root completed
	Maxillary	Mandibular	Maxillary and Mandibular	Maxillary	Mandibular	Maxillary and Mandibular
Central	3 mo	3 mo	3 ½ - 4 ½ yr	7 ¼ yr	6 ¼ yr	9 ½ - 10 ½ yr
Lateral	11 mo	3 mo	4 – 5 ½ yr	8 ¼ yr	7 ½ yr	10 – 11 yr
Canine	4mo	4 mo	5 ¾ - 6 yr	11 ½ yr	10 ½ yr	12 ¾ yr – 13 ½ yr
1st Premolar	20 mo	22 mo	6 ¾ - 7 yr	10 ¼ yr	10 ½ yr	13 ½ yr
2nd Premolar	27 mo	28 mo	7 ½ - 7 ¾ yr	11 yr	11 ¼ yr	14 ½ - 15 yr
1st Molar	32 wk in utero	32 wk in utero	3 ¾ - 4 ¼ yr	6 ¼ yr	6 yr	10 ½ yr
2nd Molar	27 mo	27 mo	7 ½ - 7 ¾ yr	12 ½ yr	12 yr	15 ¾ - 16 yr
3rd Molar	8 yr	9 yr	14 yr	20 yr	20 yr	22 yr

2. Dental agenesis

a. Definition

Congenital lack of a tooth results from disturbances during the early stages of dental morphogenesis described above and may cause arrest of development. A tooth is defined to be congenitally missing if it has not erupted in the oral cavity and is not visible on radiographs.

Diagnosis of dental agenesis is based on clinical and radiographical examinations. A child by age 3 would have all his primary teeth erupted and an adolescent would have all his permanent teeth erupted by age 12 – 14 (except for third molars). (Polder et al., 2004; Thesleff, 2015) Therefore, clinical diagnosis of congenitally missing primary teeth can be done on 3 to 4-year-old children, and that of permanent teeth on 12 to 14-year-old adolescents, excluding the third molars based on clinical examination.

Radiographic diagnosis can be made at younger age depending on tooth group. All primary teeth and the crypts of first permanent molars are visible by radiograph at birth. The crowns of first premolars, second premolars, and second permanent molars start to mineralize near the second birthday, and all permanent tooth crowns except the third molars have begun their mineralization by the age of six. (Thesleff, 2000)

Differences exist in mineralization stages among children depending on race, gender, and even on family and on the individual. The second premolars may show late onset of mineralization, and give a false-positive diagnosis of hypodontia in radiographs. Therefore, diagnosis of tooth agenesis in the permanent dentition should be made after the age of 6 excluding third molars, and after 10 years of age if third molars are also studied. (Lowder and Mueller, 1998; Polder et al., 2004; Thesleff, 2015)

b. Etiology

Many theories of the etiology of dental agenesis have been suggested in the literature, especially before the recent extensive genetic studies, and obviously both genetic and environmental factors may contribute.(Polder et al., 2004; Schalk-Van Der Weide and Bosman, 1996; Vastardis, 2000).

When trying to elucidate the causal factors underlying agenesis, it is necessary to analyze carefully the pattern of hypodontia. First, the developmental level at which defective tooth development occurred can be very informative. Failure of initiation

(formation of an epithelial thickening) is likely to be caused by a defect other than the one responsible for arrest of development at cap or bell stage.(Coster et al., 2009)

i. Environmental factors

Many environmental factors may cause arrested tooth development. Traumas in the dental region such as fractures, surgical procedures on the jaws, and extraction of the preceding primary tooth are the main environmental causes of dental agenesis followed by multiagent chemotherapy and radiation therapy that may affect irreversibly the developing teeth with various severities depending on age of patient and dosage. (Nieminen, 2009).

Treatment of malignant diseases in children at an early age shows arrest in root development with short V-shaped roots, roots with premature apical closure, enamel hypoplasia, microdontia, and hypodontia. Irradiation produces more severe effects than those caused by chemotherapeutic agents (Arte, 2001; Näsman et al., 1997). Thalidomide R (N-phtaloyglutamimide) consumption by women during pregnancy have been reported to cause congenitally missing teeth in their children. No definite etiologic relationship has been found between hypodontia and systemic diseases or endocrine disturbances. (Arte, 2001; Thesleff, 2000)

ii. Genetic factors

Although tooth agenesis is occasionally caused by environmental factors, in the majority of cases hypodontia has a genetic basis. The classic family study of Grahnen in

Sweden done in 1956 on a total of 685 family members of 171 probands affected with hypodontia (Grahnén, 1956) showed that hypodontia in permanent dentition is primarily determined by genetic factors.

Frequency of hypodontia differs between races, and greater concordance of hypodontia appears in identical twins (Markovic, 1982). In familial hypodontia, the type of inheritance in the majority of families seems to be autosomal dominant with incomplete penetrance and variable expressivity. (Human Genome Program, 2008: Penetrance is considered incomplete if someone have a particular gene variant that is known to be associated with a disease, yet they never get the disease). In addition, peg-shaped maxillary lateral incisors are considered to be a modified manifestation or different phenotype of the same genotypes as hypodontia (Grahnén, 1956; Thesleff, 2000).

Sex-linked inheritance patterns and a polygenic or multifactorial model of inheritance have also been suggested (Peck et al., 1996; Suarez and Spence, 1974), and an autosomal recessive model in one family (Ahmad et al., 1998). Female predominance has been reported, but in most studies the difference does not reach statistical significance (Bergstrom, 1977; Kotsomitis et al., 1996; Rølling, 1980; Symons et al., 1993).

Different studies have shown evidence for the genetic basis of dental agenesis. Two mutated genes were described causing an autosomal dominant form of human non-syndromic tooth agenesis (van den Boogaard et al., 2000; Vastardis et al., 1996). A missense mutation was found by the Vastardis group in the homeodomain of MSX1 gene in chromosome 4 (4p16) in all affected members of a family with missing second premolars. Some affected individuals also lacked their maxillary first premolars, mandibular first

molars, one or both maxillary lateral incisors, or a single mandibular central incisor. All affected individuals were reported to have initially normal primary dentitions.

In a Dutch family, however, a nonsense mutation in the MSX1 gene was associated with tooth agenesis and various combinations of cleft lip and/or palate(van den Boogaard et al., 2000). Other reports have excluded MSX1 as the gene responsible for tooth agenesis (Scarel et al., 2000).

A frameshift mutation in another transcription factor gene, PAX9, in chromosome 14 (14q21-q13) was identified in a family with autosomal dominant oligodontia causing a premature termination of the protein coded by the PAX9 gene (Stockton et al., 2000). The PAX 9 mutation causes agenesis of most permanent molars, maxillary and/or mandibular second premolars as well as mandibular central incisors. In this study sample, dental agenesis was not observed in the primary dentition. MSX1 and PAX9 are transcription factors which, before being associated with human tooth agenesis, had been shown to regulate early tooth morphogenesis in the mouse. They are expressed in dental mesenchyme after initiation of tooth development in response to epithelial signals (Thesleff, 2000). Mutation and inactivation of Msx1 and Pax9 genes in mice causes arrest in dental development at the bud stage and malformations of palate, limb, and pharyngeal pouch derivatives, whereas heterozygous mice develop normal teeth which concludes that in mice the mutation was recessive. (Peters et al., 1998).

In humans, the mutation of the gene is dominant and one copy of the gene (one allele) causes dental defects (Stockton et al., 2000; Vastardis, 2000) or dental defects associated with cleft lip and palate in the case of MSX1 nonsense mutation (van den

Boogaard et al., 2000). In addition, several gene defects have been identified which cause syndromes with hypodontia or oligodontia and few others cause non-syndromic hypodontia or oligodontia MSX1, PAX9, AXIN2, WINT10A, EDA, and LRP6. (Juuri and Balic, 2017; Lammi et al., 2004; Polder et al., 2004; Stockton et al., 2000) (Table II.3-4). In grey are the most common gene associated either with syndromic or isolated dental agenesis.

The inheritance pattern of dental agenesis was reported in different studies as being: Autosomal dominant or recessive for some genes and X-linked for others. (De Coster et al., 2009a; van den Boogaard et al., 2000; Vieira et al., 2004)

Table II.3: Genes involved in syndromic dental agenesis

GENEs	Chromosome	Mutation effect	Syndrome/ condition Association	Publication	Population
Mutation in BCOR Gene	Xp11.4	Hypodontia/Oligodontia	Occulofacicardiodigital syndrome Lenz micro-phtalmia syndrome	Zhu et al., 2015 Du et al., 2018	Chinese Turkish families
Mutation in TSPEAR Gene	21q22.3	Hypodontia	Deafness and hypotrichosis	Du et al., 2018	Turkish families
Mutation in LAMB3	1q32.2	Tooth agenesis, Amelogenesis imperfeca type IA	Epidermolysis bullosa, junctional spinal disorders	Du et al., 2018	Turkish families
Mutation in DLX3	17q21.33	Hypodontia, amelogenesis imperfecta	Down syndrome, tricho-dento-osseous syndrome	Price et al., 1998 Whitehouse et al., 2019	American families (Virgina) American families
Mutations in EDA	Xq13.1	Enamel hypo-mineralisation, hypodontia, hypoplasia	X-linked anhidrotic ectodermal dysplasia syndrome	Tao et al., 2006 Parveen et al., 2019 Zeng et al., 2016 Zeng et al., 2017 Andreoni et al., 2021	Mongolian family Pakistan, Egypt, Saudi Arabia, and Syria Chinese Chinese Italian
Mutation in EDARADD	1q42.3	Enamel hypo-mineralisation, hypodontia, hypoplasia	Autosomal anhidrotic ectodermal dysplasia syndrome	Martínez-Romero et al., 2019 Zeng et al., 2016	Spannish Chinese
Mutation in EDAR	2q13	Enamel hypo-mineralisation, hypodontia, hypoplasia	Autosomal anhidrotic ectodermal dysplasia syndrome	Parveen et al., 2019 Zeng et al., 2016 Martínez-Romero et al., 2019 Andreoni et al., 2021	Pakistan, Egypt, Saudi Arabia, and Syria Chinese Spannish Italian

Mutation in WNT10A	2q35	Oligodontia	Autosomal anhidrotic ectodermal dysplasia syndrome	Parveen et al., 2019 Zeng et al., 2016 Zeng et al., 2017 Martínez-Romero et al., 2019	Pakistan, Egypt, Saudi Arabia, and Syria Chinese Chinese Spannish
Mutation in IRF6	1q32.2	Missing incisors and/or premolars. Cleft lip with/without palate + pits in the lips.	Van der Woude and popliteal pterygium syndromes	Kondo et al., 2002 Ghassibé et al., 2004	Northern European European
Mutation in Tp63	3q28	Agenesis of primary and permanent incisors	RIH syndrome Remote intracranial hemorrhage	van Bokhoven et al., 2001 Jin et al., 2019	Netherlands, Belgium, US, Italy, Israel, Turkey, Austria, Germany, and the UK Chinese family
Mutation in SHH	7q36.3	Agenesis of 1 maxillary central incisor	Hyloprosencephaly	Frazier-Bowers et al., 2003	Vietnam
Mutation in OFD1	Xp22.2	Congenitally missing and supernumerary teeth + enamel hypoplasia	Oro-facial-digital syndrome type 1	Romero et al., 2007	Spanish
Mutation in PITX2	4q25	Hypodontia, microdontia and/or conical shaped teeth	Rieger syndrome (malformed teeth and underdeveloped eyes)	Reis et al., 2012	American
Mutation in PVRL1/ nectin	11q23.3	Cleft lip/ palate and Hypodontia to oligodontia	Ectodermal dysplasia syndrome	Suzuki et al., 2000	Israel

Table II.4: Genes involved in isolated dental agenesis

GENEs	Chromosome	Mutation effect	Publication	Population
Mutation of EDARADD	1q42.3	**Isolated oligodontia**	Bergendal et al., 2011	Swedish
Mutation of TSPEAR	21q22.3	**Isolated hypodontia**	Dinckan et al., 2018	Turkish
Mutation of DKK1	10q21.1	**Isolated hypodontia**	Dinckan et al., 2018 Liu et al., 2014	Turkish Chinese
Mutation of LAMB3	1q32.2	**Isolated hypodontia**	Dinckan et al., 2018	Turkish
Mutation of LRP6	12p13.2	**Isolated hypodontia**	Ockeloen et al., 2016 Shokeh, 2014	American families Palestinian
Mutation of IRF6	1q32.2	**Isolated hypodontia + Cleft lip with or without palate**	Pegelow et al., 2008	Swedish families
Mutation of EDA	Xq13.1	**Isolated oligodontia**	Song et al., 2009 Bergendal et al., 2011	Chinese Swedish families
Mutation of MSX1	4p16.2	**Oligodontia (Severe congenital dental agenesis)**	Nieminen et al., 1995 Vastardis et al., 1996 Bergendal et al., 2011 Silvia Bowers et al., 2011	American families Swedish American
Mutation of PAX9	14q13.3	**Oligodontia (Severe congenital dental agenesis)**	Stockton et al., 2000 Bergendal et al., 2011 Silvia Bowers et al., 2011	American (Texas) Swedish American

			Koskinen et al., 2019	Finnish family
Mutation of AXIN2	17q24.1	**Dental agenesis and colorectal cancer Oligodontia**	Callahan et al., 2009; Hartsfield et al. 2011 Lammi et al., 2004 (Thesleff) Bergendal et al., 2011 Silvia Bowers et al., 2011	Finnish and German families Finnish Families Swedish American
Mutation of WNT10A	2q35	**Congenital dental agenesis Hypodontia and microdontia Isolated Hypodontia**	Du et al., 2018; Dinckan et al., 2018 Kantaputra and Sripathomsawat, 2011 Abdalla et al., 2014 Zeng et al., 2017 Parveen et al., 2019	Turkish American family Egyptian Chinese Pakistan, Egypt, Saudi Arabia, and Syria

c. Types of dental agenesis

i. Non-syndromic hypodontia in primary dentition

Abnormality in the number of teeth in primary dentition is not as common as in the permanent dentition, with no significant difference exists between both genders. The prevalence of dental agenesis in primary dentition is shown in Table II.5 (Grahnen, 1961; Järvinen and Lehtinen, 1981; Ravn, 1971; Whittington and Durward, 1996).

Mostly one or two teeth are missing, and the majority of cases represent unilateral hypodontia (Coster et al., 2009). A strong correlation exists between hypodontia in the primary and permanent dentitions. Children with hypodontia in the primary dentition nearly always show hypodontia of the successors.(Coster et al., 2009; Thesleff, 2000)

Table II.5: Prevalence of hypodontia in primary dentition in various countries

Author	Year	Country	Prevalence	Tooth most frequently missing
Grahnen and Granath	1961	Sweden	0.4%	Upper lateral incisor
Ravn	1971	Denmark	0.6%	Upper lateral incisor
Brook	1994	Britain	0.3%	---

Jarvinen and Lehtinen	1981	Finland	0.9%	Upper lateral incisor
Magnusson	1984	Iceland	0.6%	Upper lateral incisor
Whittington and Durward	1996	New Zealand	0.4%	Upper lateral incisor
Yonezu et al.	1997	Japan	2.4%	Lower lateral incisor
Carvalho et al.	1998	Belgium	0.4%	Upper lateral incisor

ii. Non-syndromic hypodontia in permanent dentition

- *Prevalence and characteristics*

Various studies have highlighted the prevalence of hypodontia (early studies shown in table II.6) in different countries, showing some variation in populations, on continents and among races (Grahnen, 1961; Haavikko, 1971; Lynham, 1990; Muller et al., 1970; Niswander and Sujaku, 1963)

Table II.6: Prevalence of hypodontia in permanent dentition in various countries

Author	Year	Country	Prevalence
Muller et al.	1970	USA	2.8%
Dolder	1936	Switzerland	3.4%
Muller et al.	1970	North America	3.5-3.7%
Grahnen and Haavikko	1961-1971	Europe	6-8%
Thompson and Popovich	1974	Canada	7.4%
Lynham	1990	Australia	6.3%
Niswander and Sujaku	1963	Japan	6.6%

Recent studies showed similar prevalence values: 4-8% in European Caucasian population, 3.2 -4.6% in North American population, 5.6-11.4% in Spanish population, and 5.5-7.6 in Australian population. (Polder et al., 2004; Tallón-Walton et al., 2010).

Hypodontia is more prevalent in permanent than primary dentition. A higher but not statistically significant predominance in females was reported. (Järvinen and Lehtinen, 1981; Polder et al., 2004; Tallón-Walton et al., 2010)

Early and recent studies confirm that the most frequently missing teeth (excluding third molars) are the mandibular second premolars followed by the maxillary lateral incisors and the maxillary second premolars. (HAAVIKKO, 1971; Polder et al., 2004; Symons et al., 1993; Wang et al., 2018)

iii. Non-syndromic oligodontia in permanent dentition

The prevalence of non-syndromic oligodontia is low compared to the hypodontia and to its higher presence in combination with syndromes. The difference in the frequency of oligodontia between males and females is not statistically significant, nor is the difference in distribution of missing teeth over maxilla/mandible and left/right sides. (Rølling and Poulsen, 2001; Schalk-Van Der Weide and Bosman, 1996).

Isolated oligodontia is inherited in an autosomal dominant form with reduced penetrance. Oligodontia and hypodontia have similar associated anomalies with a tendency toward delayed tooth formation, reduced size of teeth, and taurodontism. (Schalk-Van Der Weide and Bosman, 1996)

iv. Anodontia

Anodontia is defined as congenital lack of all teeth and its isolated form is extremely rare. Some case reports of anodontia have suggested autosomal recessive inheritance. Anodontia occurs as an extreme dental phenotype in ectodermal dysplasia syndromes. (Schalk-Van Der Weide and Bosman, 1996; Stockton et al., 2000). Incisors and premolars are the most frequently missing teeth. Therefore, incisor premolar hypodontia (IPH) is a term used in different studies to describe this form of the anomaly. (Arte, 2001)

v. Syndromic hypodontia

Dental abnormalities are seen in several syndromes together with malformations of other organs. Several syndromes have been described in the literature to be associated with hypodontia or oligodontia (De Coster et al., 2009a). Some of these syndromes are: Ectodermal dysplasia, oral-facial-digital syndromes, syndromes with oral-facial clefting CL/CLP such as Pierre-Robin sequence and Van Der Woude syndrome. In cases of clefting, the most frequent tooth to be missing is the maxillary lateral incisor in the cleft area both in primary and permanent dentitions with higher prevalence in the permanent dentition (De Coster et al., 2009a). Pierre-Robin sequence is associated with the following peri and intra-oral manifestations: CL/CLP, micrognathia, glossoptosis, and 50% prevalence of hypodontia most frequently in the mandibular arch. Van Der Woude syndrome (VWS) one of the most common human autosomal dominant disorders is associated with CL/CLP (1%), pitting of the lower lip mucosa and hypodontia (70%) (De Coster et al., 2009b). Other syndromes were described in earlier studies: Ectodermal dysplasias (ED), Rieger syndrome, Holoprosencephaly, Down syndrome, Wolf-Hirschhorn

syndrome, Kabuki syndrome, Diastrophic dysplasia (DTD), Hemifacial microsomia, and Recessive incisor hypodontia (RIH) (Gorlin et al., 1995; Mhanni et al., 1999; Shapira et al., 2000; Wallis and Muenke, 2000; Wright et al., 1997)

3. Associated dental anomalies to hypodontia

Abnormal function of the genes responsible for the dental development will cause disruption of one or more signaling cascades, which may result in a variety of dental anomalies that can either be generalized or local, and either numerical, morphological and/or structural (De Coster et al., 2009b).

a. Delayed formation and eruption of teeth

Delayed formation and eruption of premolars and molars were reported in children with agenesis of the lower third molar(s) or third molar(s) together with some other teeth (Garn and Burdi, 1971). A mean delay of 1.8 years for boys and 2.0 years for girls in relation to chronological age has been reported.(Rune and Sarnäs, 1974) A tendency to delayed eruption was found in teeth contralateral to the missing teeth (Rune and Sarnäs, 1974). Great individual variation in tooth formation has been noticed in oligodontia patients (Schalk Van Der Weide, 1992). Delay diversity was shown between patients and the delay was more obvious in males than females. (SCHALK VAN DER WEIDE, 1992)

b. Reduction in tooth size and form

Early studies have reported reduction in the mesiodistal and occluso-gingival dimensions of teeth crowns in individuals with hypodontia (Garn and Lewis, 1970; GRAHNEN, 1961a; McKeown et al., 2002). Correlation was present between the number of teeth missing and the crown-size reduction, so that the increased number of missing teeth, the higher percentage of clinically apparent microdontia in remaining teeth crowns (Brook, 1984; Garn and Lewis, 1970; McKeown et al., 2002).

The most frequent example of crown-size reduction associated with hypodontia is the "peg-shaped" maxillary lateral incisor, a mesiodistally and occluso-gingivally reduced tooth (Baccetti, 1998). Significant reciprocal correlation between agenesis of second premolars and decreased overall size of upper lateral incisors (Baccetti, 1998). Different studies considering the frequency of reduced crown length and width of maxillary laterals in cases of missing maxillary premolars and/or lateral incisors suggested that the agenesis and peg-shaping of the maxillary lateral incisors are different phenotypes of one dominant autosomal gene mutation with incomplete or reduced penetrance (Baccetti, 1998; Brook, 1984; McKeown et al., 2002).

c. Malposition of teeth

i. Ectopic maxillary canines

Baccetti et al. reported on non-orthodontically treated individuals that palatally displaced canines had significant reciprocal correlation with reduced size of maxillary

lateral incisors and agenesis of second premolars (Baccetti, 1998). Significantly higher frequencies were observed in the experimental group than the control group (Baccetti, 1998).

Other earlier studies have shown that hypodontia was significantly associated to ectopic permanent canines (palatally displaced), maxillary canine-first premolar transposition or mandibular lateral-canine transposition (Peck et al., 1996; Pirinen et al., 1996; Shapira et al., 2000).

ii. Ectopic eruption of other teeth

In the same aforementioned study, Baccetti et al. reported that ectopic eruption of the first permanent molar(s) was significantly correlated with agenesis of second premolars and reduced size of maxillary lateral incisors (Baccetti, 1998; Shapira et al., 2000).

d. Infra-position of primary molar(s)

Infra-position or infra-occlusion is defined by the lower position of the primary molars compared to the adjacent permanent teeth and this is caused by ankyloses of the primary tooth.(Baccetti, 1998) A reciprocal correlation exists between infra-position of primary molars and agenesis of premolars where absence or agenesis of the succedaneous premolars will not cause root resorption of the primary molars but ankylosis in some cases. (Baccetti, 1998; Symons et al., 1993)

e. Short roots of teeth

Short roots of teeth have a reciprocal association with dental agenesis and it affects mostly the central and lateral incisors and premolars. (Baccetti, 1998; Brook, 1984)

f. Taurodontism

Studies were done on siblings to investigate the association between taurodontism and hypodontia as well as oligodontia and resulted in: mandibular molars are the most affected and more in oligodontia than hypodontia. (Rølling and Poulsen, 2001; SCHALK VAN DER WEIDE, 1992; Schalk-Van Der Weide and Bosman, 1996)

g. Rotation of premolars and/or maxillary lateral incisors

The most relevant study that illustrated the rotation of the premolars and the maxillary lateral incisors associated with dental agenesis was conducted by Baccetti in 1998 (Baccetti, 1998). In this study the authors compared a group of affected patients with dental agenesis combined to different dental anomalies with the control group (normal patients). The study revealed that the occurrence of tooth rotation in association with agenesis of second premolars and/or maxillary lateral incisors was significantly higher than in the control group for all the categories of tooth rotation.(Baccetti, 1998) Its frequency is higher with the agenesis of maxillary lateral incisors. It was reported as well that rotation of

the contralateral lateral incisor is associated with the agenesis of unilateral maxillary lateral incisor and the same for the maxillary premolars. (Baccetti, 1998)

h. Enamel hypoplasia and hypocalcification

Different studies highlighted the association between hypodontia or oligodontia and enamel imperfections and defects (enamel hypoplasia, hypocalcification, and dentino-genesis imperfecta) (Baccetti, 1998; Garib et al., 2010; Kotsomitis et al., 1996; Thesleff, 2000). Bacceti in his study (1998) included enamel hypoplasia in seven types of dental anomalies and concluded that it presented significant associations with agenesis of the second premolars, small size of the upper lateral incisors, infraocclusion of the primary molars, and palatal displacement of the upper canines. (Baccetti, 1998)

4. Dental Supernumerary or "Hyperdontia"

a. Definition and prevalence

Supernumerary teeth known for "hyperdontia" refers to the presence of extra teeth compared to a normal dentition (Wang et al., 2018). Hyperdontia may occur in both dentitions, unilaterally or bilaterally and in both jaws (GRAHNEN, 1961b; Rajab and Hamdan, 2002). Hyperdontia in the primary dentition is overlooked because supernumerary teeth are often of normal shape, erupt normally and appear to be in proper alignment and can be mistaken for gemination or fusion anomalies. Anterior primary supernumerary teeth would erupt and exfoliate normally prior to detection. (Nazif et al., 1983).

Hyperdontia may occur in any region of the dental arch with a particular predilection for the premaxilla (Nazif et al., 1983; Rajab and Hamdan, 2002). The reported prevalence of supernumerary teeth in the general Caucasian population for the permanent dentition ranges from 0.1 to 3.8%. (MCKIBBEN, 1971; Paterson and Thomas, 2000; Ravn, 1971; Stellzig et al., 1997). Supernumerary teeth seem to be more common in Mongoloid racial groups, with a frequency higher than 3% (Davis, 1987; Niswander and Sujaku, 1963). The prevalence of supernumerary teeth is lower in the primary dentition and is reported to range between 0.3 and 0.8% (Nazif et al., 1983; Ravn, 1971).

Although no difference in the sex distribution exists in the primary dentition, supernumeraries occur more frequently in the permanent dentition of boys than girls. (Brook, 1984; Ravn, 1971) The frequency of supernumerary teeth was reported as follow: upper lateral incisors (50%), mesiodens (36%), upper central incisors (11%), followed by bicuspids (3%). Whereas in the mandible, the premolar region was the more vulnerable for hyperdontia followed by the anterior region (Fukuta et al., 1999; Rajab and Hamdan, 2002; TANAKA, 1998).

Single supernumerary teeth occur in 76-86% of cases, double supernumeraries in 12-23% of cases and multiple supernumeraries in less than 1% of cases (GRAHNEN, 1961b; Rosenzweig and Garbarski, 1965).

b. Etiology

While the etiology of hyperdontia remains unclear, heredity is believed to be a major factor behind supernumerary tooth formation. It has been suggested that supernumerary teeth may be associated with autosomal recessive heredity, with lower penetrance in females (Niswander and Sujaku, 1963). However, few case reports have proposed a low frequency of autosomal dominant inheritance (Batra et al., 2005; Orhan et al., 2006; Wang et al., 2007). Several articles support the idea that genetic component is needed for development of supernumerary teeth (Anthonappa et al., 2013; Fleming et al., 2010; Kangas et al., 2004).

Originally, it was theorized that supernumerary teeth formation was nature's way of restoring teeth that had been lost during the process of evolution, a third incisor, a third premolar, or a fourth molar (Anthonappa et al., 2013).

Several theories behind the etiology of supernumerary teeth have been investigated in previous studies. One of these theories was *dichotomy*, which claims that the developing tooth bud may be divided to form a supernumerary tooth. Another theory, the *hyperactivity of the dental lamina* has also been suggested as a possible factor (Anthonappa et al., 2008; Khambete and Kumar, 2012).

Genes associated with syndromic and isolated supernumerary teeth are reported in the following tables. (Table II.7-8). In grey are the most common gene associated either with syndromic or isolated supernumerary teeth.

Table II.7: Genes involved with syndromic supernumerary teeth

GENEs	Chromosome	Mutation effect	Syndrome/ condition	Publication	Population
Mutation FAM20A	17q24.2	Supernumerary teeth + amelogenesis imperfecta	Autosomal anhidrotic ectodermal dysplasia syndrome	Kantaputra et al., 2014	Thailand
Mutation of IKBKG/NEMO	Xq28	Multiple supernumerary teeth + cleft palate	Bloch-Sulzberger syndrome: "Incontinentia pigmenti (IP)"	Aradhya et al., 2001 Hull et al., 2015	US/UK 1 Patient: Czech Republic (mother) and Tanzanian Indian (father)
Mutation of RUNX2	6p21.1	Multiple supernumerary teeth	Cleidocranial dysplasia	Suda et al., 2007 Ma et al., 2018	Japanese Chinese
Mutation of EVC, EVC2	4p16.2	Supernumerary teeth and polydactyly	Ellis-Van Creveld	Temtamy et al., 2008	Egyptian
Mutation of APC	5q22.2	Multiple impacted and supernumerary teeth and jaw osteomas	Gardner syndrome	Yu et al., 2018	Chinese
Mutation in IL11RA	9p13.3	Multiple supernumerary teeth, delayed tooth eruption, maxillary hypoplasia and digit abnormalities	Craniosynostosis Crouzon-like craniosynostosis syndrome	Nieminen et al., 2011 Korakavi et al., 2019	Pakistani families Finnish Europeans
Mutation of FGFR2	10q26.13	Mesiodens + delayed dental development and cleft palate	Crouzon syndrome	Glaser et al., 2000	British Families
Mutation of NHS	Xp22.2	Supernumerary teeth and dental dysmorphology	Nance-Horan	Burdon et al., 2003	Australian
Mutation of PTPN11	12q24.13	Supernumerary teeth	Noonan syndrome	Bentires-Alj et al., 2004	American (Boston)
Mutation of OFD1	Xp22.2	Supernumerary teeth and abnormal size and shape	Oro-facial-digital type I	Ferrante et al., 2001	Italian families
Mutation of RECQL4	8q24.3	Supernumerary teeth, dental dystrophy, hair and skin problems	Rothmund-Thomson syndrome	Kitao et al., 1999	Japanese
Mutation of DYRK1A	21q22.13	Supernumerary teeth, neonatal teeth, extreme calculus, delayed primary dentition	DYRK1A syndrome	Van Bon et al., 1993 Bwm et al., 2015	American
Mutation of SOX2	3q26.33	Supernumerary impacted teeth	Anophthalmia syndrome	Numakura et al., 2010	Japanese patient
Mutation of TRPS1	8q23.3	Supernumerary teeth and enamel hypermineralisation	Trichorhinophalangeal	Kantaputra et al., 2008	Thailand

Table II.8: Genes involved with isolated supernumerary teeth

GENEs	Chromosome	Mutation effect	Publication	Population
Mutation of AGRN	1p36.33	**Isolated supernumerary teeth**	**Takahashi et al., 2017**	**Japanese families (19 Genes in 4 families: 2 Generations per family, 2 affected members in 3 families and 4 affected members in the 4th family)**
Mutation of ATXN1	6p22.3			
Mutation of CDH26	20q13.33			
Mutation of CFB	6p21.33			
Mutation of EFCAB5	17q11.2			
Mutation of EXOC3L4	14q32.32			
Mutation of FANCE	6p21.31			
Mutation of FMNL1	17q21.31			
Mutation of FXYD4	10q11.21			
Mutation of HMCN1	1q25.3			
Mutation of IGSF9B	11q25			
Mutation of MGA	15q15.1			
Mutation in KIAA1614	1q25.3			
Mutation of PLCH2	1p36.32			
Mutation of PKD1L2	16q23.2			
Mutation of RNF207	1p36.31			
Mutation of TEX15	8p12			
Mutation of TKTL1	Xq28			
Mutation of TUSC1	9p21.2			
Mutation of SOSTDC1	7p21.2	**Isolated supernumerary teeth**	**Arikan et al., 2018**	**Turkish**

c. Classification

Supernumerary teeth may be classified according to morphology and location (Brook, 1984). In the primary dentition, the morphology is usually normal or conical. The morphology of supernumerary teeth in the permanent dentition is more variable, and four morphological types have been described: *Conical*, *Tuberculate*, *Supplemental*, and *Odontoma*. (Rajab and Hamdan, 2002). According to their locations, supernumerary teeth are classified as follow: Mesiodens, paramolar and distomolar. (Rajab and Hamdan, 2002) Mesiodens is the most frequent type of supernumerary tooth, multiple supernumerary teeth are rare in individuals with no syndromes associated (Rajab and Hamdan, 2002; Varela et al., 2009).

d. Concomitant hypo/hyperdontia "CHH"

The condition of Hypodontia and hyperdontia occurring in the same individual has been described as "concomitant hypodontia and hyperdontia" (CHH) by Camilleri or simply "concomitant hypo-hyperdontia" (Camilleri, 1967). The prevalence of CHH is very rare, while hypo-hyperdontia distributed in one family into different generations or within one generation is more prevalent although rare studies were published on these cases. (Camilleri, 1967)

B. Genome, genotype and genes

1. Human genome

An organism's genome is defined as the complete set of genetic instructions for that organism. The human genome is made up of a double helix of deoxyribonucleic acid (DNA) comprised of 3.2 billion chemical nucleotide base pairs. The genetic instructions, or DNA code(s), are created by the particular side-by-side arrangement (linear pattern, order, and number) of purines: adenine (A) and guanine (G), and pyrimidine nucleotides: thymine (T) and cytosine (C) along the paired double helix, where A base pairs with T, and C base pairs with G. This genetic information (DNA) is normally packaged in each human cell into 46 smaller units (ranging in length from 50 to 250 million base pairs each) called chromosomes, which are arranged in 23 pairs (Human Genome Program, 2008).

A chromosome is made up of the double helical DNA that is wrapped around proteins called histones. Those proteins enable the DNA units to be tightly packed into the nucleus of the cells and also play an important role in regulating when and where the cells will use portions of the genetic information contained in the genome (Golbabapour et al., 2011). Each human being inherits a total of 46 chromosomes; 22 homologous pairs of chromosomes called autosomes and one pair of sex chromosomes that are homologous (X, X) in females and only partly homologous (X, Y) in males, which make the individual unique. Each pair is formed by one chromosome that is a copy of the original maternal chromosome and another chromosome that is a copy of the original paternal chromosome. (Human Genome Program, 2008)

2. Genes

Chromosomes are microscopic elements present in the eukaryotic cell nucleus and are the carriers of genetic information. Each chromosome contains many genes, which represent the smallest physical and functional units of inheritance and allow their distribution. Genes are specific sequences of bases that encode instructions for the synthesis of a specific polypeptide via a messenger RNA intermediate (mRNA) or the synthesis of a specific RNA molecule (e.g., transfer RNA, ribosomal RNA, and noncoding regulatory RNA molecules such as microRNA or long noncoding RNA). In fact, most codons (sequence of three nucleotides) in the mRNA lead to the addition of an amino acid to a growing polypeptide chain, which may ultimately become a protein (Figure II.2). Each person normally inherits two copies of every gene within the genome: one gene copy on the autosome or sex chromosome of maternal origin and the other gene copy on the autosome or sex chromosome of paternal origin (Hartsfield, 2011).

Our genes only make up 2% of the estimated 3.2 billion base pairs present in the human genome and the average gene is 3000 nucleotide base pairs in length. The remainder consists of non-coding regions (Human Genome Program, 2008). Every gene resides in a specific location referred to as a locus. Genes at the same locus on a pair of homologous chromosomes are called alleles, these terms imply that for a given character transmitted in a Mendelian way, each individual has two physical versions of the same hereditary element "gene".(Allen, 2003) One allele would be a copy of the maternal allele and the other a copy of the paternal allele.(Allen, 2003) When the two alleles are identical, the individual is said to be homozygous for that locus. When the two alleles are different in the DNA sequence,

the individual is said to be heterozygous for that locus (Hartsfield, 2011). The gene is simultaneously a unit of function and transmission, a unit of recombination, and of mutation (Allen, 2003).

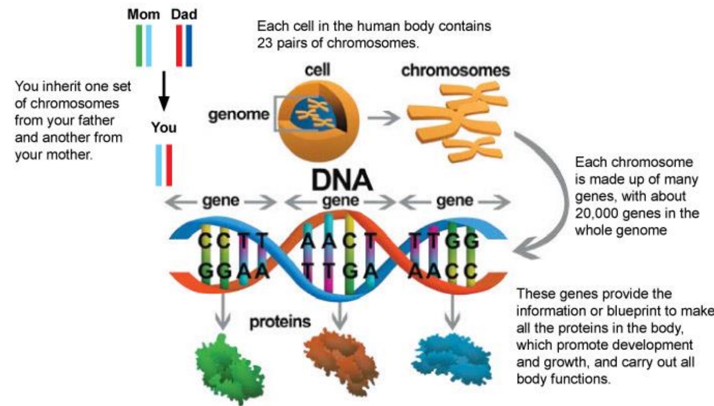


Figure II.2: From DNA, gene to protein

3. DNA variations

The human genome is 99.9% identical from one person to another. Thus, there is only an estimated 0.1% variation within the entire DNA code between two people that makes each individual unique. The DNA variation is due to either normal inherited variations or sporadic mutations.

The most common DNA variation in the human genome is called Single Nucleotide Polymorphism (SNP; pronounced “snip”). SNP describes the occasion when more than one nucleotide base (A, G, T, or C) can be inherited at a specific location in the DNA code upon comparing the DNA codes at that same position among many individuals. In other words, it is a variation in a single nucleotide that occurs at a specific position in the genome. There are over 10 million SNPs that have been identified in the human genome to date; 1 SNP occurs every 300 nucleotides. An example of a SNP is a frameshift mutation

(also called framing error or reading frame shift) caused by INDELS (Insertions or Deletions) of a number of nucleotides in a DNA sequence that is not divisible by three. Due to the triplet nature of gene expression by codons, the insertion or deletion can change the reading frame (grouping of codons), resulting in a completely different translation from the original.

Nonsense mutation is also an example of a SNP, defined as a point mutation in a DNA sequence that results in a premature stop codon (or termination codon) within the mRNA that signals a termination of translation into proteins. Those two types of mutations are called “disruptive mutations”, having a high putative impact on protein function and structure. Another example of a SNP is a missense mutation defined as a point mutation in which a single nucleotide changes results in a codon that codes for a different amino acid. This type of mutation has a moderate putative impact on protein function and structure (Human Genome Program, 2008).

4. Genotype

A genotype refers to the combination of alleles at a given locus within the genome that codes for a particular trait. Two organisms whose genes differ at even one locus are said to have different genotypes. The transmission of genes from parents to descendants is controlled by precise molecular mechanisms which was discovered by Gregor Mendel (Allen, 2003; Hartsfield, 2011; Chandra S. Pareek et al., 2011).

5. Phenotype

The phenotype can be thought of as a clinical expression of an individual's specific genotype. It is the observable properties, measurable features and physical characteristics of an individual (Baltimore, 2001). It is created by summation of the effects arising from an individual's genotype and the environment in which the individual develops over a period of time.

Dental agenesis is a trait, which is a particular aspect or characteristic of the phenotype that has a specific mode of inheritance. The genetic influences on traits are monogenic (predominantly single gene with the possibility of other smaller genetic and environmental factors) or complex (many genetic and environmental factors) (Hartsfield, 2011; Chandra S. Pareek et al., 2011).

6. Modes of inheritance and penetrance

a. Modes of inheritance

The nature of family-based (familial) traits can be studied by constructing family trees called pedigrees in which, it shows how individuals within a family are related to each other and indicate which individuals have a particular trait or genetic condition. The standard way to read pedigrees, squares denote males and circled denotes females. The affected individuals having a particular genetic trait will have their symbol filled in black. Three generations are usually drawn in pedigrees and numbered following the roman

numerals. The first or top generation “I” represents the grandparents, the second generation “II” represents the parents or children, aunts, uncles. The third generation “III” represents the siblings and cousins or grandchildren. It is important when we draw pedigrees that we try to put in as much information as possible (Figure II.3). (Allen, 2003; Pemberton, 2008)

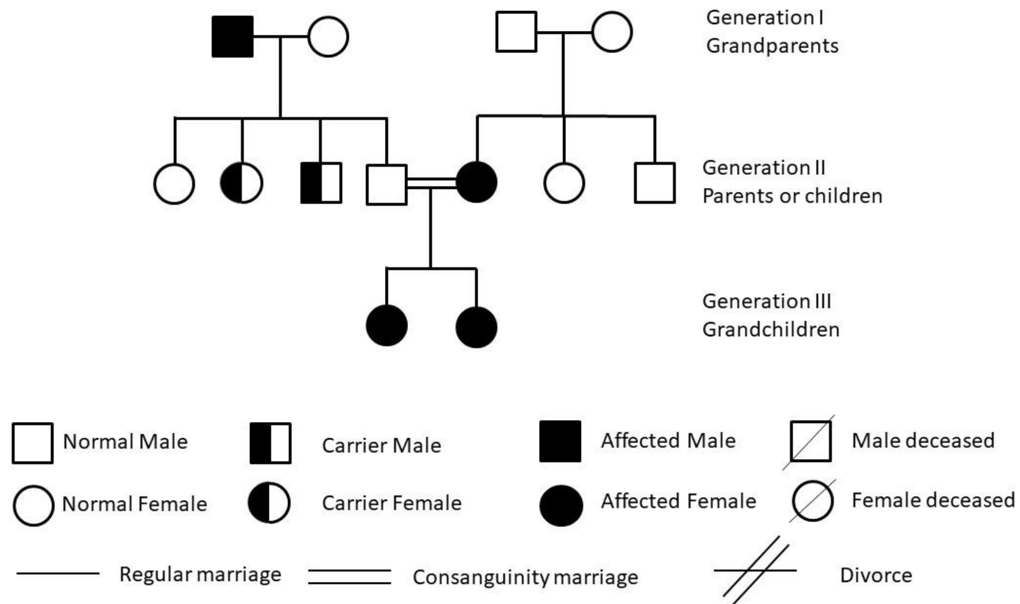


Figure II.3: Pedigree template

i. Autosomal dominant

The mode of inheritance is autosomal dominant when a trait is present as the result of only one copy of a particular allele (example: “A”) in a heterozygous allele pair

(example: “Aa”). The trait is also expressed in the presence of a homozygous allele pair (example: “AA”) (Figure II.4) (Hartsfield, 2011)

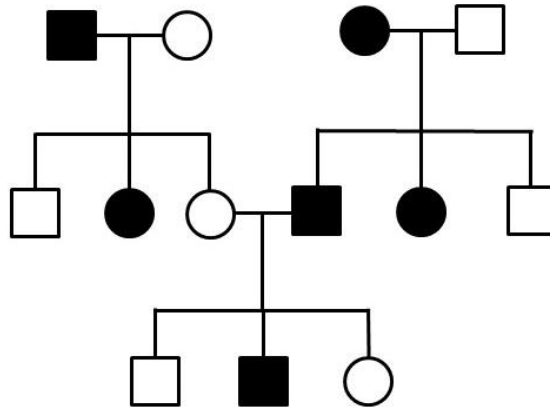


Figure II.4: Three-generation pedigree of a family with an autosomal dominant trait

ii. Autosomal recessive

The mode of inheritance is autosomal recessive when a trait is only present when both alleles at the locus are the same (example: “aa”); in other words, when the individual is homozygous for “a”. The symbols for presumed carriers (heterozygotes) of the autosomal recessive gene are filled in halfway (Figure II.5) (Hartsfield, 2011).

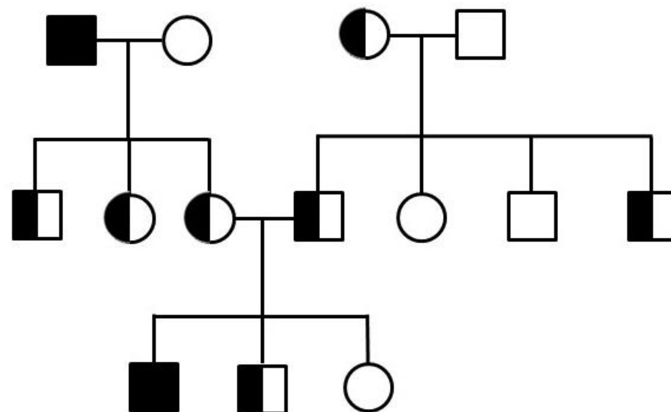


Figure II.5: Three-generation pedigree of a family with an autosomal recessive trait

iii. X-linked recessive

The mode of inheritance is X-linked recessive when a mutation in a gene on the X chromosome causes the phenotype to be expressed in males (who are necessarily hemizygous for the gene mutation because they have one X and one Y chromosome) and in females who are homozygous for the gene mutation. The symbols for presumed female carriers (heterozygotes) of the X-linked recessive gene have a dot in the middle of the circle (Figure II.6) (Hartsfield, 2011).

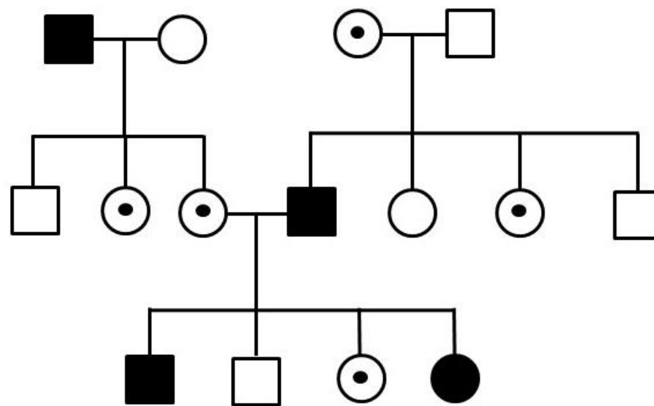


Figure II.6: Three-generation pedigree of a family with an X-linked recessive trait

iv. X-linked dominant

The mode of inheritance is X-linked dominant when a mutation in a gene on the X chromosome causes the phenotype to be expressed in males (who are necessarily hemizygous for the gene mutation because they have one X and one Y chromosome) and in females who are homozygous or heterozygous for the gene mutation (Figure II.7). (Hartsfield, 2011)

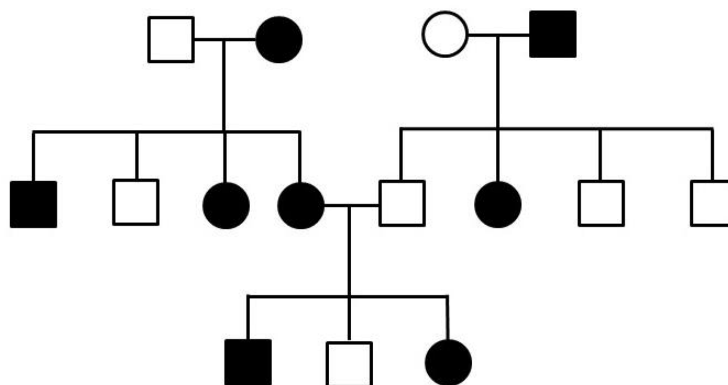


Figure II.7: Three-generation pedigree of a family with an X-linked dominant trait

Table II.9: Summary of the modes of inheritance

Males and females affected	Equally	Equally	Males more than females
Phenotype appearance	Every generation	Typically appears in one generation and not in the individual's offspring or parents	----
Probability of inheritance	Offspring have a 50% chance of inheriting the trait	Offspring have a 25% chance of inheriting the trait if both parents are carriers	Carrier females have 50% chance of having an affected son and 50% chance of having a carrier daughter

b. Modes of penetrance

Penetrance is the proportion of individuals carrying a particular variant of a gene that also expresses an associated trait.

- *Complete penetrance:* when all individuals who have the trait-causing mutation have clinical symptoms of the trait. In other words, whoever is carrying the gene variant will develop the disease.

- *Highly penetrance:* when the trait is almost always apparent in an individual carrying the allele. (Baltimore, 2001; Zlotogora, 2003)
- *Incomplete penetrance or reduced penetrance*: when some individuals fail to express the trait, even though they carry the allele. Those individuals are able to have offspring with the trait. In other words, if some people have a particular gene variant that is known to be associated with a disease, yet they never get the disease.
- *Low penetrance:* it will only sometimes produce the trait with which it has been associated at a detectable level (U.S. National Library Of Medicine, 2016). (Baltimore, 2001; Zlotogora, 2003)

c. Measures of heritability

Heritability is a static used in genetics to estimate how much variation in a phenotypic trait in a population is due to genetic variation among individuals in that population. Heritability increases when genetic factors are contributing more variation or when non-genetic (environmental) factors are contributing less variation.

H^2 is a common measure of heritability, which reflects a specific mathematical formula that embeds all the genetic contributions to a population's phenotypic variance. The values of H^2 range between 0 and 1. A trait with a heritability estimate of 1 would be expressed with complete positive correlation to genotypic factors theoretically, as measured by comparing the concordance of the phenotype to the percentage of genes in common among twins or other siblings. By comparison, a trait with a heritability of 0.5 would have half its variability of concordance (from individual to individual) positively correlated with

the percentage of genes in common. $H^2 \geq 0.5$ reflects a high heritability and $H^2 \leq 0.2$ suggests a low heritability (Allen, 2003; Baltimore, 2001; Hartsfield, 2011).

7. Sanger and next generation sequencing techniques

DNA sequencing is the process of determining the precise order of nucleotides (adenine, guanine, cytosine and thymine) within a DNA molecule.

a. Sanger sequencing

Developed by Frederick Sanger and his colleagues in 1977, the Sanger sequencing technique is based on the selective incorporation of chain-terminating dideoxy-nucleotides by DNA polymerase during in vitro DNA replication. It requires a single-stranded DNA template (DNA to be sequenced), a DNA primer (starting point for DNA synthesis on the strand of DNA to be sequenced), a DNA polymerase, normal deoxy-nucleoside triphosphates (dNTPs), and modified dideoxynucleoside triphosphates (ddNTPs) that terminate DNA strand elongation (chain terminators).

Four individual's DNA synthesis reactions are performed. The four reactions include normal A, G, C, and T dNTPs and each contains a low level of one of four ddNTPs: ddATP, ddGTP, ddCTP, or ddTTP. The four reactions can be named A, G, C and T, according to which of the four ddNTPs was included. The DNA to be sequenced is added to the 4 reactions. Most of the time, DNA polymerase will add a proper dNTP to the growing strand it is synthesizing in vitro, but at random locations, it will instead add a

ddNTP. When it does, that strand will be terminated at the ddNTP just added. If enough template DNAs are included in the reaction mix, each one will have the ddNTP inserted at a different random location, and there will be at least one DNA terminated at each different nucleotide along its length for as long as the in vitro reaction can take place. The ddNTPs that terminate the strands have specific fluorescent labels covalently attached to them. After the reaction is over, it is subject to capillary electrophoresis.

All the newly synthesized fragments, each terminated at a different nucleotide and so each a different length, are separated by size. Smaller fragments will migrate more. As each differently sized fragment exits the capillary column, a laser excites the fluorescent tag on its terminal nucleotide. From the color of the resulting fluorescence, a computer can keep track of which nucleotide was present as the terminating nucleotide. The computer also keeps track of the order in which the terminating nucleotides appeared, which is the sequence of the DNA used in the original reaction (Sanger et al., 1977).

b. Next generation sequencing “NGS”

In 2009, Next Generation Sequencing (NGS) or deep sequencing platform appeared on the market as a revolutionary technology that performs sequencing of millions of small DNA fragments in parallel “massive parallel sequencing”. NGS can be used to sequence entire genomes (Whole Genome Sequencing: WGS) or constrained to specific areas of interest, including a whole exome (all 22 000 coding genes) (Whole Exome Sequencing: WES) or small numbers of individual genes.

NGS has many advantages over the traditional sequencing technique known as Sanger sequencing: much faster, captures a broader spectrum of mutations, produces more data, genomes can be interrogated without bias, needs significantly less template DNA and is cost effective. However, the Sanger method remains in wide use for smaller-scale projects, validation of NGS results and for obtaining especially long contiguous DNA sequence reads (> 500 nucleotides) (Behjati and Tarpey, 2013; Chandra Shekhar Pareek et al., 2011). The technique of NGS will be described in details in the material and methods section.

8. *Terms related to the genetic analysis*

a. Quality score

The quality score (Q score) is used to measure base calling accuracy, one of the most common metrics for assessing sequencing data quality. It reveals how much of the data from a given run is usable in a resequencing or assembly experiment. Sequencing data with lower quality scores can result in a significant portion of the reads being unusable, resulting in wasted time and expense. Low Q scores can lead also to increased false-positive variant calls, resulting in inaccurate conclusions and higher costs for validation experiments. For example, Q20 means that 90% of the fragments have 20 copies read and more and Q30 means that 90% of the fragments have 30 copies read and more (Human Genome Program, 2008).

b. Filter status

Prior to cluster analysis or genetic network analysis it is customary to filter, or remove genes considered being irrelevant from the set of genes to be analyzed. The filter status is marked “PASS” if this position has passed all filters, i.e., a call is made at this position. Otherwise, if the site has not passed all filters, a specific code for filters that fail is written (Human Genome Program, 2008).

c. Putative impact

The putative impact aims to check if the variant has a deleterious effect on protein function and structure or not. It is classified into high, moderate or low (Human Genome Program, 2008).

d. Allele count and Minor Allele Frequency (MAF)

The allele count is defined as the count of each alternate allele for each site across all samples. The MAF determines the frequency of a minor variant of the gene at a particular locus in the normal population, obtained by dividing the number of appearances of the minor variant by the total number of alleles. An MAF of 0% indicates that the variant is not present in the normal population (new variant) and an MAF of 100% indicates that the variant is present in the normal population (Human Genome Program, 2008).

e. Approximate read depth

The approximate read depth (sequencing depth or coverage) is obtained by using the following formula: number of reads x read length / assembly size; number of reads meaning the number of times that a given nucleotide in the genome has been read in the reconstructed sequence and assembly size meaning the size of the genome that is sequenced. Deep sequencing refers to the general concept of aiming for high number of replicates reads of each region of a sequence (Human Genome Program, 2008).

f. Inbreeding coefficient

The inbreeding coefficient (F) is the probability of auto-zygosity. Inbreeding is evident when alleles in an individual are identical by descent (IBD) in other words (F) is the probability that a zygote obtains the two identical alleles at a locus from consanguineous parents (related parents). In contrast identical alleles arising from mutations don't count as being IBD.I (Human Genome Program, 2008).

g. Yield

The yield is defined as the total number of sequences for one individual. The total yield is calculated by using the following formula: total number of reads * average read length (Human Genome Program, 2008).

h. GC-content

GC-content (or guanine-cytosine content) is the percentage of nitrogenous bases on a DNA molecule that are either guanine or cytosine (from a possibility of four different ones, also including adenine and thymine). DNA with high GC-content is more stable than DNA with low GC-content (Human Genome Program, 2008).

C. Inheritance pattern

The inheritance pattern of dental agenesis was reported in different studies as being: Autosomal dominant or recessive for some genes and X-linked for others. (De Coster et al., 2009a; van den Boogaard et al., 2000; Vieira et al., 2004)

A study conducted by wang et al. in 2017 on 21 CHH subjects revealed that a recessive inheritance or a dominant inheritance with incomplete penetrance was observed. The variability in the pattern of missing teeth combined with the supernumerary suggested a significant influence from environmental factors and potential genetic modifiers on the disease expressivity and phenotype (Wang et al., 2018).

This hypothesis was supported by the phenotypic variance from identical twin cases included in this study. Despite their identical genetic backgrounds, one boy had 3 supernumeraries and missing tooth number 29, while the other had 2 supernumeraries and missing tooth number 20, this confirms the environmental contribution to the condition. Isolated hyperdontia and CHH might result from the same genetic mutation but be

phenotypically affected by environmental factors and potential genetic modifiers. (Wang et al., 2018)

D. Genetic studies

PAX9 has been identified as a key controlling factor during dental morphogenesis process and highly expressed specifically at the prospective sites of all teeth prior to any morphological signs of odontogenesis (Vieira et al., 2004). MSX1 plays a major role in the development of ectodermal derivatives and strongly expressed in the dental mesenchyme (Vieira et al., 2004). Signaling molecules that determine the position and the shape of the teeth are MSX1, MSX2, DLX1, DLX2, BARX1, and PAX (Miletich and Sharpe, 2003).

PAX9 and MSX1 have been reported to have an important regulatory role in the maintenance of BMP4 expression and signaling *(BMP signaling regulates the progression of tooth development from the lamina to the bud stage by controlling Cyclin-D1 expression and thereby cell proliferation in the dental epithelium, and by maintaining the odontogenic fate of dental epithelium),* therefore it is suggested that they have a role in odontogenic potential shifts (Miletich and Sharpe, 2003).

A variety of dental anomalies either morphological, numerical, and/or structural in nature may result due to abnormal function of these specific proteins. Depending on the molecule and its timing of required expression in either (or both) the oral epithelium and adjacent mesenchyme, tooth primordia may be absent (Wnt, p63), or tooth development may be arrested at the bud stage (Lef1, Msx1, Msx2, Pax9, Pitx2) or at the cap/bell stage

(Cbfa1/Runx2) *(Table II.10)* (Das et al., 2002; D'Souza et al., 1999; Lin et al., 1999; Sarkar and Sharpe, 1999; Satokata et al., 2000; Zhao et al., 2000).

Studies in mutant mice have demonstrated that tooth development is arrested at the bud stage in both PAX9 and MSX1, suggesting they have similar, but non-redundant roles in signal progression to the cap stage of tooth development.(Das et al., 2002; Vieira et al., 2004, p. 9).

Table II.10: Protein factors involved in specific stage of tooth development

Stage of tooth development	Protein factors involved in signaling from epithelium	Protein factors involved in signaling from mesenchyme
Initiation Stage ↓ Bud Stage	Fgfs, Bmps, Shh, Pitx2 and Wnts	Pax9, Ptc, Msx1, Msx2, Bmp4, Lhx6, Lhx7, Lef1, Dlx1, Dlx2, Gli1, Gli2, Gli3 and Barx1
Bud Stage ↓ Cap Stage	Bmp, Fgf, Wnts, Shh, Pdgf, p21, Msx2, Lef1 and Tgf-β	Pax9, Bmp, Dlx1, Dlx2, Lhx6, Lhx7, Msx1, Lef1, Gli1, Gli2, Gli3, Barx1 and Fgfs
Cap Stage ↓ Bell Stage	Bmp, Fgf, Wnts, Shh, Pdgf, p21, Msx2, Lef1 and Tgf-β	Pax9, Bmp, Dlx1, Dlx2, Lhx6, Lhx7, Msx1, Lef1, Gli1, Gli2, Gli3, Barx1, Bmp4, Msx2 and Fgfs

Different studies revealed defects in various genes that encode transcription factors as MSX1 and PAX9, or genes that code for a protein involved in the canonical Wnt signaling "AXIN2", and a transmembrane receptor of fibroblast growth factors (FGFR1). Protein products of genes that encode transcription factors MSX1 and PAX9, are responsible for the crosslink between dental tissues and are essential for the establishment of the odontogenic potential of the mesenchyme (Peters and Balling, 1999).

MSX1 and PAX9 interact during odontogenesis at both the gene and protein level and are intimately involved in the genetic networks regulating tooth development. PAX9 forms a physical association with MSX1, which enhances the ability of PAX9 to activate both MSX1 and mesenchymal Bmp4 gene expression during tooth development. This interaction dictates morphogenesis of the dental organ, more in particular, the transition from bud to cap stage during tooth morphogenesis and enamel knot induction at the late cap stage (Ogawa et al., 2005).

Besides Bmp4 downregulation, PAX9 mutations could result in a selective reduction in PAX9 binding to sites that regulate MSX1 expression levels. Mutations in either PAX9 or MSX1 might also lead to defective protein–protein interactions, both at the gene and protein levels that disrupt normal downstream functions important for tooth development (Ogawa et al., 2005).

CHAPTER III

MATERIAL AND METHODS

A. Target population

1. General characteristics

This is a prospective case-control study including 16 Mediterranean families known to include subjects diagnosed with hypodontia/hyperdontia over 2 generations. The approached probands were either ongoing or had previous orthodontic treatment at the Division of Orthodontics and Dentofacial Orthopedics of the American University of Beirut Medical Center, AUBMC. The pedigrees were drawn for the families in concern.

Nine families, including 37 subjects, accepted to undergo a detailed data and biospecimen collection for the genetic analysis. IRB approval was granted before initiation of the study for both levels of investigation (Protocol Number: BIO-2019-0464). Privacy of the subjects was only accessed by the research group members; therefore, there was no potential risk of breach of confidentiality. The research was funded by the Medical Practice Plan (MPP) and the University Research Board (URB).

2. Inclusion criteria

The approached families were part of the Mediterranean population and comprised several affected individuals over at least 2 generations. The inclusion criteria were congenital agenesis of at least one tooth, not including third molars, as verified by clinical examination, radiographs and dental history. Individuals with supernumerary of at least one

tooth were also enrolled in a separate category. Non-affected relatives for both groups were additionally included as controls.

3. Exclusion criteria

Instances of tooth agenesis adjacent to a cleft site were not included, because the absence of such teeth is likely to be the consequence of local developmental anomalies at the cleft site. Subjects having congenital disorders and syndromes were excluded.

B. Families selection and recruitment process

Twenty-Six probands from 16 families, belonging to the Mediterranean population and diagnosed with hypo/hyperdontia, were identified from the pool of patients at the Division of Orthodontics and Dentofacial Orthopedics at AUBMC due to a previous or ongoing orthodontic treatment.

The recruitment process for the detailed data and biospecimen collection came as follows:

a- The probands were first approached by their treating orthodontist, then subsequently addressed by the study coordinator and asked if they are willing to participate in the research project.

b- Subjects who agreed to enroll were detailed more about the study, handed a written consent form and given enough time to sign it in a private environment. Consent forms were adapted to each age category. (Child between 7-12 years, adolescent between 13-17 years, adult above 18 years and parental consents). The consent form enclosed

information about the aims of the study, procedure, risks and benefits and a confidentiality section.

c- Detailed information on demographics, medical history, affection status of other individuals within the family etc. was gathered

d- Flyers (invitation to participate in a study) were handed to the probands to distribute them to their affected and non-affected relatives. The flyers included general information about the study.

e- Interested relatives were requested to contact the research team (at (01) 350 000 ext. 5706) to come to the Division of Orthodontics and Dentofacial Orthopedics, AUBMC.

f- Affected and non-affected subjects underwent the data and biospecimen collection procedure that consisted of a thorough clinical examination, a panoramic radiograph and the collection of 5cc of blood.

Nine Mediterranean families (Lebanese) including 37 subjects (14 males, 23 females; 25 affected, 12 non-affected) consented to participate and undergo the data and biospecimen collection. Each one of the 37 selected subjects was assigned a specific code that includes the family code (A-I) followed by a number (1-6). Among those families:

- 5 (A, B, C, D and E) had isolated dental agenesis of <6 teeth missing,
- 2 families (F and G) had members with either dental agenesis of <6 teeth missing or supernumerary,
- In family H, one of the two members who had dental agenesis, presented with oligodontia (≥ 6 teeth missing). Another member within the same family had hyperdontia.

- Family I exhibited 3 affected members with Hyperdontia (Table III.1-III.2).

Table III.1: Distribution of the phenotypes in the 9 recruited families

Family	AFFECTED		NON-AFFECTED
	HYPO	**HYPER**	
A	2		1
B	2		2
C	2		2
D	3		1
E	3		1
H	2 *(1 oligo)*	**1**	0
	14		**7**
F	2	**1**	1
G	3	**1**	2
	5	**3**	**3**
I		3	2
			5
Total	**19**	**6**	**10 (12)**
TOTAL	**37**		

individuals having hypodontia

individuals having hyperdontia

Table III.2: Summary of the demographic characteristics of the 9 recruited families

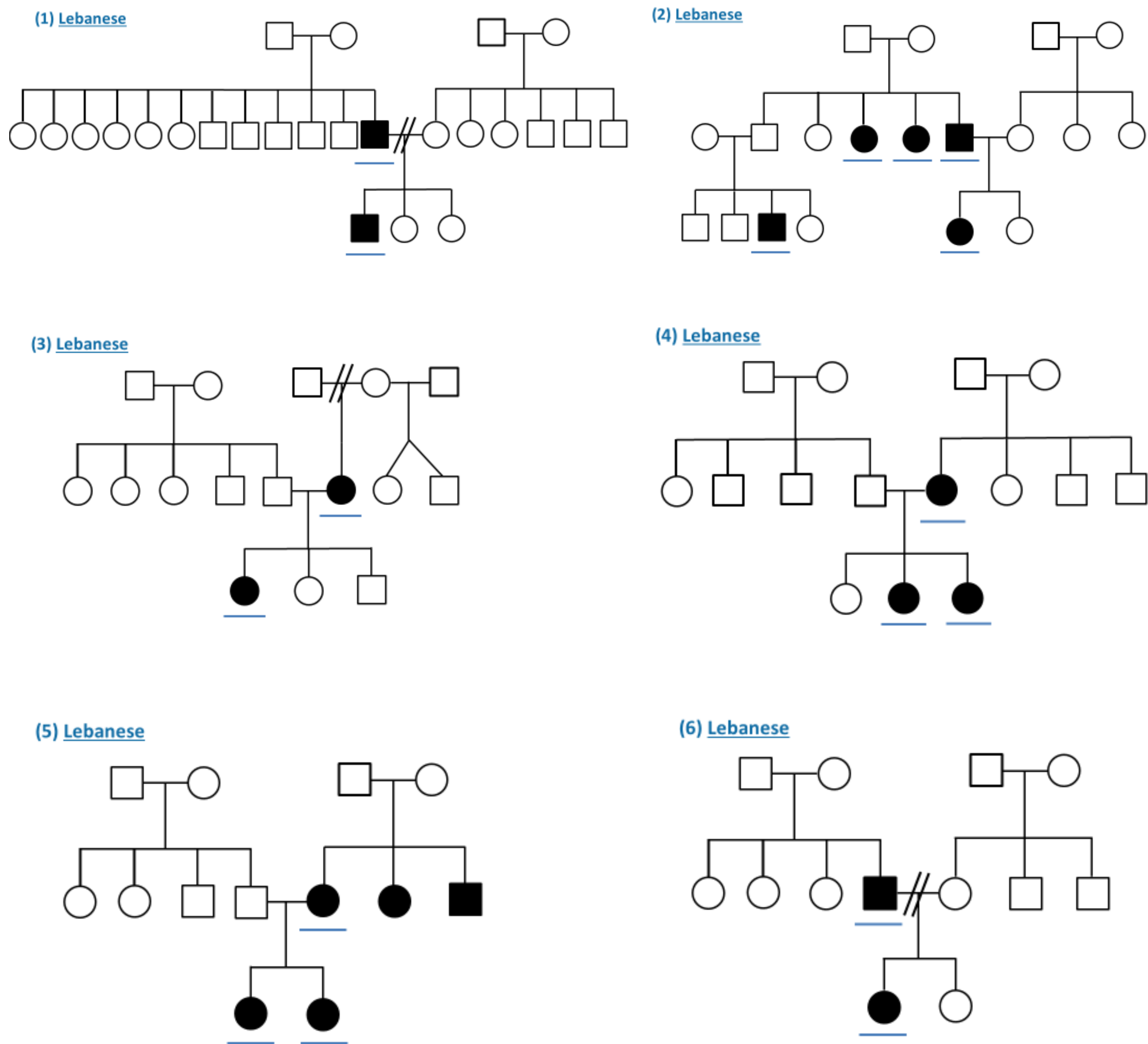
	Ethnicity	Blood collection				Panoramic x-ray	
		Number of males	Number of females	Number of affected individuals	Number of non-affected individuals	Number of males	Number of females
Family (A)	Lebanese	2	1	2	1	2	1
Family (B)	Lebanese	1	3	2	2	1	3
Family (C)	Lebanese	2	2	2	2	2	2
Family (D)	Lebanese	---	4	3	1	---	4
Family (E)	Lebanese	1	3	3	1	1	3
Family (F)	Lebanese	2	2	3	1	2	2
Family (G)	Lebanese	2	4	4	2	2	4
Family (H)	Lebanese	1	2	3	---	1	2
Family (I)	Lebanese	3	2	3	2	3	2
Total		**14**	**23**	**25**	**12**	**14**	**23**
TOTAL		**37**		**37**		**37**	

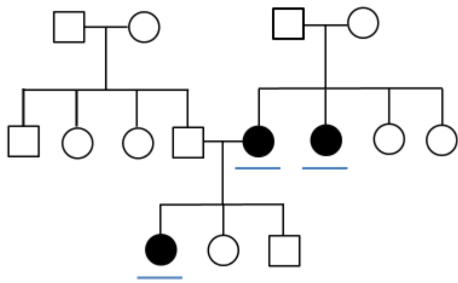
C. Families' structure (pedigrees)

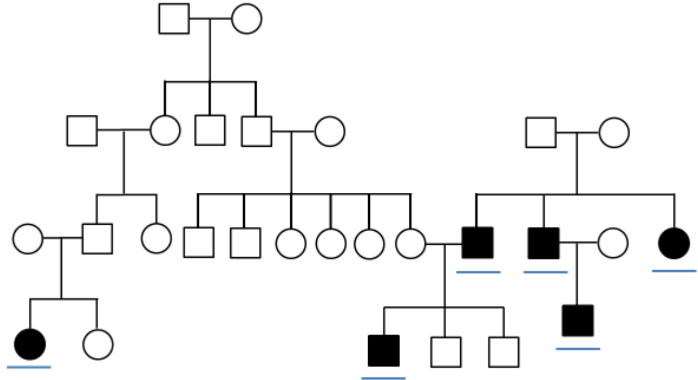
1. Pedigrees of the 16 approached families

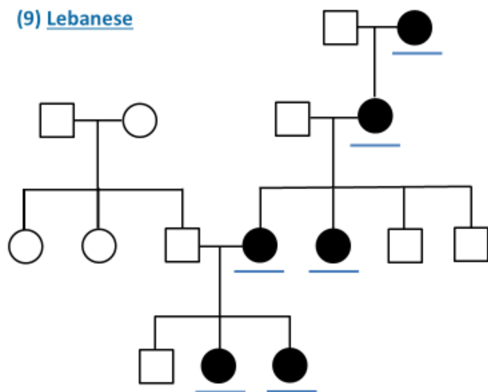
Pedigrees of the 16 Mediterranean families that were approached are illustrated below

a. <u>Families with distributed hypodontia (Figure III.1)</u>









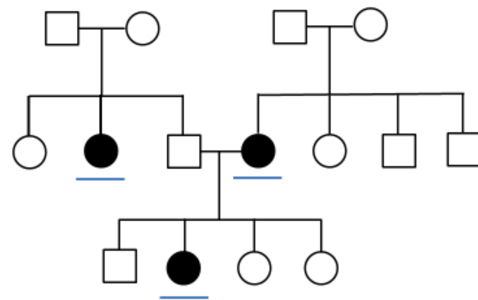
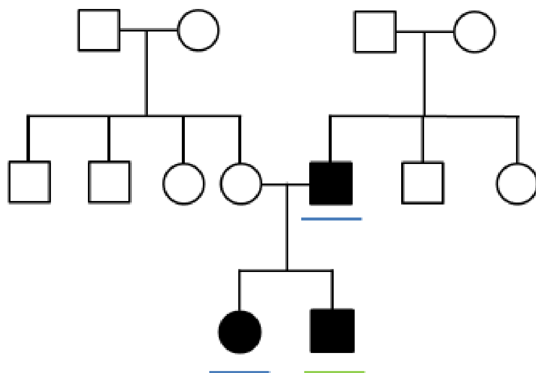
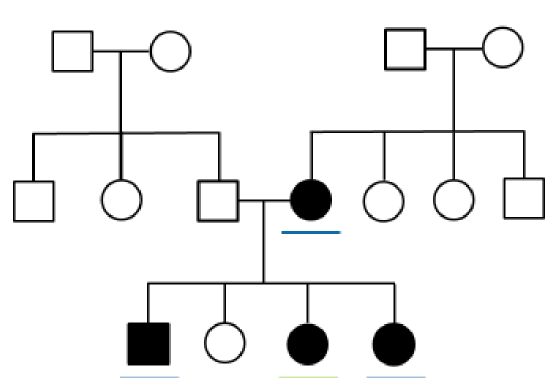


Figure III-1: Pedigrees of 10 families with affected individuals with hypodontia (underlined in blue)

b. Families with distributed hypodontia and hyperdontia (Figure III.2):





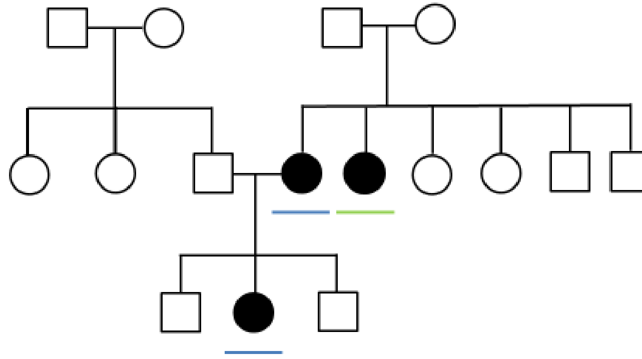
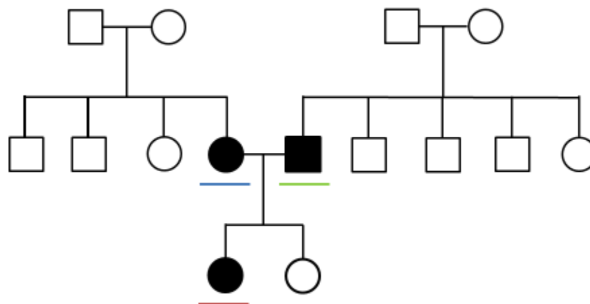


Figure III.2: Pedigrees of 3 families with distributed hypodontia (underlined in blue) and hyperdontia (underlined in green)

c. Families with distributed oligodontia, hypodontia and hyperdontia (Figure III.3)



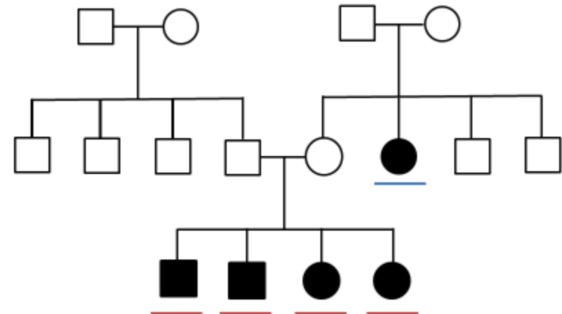


Figure III.3: Pedigrees of 2 families with distributed oligodontia (underlined in red), hypodontia (underlined in blue) and hyperdontia (underlined in green)

d. Family with distributed hyperdontia (Figure III.4)

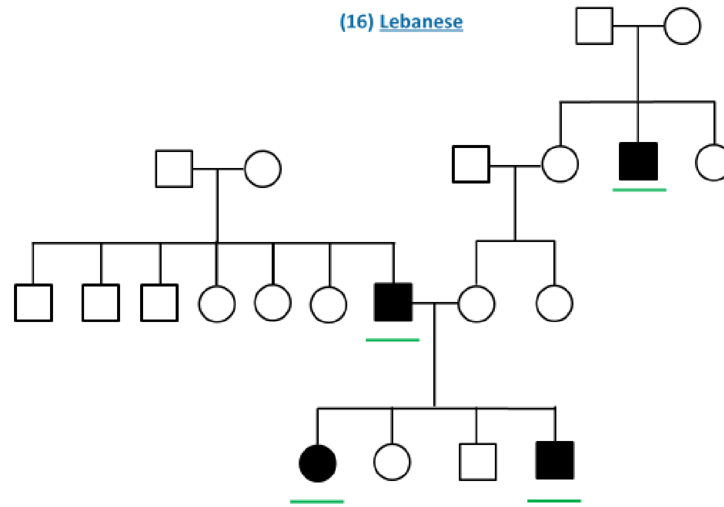


Figure III.4: Pedigree of the family with distributed hyperdontia (underlined in green)

2. Pedigrees of the 5 enrolled families

The Pedigrees of the families who accepted to enroll in the study are illustrated below (Figures III.5-13), with the subjects for whom blood was collected and panoramic x-ray was taken underlined in orange. The selected subjects are numbered using Arabic numerals. Double oblique lines indicate divorce. All 9 families are Lebanese.

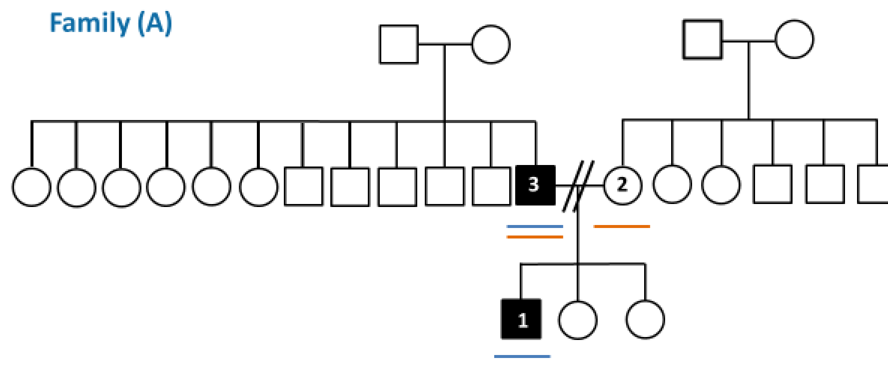


Figure III.5: Pedigree of the family (A)

Family's structure: the pedigree illustrated 3 generations with a total of 25 individuals with 2 affected males only. The mode of inheritance is autosomal dominant. Blood was collected from 3 subjects (2 affected, 1 non-affected) and a panoramic x-ray was taken for them.

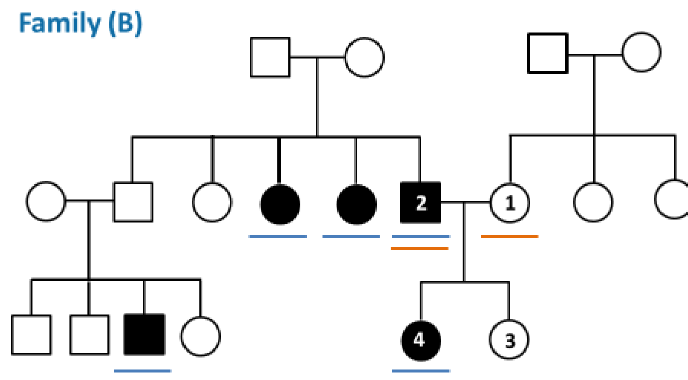


Figure III.6: Pedigree of the family (B)

Family's structure: the pedigree comprised 3 generations with a total of 19 individuals including 5 affected of which 3 were females and 2 were males. The mode of inheritance is autosomal dominant. Blood was collected from 4 subjects (2 affected, 2 non-affected).

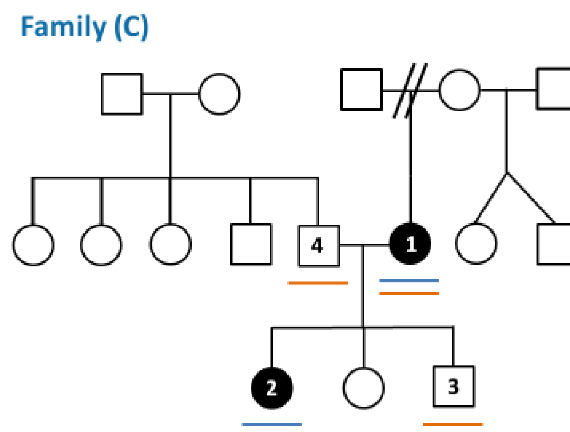


Figure III.7: Pedigree of the family (C)

Family's structure: the pedigree consisted of 3 generations with a total of 16 individuals (2 affected and 14 non-affected). The mode of inheritance is autosomal dominant. Blood was collected from 4 subjects (2 affected, 2 non-affected) and a panoramic x-ray was taken on them.

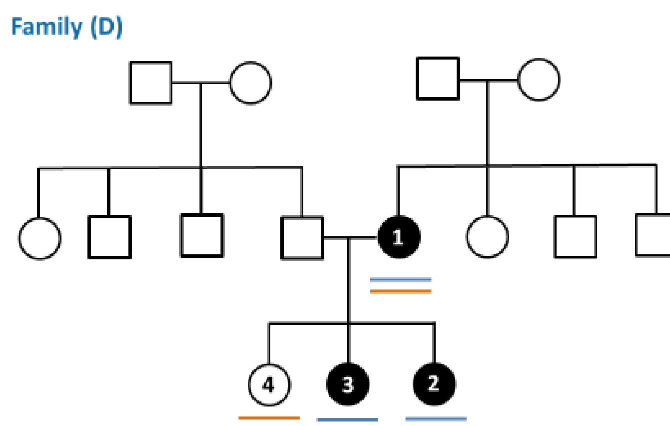


Figure III.8: Pedigree of the family (D)

Family's structure: The pedigree of 3 generations included a total of 15 individuals with 3 affected females. The mode of inheritance is autosomal dominant. Blood was collected from 4 subjects (3 affected, 1 non-affected) and a panoramic x-ray was taken on them.

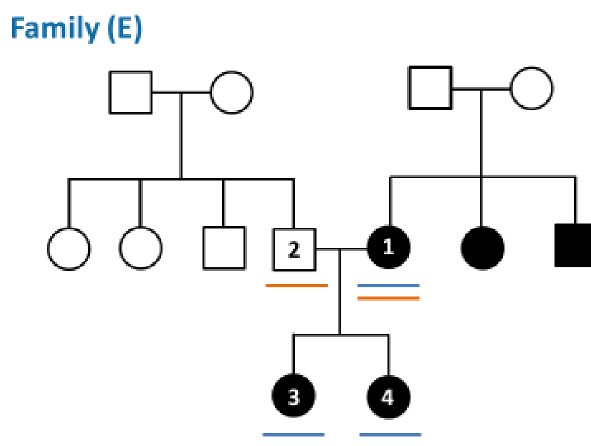


Figure III.9: Pedigree of the family (E)

Family's structure: pedigree comprised of 3 generations with a total of 13 individuals (5 affected and 8 non-affected). The mode of inheritance is autosomal dominant. Blood was collected from 4 subjects (3 affected, 1 non-affected) and a panoramic x-ray was taken on them.

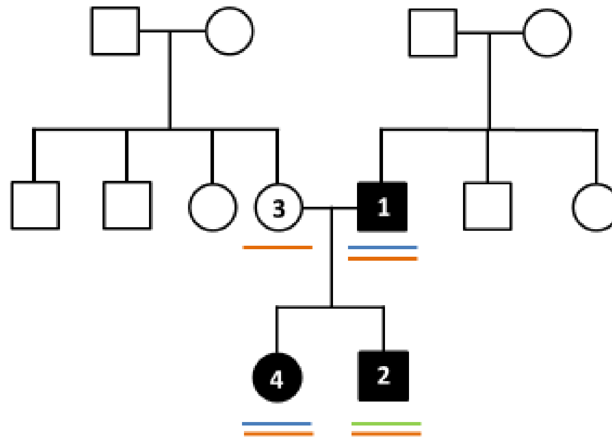


Figure III.10: Pedigree of the family (F)

Family's structure: the pedigree comprised of 3 generations with a total of 13 individuals including 3 affected (2 males and 1 female) and 10 non-affected. The mode of inheritance is autosomal dominant. Blood was collected from 4 subjects (3 affected, 1 non-affected) and a panoramic x-ray was taken on them.

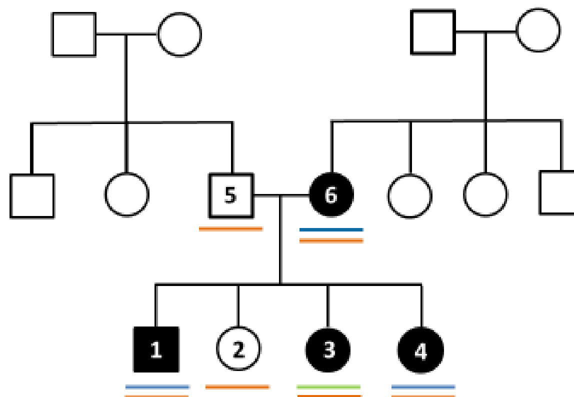


Figure III.11: Pedigree of the family (G)

Family's structure: the pedigree enclosed 3 generations with a total of 15 individuals with 3 affected females versus 1 affected male. The mode of inheritance is autosomal dominant. Blood was collected from 6 subjects (4 affected, 2 non-affected) and a panoramic x-ray was taken on them.

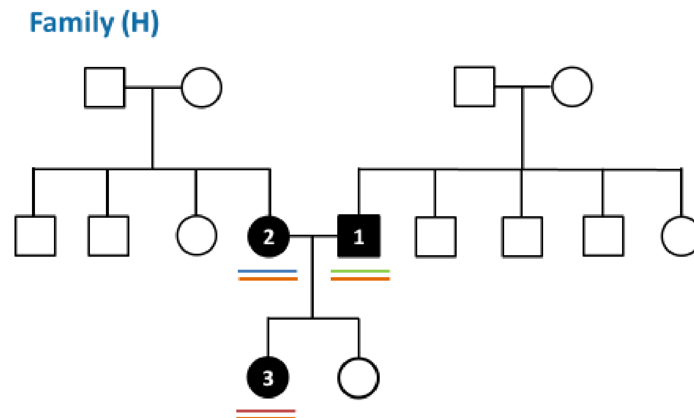


Figure III.12: Pedigree of the family (H)

Family's structure: the pedigree illustrated 3 generations with a total of 15 individuals with 2 affected males and 1 affected female. The mode of inheritance is autosomal dominant. Blood was collected from 3 affected subjects only and a panoramic x-ray was taken for them.

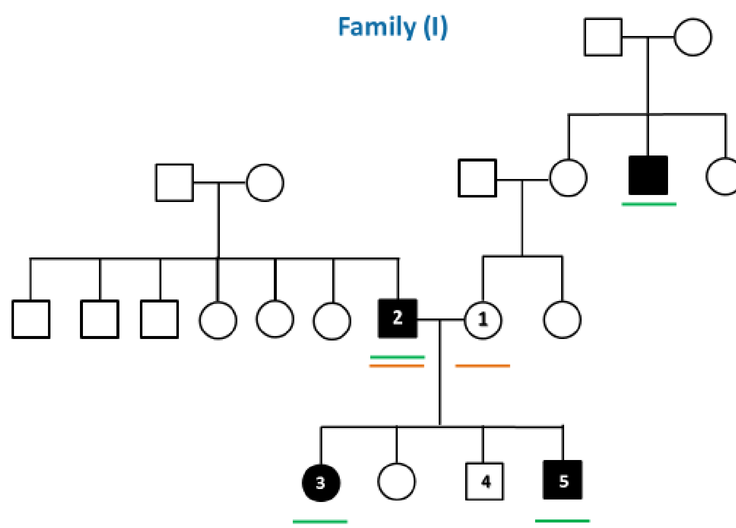


Figure III.13: Pedigree of the family (I)

Family's structure: the pedigree comprised of 4 generations with a total of 21 individuals with 4 affected (3 males and 1 female). The mode of inheritance is autosomal dominant. Blood was collected from 5 subjects (3 affected, 2 non-affected) and a panoramic x-ray was taken on them.

In summary, 5cc of blood was withdrawn from 37 subjects (25 affected, 12 non-affected) and a panoramic x-ray was taken on all of them ~~the 37 subjects~~. It should be noted that consanguinity was not present, and an autosomal dominant mode of inheritance was noted in all 9 families.

D. Clinical examination and dental measurements

1. Initial measurements

Dental measurements were performed in 8 families having hypodontia, on panoramic radiographs (2D) and, either cast models or 3D intraoral images using the 3 Shape Trios® intra-oral scanner. Both diagnostic tools were used as the 3D intraoral imaging technique as well as cast models provide higher accuracy versus the 2D radiographic imaging, by properly calibrating the latter to the measurements retrieved from scans and cast models. These measurements included:

✓ Arch circumference discrepancy (ACD)

✓ Arch circumference available (ACA) and required (ACR)

✓ Crown length (CL) and crown width (CW)

✓ Root length (RL).

Patients who had a recent panoramic radiograph taken within the range of 6 months to 1 year did not have to take a new one. The panoramic radiograph was taken at our division and housed in the departmental radiologic software (CLINIVIEW 9.3).

Teeth dimensions were measured by one investigator (JB) using:

- CLINIVIEW 9.3 software for the panoramic radiographs (following calibration) (Figure III.14)
- 3Shape Ortho Analyzer® for the 3D intraoral scans (Figure III.15)
- Digital Caliper for the cast models (Figure III.16)

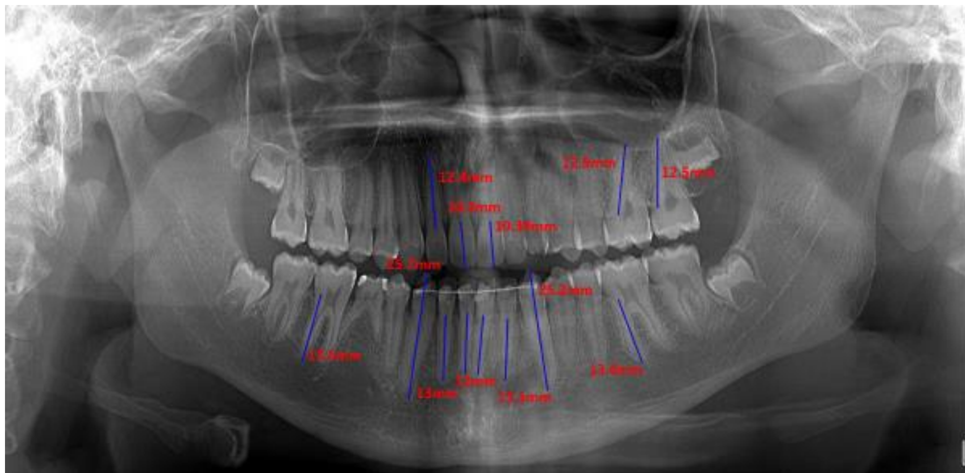


Figure III.14: Crown length, Root length for different teeth measured on the panoramic x-ray using Cliniview.

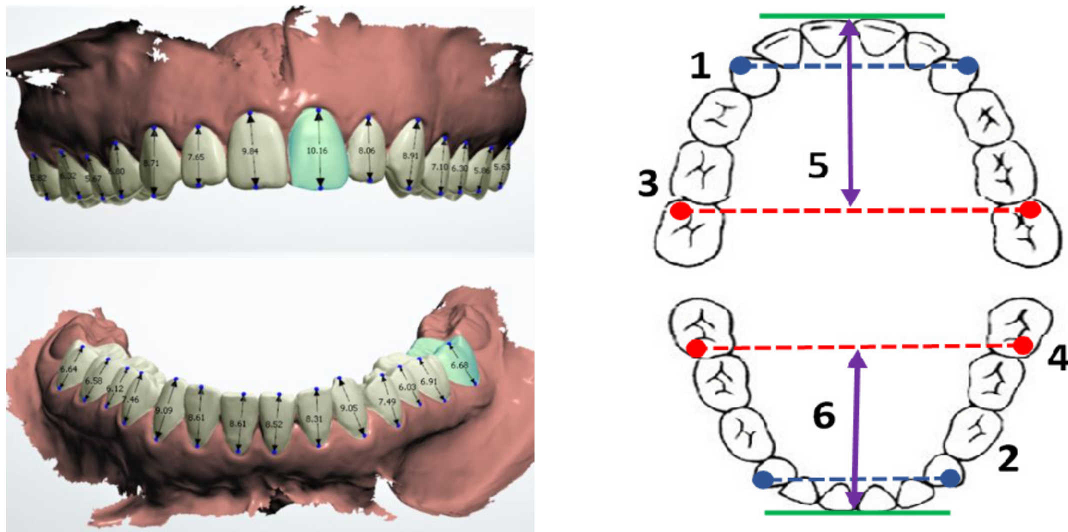


Figure III.15: Crown length, and width as well as arch dimensions measured on the 3D image obtained using 3Shape Ortho Analyzer®

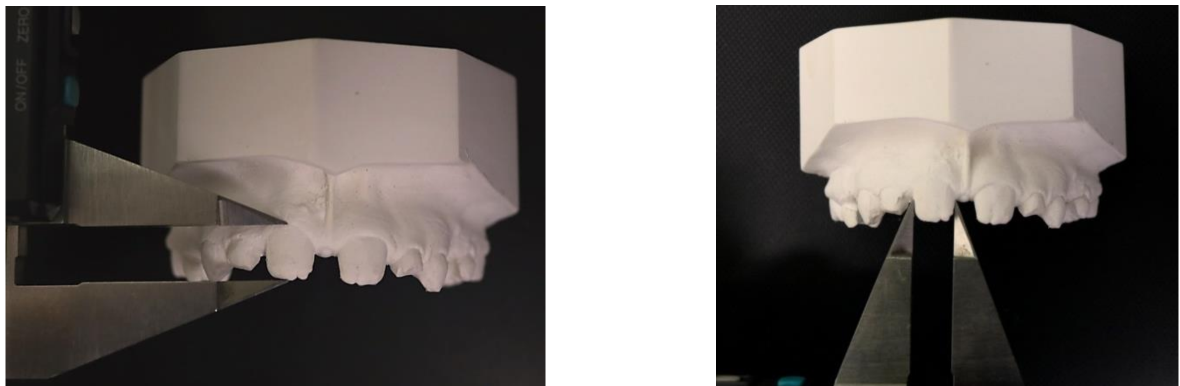


Figure III.16: Crown length, width measured on the cast models using digital caliper.

The total sample from the 8 studied families with hypodontia comprised 28 subjects (18 affected and 10 non-affected relatives). Each of both groups were matched for age and gender with corresponding controls; these matched controls had the full set of their permanent dentition (meaning no agenesis was noted). Subsequently, the subgrouping came as follow:

- Group A (N=18): affected patients with dental agenesis
- Group CA (N=18): matched control group for group A (no agenesis)
- Group N (N=10): non-affected relatives of group A
- Group CN (N=10): matched control group for group (no agenesis)

2. *Repeated measurements*

Intra-examiner reliability of the measurements was assessed by choosing randomly 28 patients (50% of the total sample) 1 month after initial digitization. Spearman correlation test was performed for intra-class examiner and gave an average correlation coefficient of 0.97.

3. *Statistical analysis*

The differences between the radiographic and the intra-oral measurements among CA/A and CN/N were evaluated using *Paired t-test* (since the groups are matched). In order to assess variable differences between A and N, a two-sample independent *t-test* was employed. The results were considered statistically different if the p value was ≤ 0.05.

E. Genetic procedure

1. Blood collection

The Inheritance pattern of oligo/hypo and hyperdontia were potentially explored by isolating genomic DNA from whole blood cells of both affected and non-affected individuals and then running the analysis, which was performed by Dr. Georges Nemer (Professor and head of Basic Science Affairs and genetic unit at FM) who has ample expertise in this field. Both affected and non-affected individuals were included in order to facilitate the analysis by associating the genotype to the underlying phenotype.

For this purpose, 5cc of blood was withdrawn from affected and ~~some~~ non-affected individuals of each family, using needles and Capillary Blood Collection tubes (CBC tubes) with a purple/lavender top color (i.e., the interior of the tube wall is coated with EDTA K2 or K3) by applying the following protocol:

1. Positioning of the patient in a chair.
2. Selection of a suitable site for venipuncture, by placing a tourniquet 3 to 4 inches above the selected puncture site on the patient.
3. Palpation for a vein.
4. When a vein is selected, cleansing of the area using alcoholic pad, in a circular motion, beginning at the site and working outward.
5. Allowing the area to air dry.
6. Asking the patient to make a fist, then grabbing the patient's arm using the thumb and swiftly inserting the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface.

7. Removal of the tourniquet when the last tube is filling.
8. Removal of the needle from the patient's arm using a swift backward motion.
9. Placement of gauze immediately on the puncture site, applying and holding adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes taping a fresh piece of gauze or Band-Aid to the puncture site (Figure III.17).

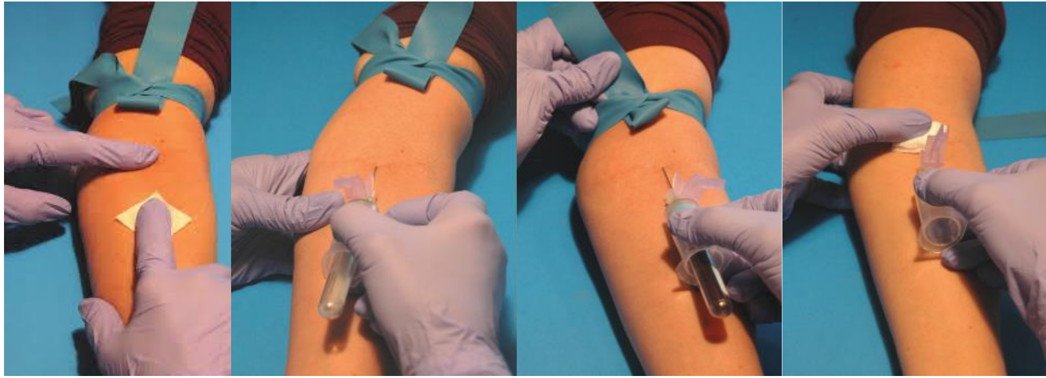


Figure III.17: Different steps for blood withdrawal

Then, the blood samples were preserved in the refrigerator at a temperature of -4°C, awaiting DNA extraction (done within 0 to 10 days maximum after blood withdrawal). The potential risks associated with blood withdrawal include bruising, pain, hematoma, and slight possibility of infection or fainting. However, blood withdrawal was done at the hospital (AUBMC), by a specialized nurse or physician, using a clean needle. The AUBMC Laboratory Medicine rules and regulations were followed, including any information provided by this department on a routine basis. Therefore, these potential risks are considered minimal and the benefits of this study outweigh its potential risks. In fact, these potential risks didn't occur to anyone of the participants in this study.

2. DNA extraction

Genomic DNA was isolated using the Qiagen Blood-Midi kit (Qiagen Science Inc., Germantown, MD), as per the manufacturer recommendations. The DNA extraction process used in this study is quite short and the DNA at the end tends to be purer though less concentrated.

It includes the following steps:

1. In a 15ml falcon tube (conical centrifugation tube), put: 2ml blood, + 200 µl protease (5.5ml H2O to the powder) (to denature the proteins and keep the DNA intact). + 2.4ml lysis buffer (breaks open cells and nuclear membranes but also exposes the DNA to proteins).
2. Mix by inversion 15 times, vortex for 1 min to mix the liquid and then incubate at 70°C for 10 min (boil the water on the heater).
3. Add 2ml of ethanol 100% to precipitate the DNA from the lysed cells.
4. Mix by inversion 10 times and vortex for 30 sec.
5. Remove half of the volume (≈ 3ml) and pour into the purifying falcon tube (with membrane).
6. Centrifuge at 3600 for 10 min at 15°C to separate the DNA from the reagents and proteins during the cell lysis step.
7. Decant the supernatant and add the remaining volume of the lysed blood + 3.6ml in the purifying tube.
8. Centrifuge at 3600 for 10 min at 15°C.
9. Add 2ml of washing buffer 1 to clean the reagent then centrifuge at 3600 for 10 min.

10. Add 2ml of washing buffer 2 also to clean the reagent, then centrifuge at 3600 for 10 min. At this stage, the DNA moved to the bottom of the tube.
11. Air-dry for 7 min to evaporate the ethanol.
12. Transfer to a new falcon tube, add 150µl of dilution buffer and incubate for 5 min at room temperature.
13. Centrifuge for 10 min at 3600. Repeat the elution step if the quantity is small.
14. Quantify with a nanodrop to know the concentration of DNA in the blood.
15. Store at -20°C with an elution buffer to stabilize the DNA while protecting it from degradation.

Steps 1 to 3 constitute the cell lysis stage. In steps 4 to 11, the cellular debris are eliminated (DNA purification process): once the hydrolytic enzymes have been destroyed and the DNA precipitated, the DNA purification process begins. In essence, the cellular components, including DNA, are placed into a spin column and the spin column is washed of all components except the DNA. Upon centrifugation, the material will pass through the filter, which attracts the DNA and allows debris to pass through. This will be followed by two wash steps with two buffers (AW1 and AW2). Steps 12 and 13 constitute the DNA elution stage that consists of removing the DNA from the filter. This is done by adding the elution buffer and then spinning the tube with the DNA embedded in the filter, which will pull the elution buffer through the matrix, thus pulling the DNA into the collection tube.

3. Genetic analysis

Through the whole Exome Sequencing (WES) the genetic variants were identified in exonic regions of the 37 subjects that are part of the 9 selected families, including the affected ones as well as the non-affected.

For each individual, the results were first displayed on a file named “FastQ file” that can only be read on a specific software. Then, by comparing the sequences to the normal databases, an annotated file named “Variant Call Formal” (VCF) was generated for each individual that can be read on a specific software “Illumina Variant Studio”, which has the advantage of allowing a clustering analysis by family. The Variant Call Format (VCF) is a text file format that contains information about variants found at specific positions in a reference genome. The file format consists of meta-information lines, a header line and data lines. Each data line contains information about a single variant: chromosome number, position of the variant, gene name, quality score, filter status, putative impact on the proteins function and structure, allele count, total number of alleles in called genotypes, allele frequency, approximate read depth, inbreeding coefficient.

DNA was first quantified and assessed for quality using the Nanodrop at the American University of Beirut Molecular Core Facility. Then, the experimental genetic procedure was done in four main steps:

1. Library preparation
2. Cluster generation
3. Sequencing
4. Data analysis and report generation (Figure III.18).

The first 3 steps were performed at Macrogen Laboratory in South Korea (dna.macrogen.com) while data analysis was done at the department of biochemistry and molecular genetics at AUB.

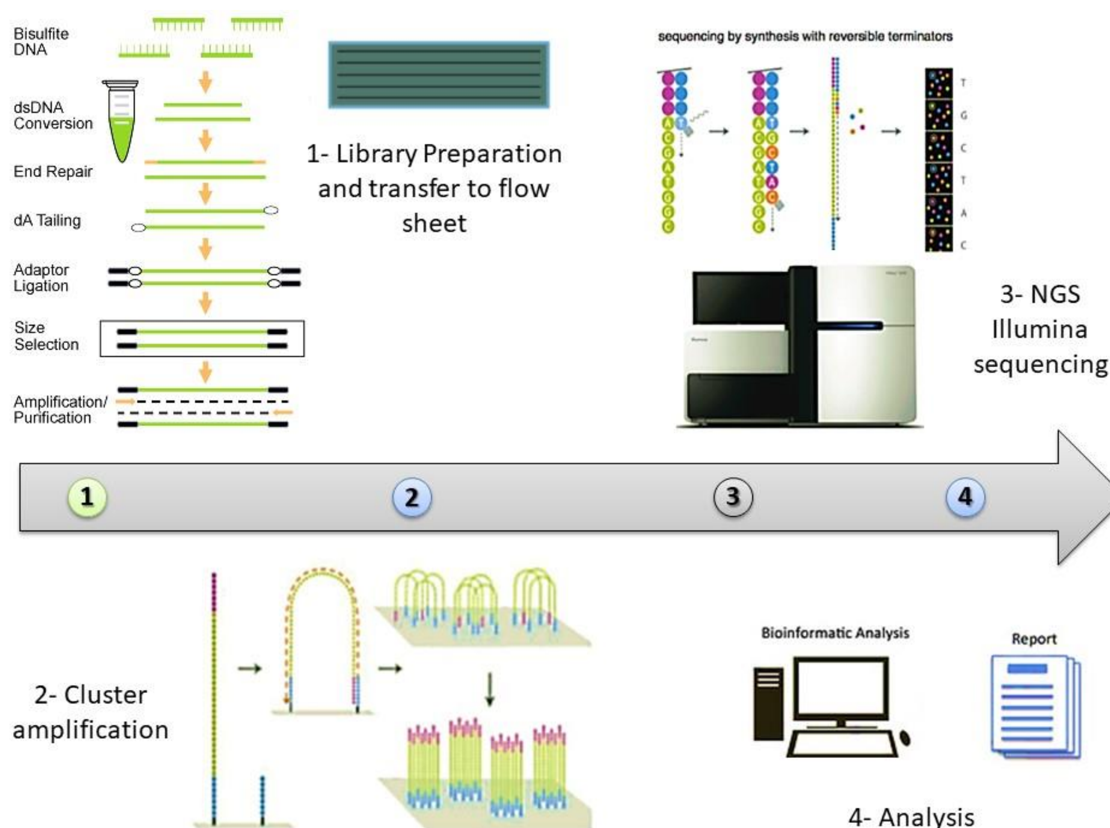


Figure III.18: Four main steps of the genetic experimental procedure following DNA extraction (Adapted from source: http://dna.macrogen.com. Accessed: November, 2020).

a. <u>Captured library preparation</u>

Following DNA extraction, a random fragmentation of the DNA was performed, followed by a library hybridization during which the DNA fragments of a few hundred base pairs were added to the target enrichment capture kit Agilent SureSelect V6-Post. The SureSelect Target Enrichment workflow is a solution-based system utilizing ultra-long -120

mer biotinylated cRNA baits- to capture regions of interest, enriching them out of a NGS genomic fragment library. The kit fragment includes primers at both ends (formed of 20 intron nucleotides) that are the same for all fragments, in addition to a unique primer (bar code) for each individual. The DNA fragments that include only exons had their both ends ligated to the kit fragment and the other DNA fragments were eliminated (Figure III.19). Then, the libraries were amplified by emulsion PCR during which DNA is amplified on micro beads. After PCR amplification, for each one of the annealed fragments, the number of copies read (read depth) was indicated. To be reliable, the read depth should be >40-50 for the preliminary analysis and then >20 if a result was not found during the preliminary analysis.

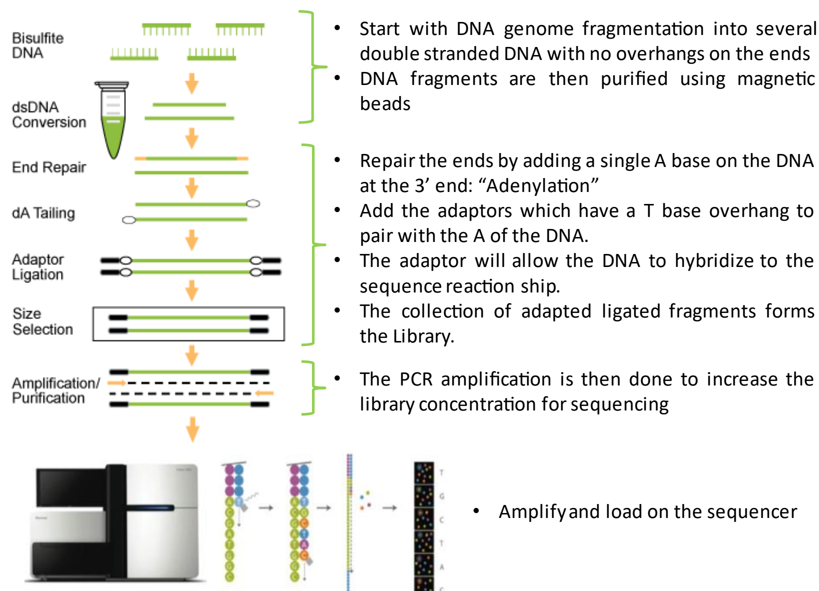


Figure III.19: Detailed procedure of the captured library preparation step. (Adapted from source: http://dna.macrogen.com. Accessed: November, 2020).

b. Cluster generation

The goal is to detect groups of genes that exhibit similar expression patterns by subdividing the genes in such a way that similar ones fall into the same sub class (cluster), whereas dissimilar ones fall in different sub-classes (clusters). Consequently, two criteria are satisfied: homogeneity - elements in the same cluster are highly similar to each other; and separation - elements from different clusters have low similarity to each other. Clusters are generated through a unique isothermal "bridge" amplification reaction occurring on the surface of the flow cell. Each cluster represents the single molecule that initiated the cluster amplification. It contains approximately one million copies, which is sufficient for reporting incorporated bases at the required signal intensity for detection during sequencing. This allows an open-ended exploration of the data, without getting lost among the thousands of individual genes.

c. Next generation sequencing (NGS)

The libraries underwent Whole Exome Sequencing (WES) on a HiSeq6000 Illumina platform to determine the exact sequence of nucleotides (adenine, guanine, cytosine and thymine). A flow cell containing millions of unique clusters is loaded into the HiSeq6000 for automated cycles of extension and imaging (Figure III.20). Sequencing-by-Synthesis is through the use of polymerase-catalyzed addition of four proprietary fluorescently labeled nucleotides with “reversible terminators”. Each sequencing cycle occurs in the presence of all four nucleotides leading to higher accuracy

than methods where only one nucleotide is present in the reaction mix at a time. This cycle is repeated, one base at a time, generating a series of images each representing a single base extension at a specific cluster. Hundreds of thousands to hundreds of millions of sequencing reactions occur simultaneously, which refers to the term “massive parallel sequencing”.

Figure III.20: Hiseq6000 Illumina sequencer (Adapted from source: http://dna.macrogen.com. Accessed: November, 2020)

d. Data analysis

Several software (GWA, Picard, GATK and Snpeff) were used to map and analyze the sequencing data with a primary focus on variant discovery and genotyping as well as strong emphasis on data quality assurance, while using the normal databases as a reference. That software annotates and predict the effects of variants on genes (such as amino acid changes). In fact, they can generate the following results: genes and transcripts affected by the variants, location of the variants, how the variants affect

protein synthesis (e.g., generating a stop codon) and comparison with other databases to find equal known variants.

Single nucleotides and deletion/insertion variants were generated using the GATK software, and during the 1st stringent filtering, only the variants having a high putative impact passed (disruptive mutation: frameshift or stop codon) and a coverage read >40-50 were analyzed; if no result was found, an analysis was performed on the variants with a read depth >20.

During the 2nd filtering, a comparison was done between individuals of the same family in an attempt to find common gene(s) between the affected ones that is(are) not noted in the genetic material of the non-affected ones. In other words, we aim to find a mutation that segregates with the phenotype. Then, the analysis was repeated by selecting only the passed variants having a moderate putative impact and a Minor Allele Frequency MAF ≤ 0.001 in the normal population and a comparison was also done between individuals of the same family in order to find common gene(s) between the affected ones. In families a large number of common genes was noted, 2nd sub-filtering was performed to reduce the number of genes. The 2nd sub-filtering is a complimentary biochemical interface done to prove that the results obtained by sequencing affect protein function. Assessment of the effects of the filtered variants on protein function was done in silico using both the Polyphen2 and SIFT softwares. During this 2nd sub-filtering, which is applicable only on the variants having a moderate putative impact, the genes having the Polyphen and SIFT predictions as “Damaging” were kept and the others were filtered out. Then, the genes having several mutations were filtered out and the potential genes were highlighted.

After the several steps of filtering, we ended up with a reduced number of possible candidate genes, so depending on their function, some of them were filtered out and the other(s) were considered as candidate gene(s) for Oligo/Hypo and Hyperdontia. In other words, the genes that do not have a role in dental development and morphogenesis were filtered out, and those that are related to the dental development, were considered as candidate gene(s) for the tested phenotypes. Then, a search was done to check if the potential candidate gene(s) is(are) found in one family were present in individuals that are part of the 7 other selected families and the candidate gene(s) found in this study were compared to those discovered by previous studies published in the literature to assess if common genes are present.

It should be noted also that this is a straightforward genetic approach to identify gene(s) implicated in the condition. No statistical power analysis is needed because the analysis is not a linkage analysis, which requires SNP genotyping across the genome, but rather a genotype-phenotype linkage based on the results of the WES. The latter takes into account all the variables (e.g., level of inbreeding) because it allows us to analyze the genotypes with a hypothesis-free strategy whereby any variant(s) would be assessed across the family as being linked to the phenotype.

CHAPTER IV

RESULTS

A. Pedigree analysis

The pedigrees drawn to represent the structure of the 16 Mediterranean families with affected individuals suggest a Mendelian inheritance pattern and segregate in an autosomal dominant manner.

The analysis of those pedigrees disclosed the following: (Tables IV.1-2):

- An equal number of reported generations per family (n=3-4).
- 11 families with female predominance.
- 4 families with male predominance.
- 1 family with equal number of reported affected males and females.
- 1 family with affected females in the first generation.
- 7 families having affected siblings in the youngest generation; 5 of the families accepted to enroll in the genetic analysis.

Table IV.1: Pedigree analysis of the 16 Middle Eastern families

	Generations	**Affected females**	**Affected males**
Total number in 16 families	51	40	19
Average	3.19	2.5	1.19

Table IV.2: Pedigree analysis of the 16 Middle Eastern families

	N	%		N	%
Families with more affected females	11	68.75	**Families with more affected males**	4	25
Families with at least 3 affected females	9	56.25	**Families with at least 3 affected males**	2	12.5
Families with no affected females	1	6.25	**Families with no affected males**	6	37.5
Families with affected females in the 1st generation	1	6.25	**Families with affected males in the 1st generation**	0	0

B. Panoramic and IO-Scanner analysis:

Statistical analysis was performed on measurements related to 56 individuals, 28 of whom were from the first 8 selected families and 28 from the control group.

1. Group A versus group CA

When comparing group A with its corresponding control (CA) for arch parameters, a significant reduction in arch dimensions (ACA) was noted, which similarly reflected on inter-molar/ canine widths and arch length, thus the constricted arches. Less crowding (ACD) was also recorded in group A, however only statistically significant in the mandible (Table IV.3).

Table IV.3: Comparison between CA&A for arch parameters

Measurements	Mean difference (CA-A)	SD	P
Maxilla			
ACD	-1.94	0.97	0.104
ACA	10.94	5.47	<0.001
ACR	12.89	6.45	<0.001
Inter-molar width	6.15	3.08	<0.001
Inter-canine width	6.81	3.40	<0.001
Arch length	6.23	3.12	<0.001
Mandible	**Mean difference (CA-A)**	**SD**	**P**
ACD	-2.11	1.06	<0.001
ACA	8.15	4.08	<0.001
ACR	6.25	3.13	<0.001
Inter-molar width	5.15	2.57	<0.001
Inter-canine width	5.71	2.85	<0.001
Arch length	4.70	2.35	<0.001

As for teeth dimensions, Group A had smaller crown widths overall however only statistically significant for maxillary R 3-4-5/ L3 and mandibular R 2-4-6-7/ L1-3-6-7 (while noting that the mean differences were within 1 SD which is not clinically substantial) (Table IV.4)

Table IV.4: Comparison between CA&A for crown width

Measurements Maxilla	Mean difference (CA-A)	SD	P
CW. MR1	0.34	0.60	0.131
CW. MR2	0.41	0.49	0.141
CW. MR3	0.32	0.40	0.027
CW. MR4	0.49	0.43	0.003
CW. MR5	0.51	0.45	0.015
CW. MR6	0.22	0.68	0.395
CW. MR7	0.29	0.72	0.136
CW. ML1	0.29	0.55	0.185
CW. ML2	0.65	0.64	0.136
CW. ML3	0.41	0.45	0.007
CW. ML4	0.14	0.34	0.348
CW. ML5	0.56	0.70	0.137
CW. ML6	0.71	1.32	0.175
CW. ML7	0.23	0.71	0.273
Mandible	**Mean difference (CA-A)**	**SD**	**P**
CW. mR1	0.33	0.37	0.07
CW. mR2	0.39	0.45	0.052
CW. mR3	0.26	0.45	0.149
CW. mR4	0.49	0.47	0.031
CW. mR5	0.62	0.62	0.066
CW. mR6	0.51	0.60	0.013
CW. mR7	0.58	0.69	0.003
CW. mL1	0.42	0.31	0.006
CW. mL2	0.34	0.41	0.09
CW. mL3	0.50	0.32	0.001
CW. mL4	0.34	0.49	0.157
CW. mL5	0.46	0.48	0.064
CW. mL6	0.49	0.54	0.008
CW. mL7	0.61	0.66	0.003

CW: crown width, M: maxilla, m: mandible, R: right, L: left

The affected group had reduced crown lengths for maxillary R 1-3/ L 1-3-6 and mandibular R/L 1-2-3-4 (Table IV.5).

Table IV.5: Comparison between CA&A for crown length

Measurements	Mean difference (CA-A)	SD	P
Maxilla			
CL. MR1	0.87	0.90	0.022
CL. MR2	0.78	0.61	0.078
CL. MR3	0.89	0.80	0.012
CL. MR4	0.43	0.75	0.083
CL. MR5	0.58	0.74	0.066
CL. MR6	0.25	0.81	0.14
CL. MR7	0.12	0.68	0.558
CL. ML1	0.92	0.80	0.007
CL. ML2	0.60	0.56	0.065
CL. ML3	0.66	0.70	0.007
CL. ML4	0.24	0.64	0.177
CL. ML5	0.43	0.63	0.121
CL. ML6	0.40	0.65	0.015
CL. ML7	-0.05	0.65	0.83
Mandible	**Mean difference (CA-A)**	**SD**	**P**
CL. mR1	0.57	0.70	0.054
CL. mR2	0.81	0.64	0.004
CL. mR3	0.89	0.60	0.001
CL. mR4	0.71	0.55	0.005
CL. mR5	0.40	0.69	0.056
CL. mR6	0.20	0.58	0.399
CL. mR7	0.05	0.73	0.719
CL. mL1	0.60	0.60	0.008
CL. mL2	0.69	0.70	0.029
CL. mL3	0.81	0.70	0.006
CL. mL4	0.61	0.60	0.038
CL. mL5	0.29	0.69	0.452
CL. mL6	0.28	0.51	0.324
CL. mL7	0.08	0.79	0.699

CL: crown length, M: maxilla, m: mandible, R: right, L: left

Significant differences for root length existed for maxillary R 2-4-5/ L 1-2-4-5-6-7 and mandibular R 2-4-6-7/ L 1-2-6. Worth noting is that the roots of patients in group A tended to be shorter in comparison with their controls, however it was not clinically momentous (Table IV.6)

Table IV.6: Comparison between CA&A for root length

Measurements Maxilla	Mean difference (CA-A)	SD	P
RL. MR1	0.01	0.45	0.936
RL. MR2	1.12	0.42	<0.001
RL. MR3	0.15	0.48	0.394
RL. MR4	0.50	0.39	0.005
RL. MR5	0.45	0.34	0.007
RL. MR6	0.22	0.30	0.079
RL. MR7	-0.13	0.20	0.078
RL. ML1	0.23	0.33	0.037
RL. ML2	1.19	0.54	0.001
RL. ML3	0.13	0.44	0.519
RL. ML4	0.49	0.35	0.002
RL. ML5	0.56	0.41	0.007
RL. ML6	0.20	0.27	0.029
RL. ML7	-0.07	0.13	0.04
Mandible	**Mean difference (CA-A)**	**SD**	**P**
RL. mR1	0.33	0.33	0.062
RL. mR2	0.35	0.44	0.063
RL. mR3	0.12	0.34	0.351
RL. mR4	0.29	0.21	0.001
RL. mR5	0.18	0.15	0.319
RL. mR6	0.54	0.20	<0.001
RL. mR7	0.31	0.28	<0.001
RL. mL1	0.48	0.41	0.009
RL. mL2	0.42	0.28	0.002
RL. mL3	0.04	0.25	0.67
RL. mL4	0.12	0.34	0.664
RL. mL5	-0.03	0.40	0.703
RL. mL6	0.60	0.25	<0.001
RL. mL7	0.07	0.26	0.496

RL: root length, M: maxilla, m: mandible, R: right, L: left

2. Group N versus group CN

The arch circumference available (ACA) was reduced in group N (by 4.32mm and 4.82mm in maxilla and mandible respectively) except not statistically significant in the maxilla. Differences in crowding (ACD) between both groups did not reach a statistical significance, while the space required (ACR) in both arches indicated significant disparity (Table IV.7).

Table IV.7: Comparison between CN&N for arch parameters

Measurements **Maxilla**	**Mean difference (CN-N)**	**SD**	**P**
ACD	-0.90	-0.90	0.217
ACA	4.32	4.32	0.056
ACR	5.22	5.22	0.027
Inter-molar width	0.21	0.21	0.819
Inter-canine width	0.23	0.23	0.800
Arch length	1.69	1.69	0.225
Mandible	**Mean difference (CN-N)**	**SD**	**P**
ACD	0.15	0.15	0.823
ACA	4.82	4.82	0.005
ACR	4.67	4.67	0.002
Inter-molar width	0.74	0.74	0.374
Inter-canine width	0.73	-0.73	0.729
Arch length	1.25	1.25	0.173

When contrasting group N with their controls, crown widths were significantly different for maxillary R 3-4-5-7/ L 4 and all mandibular teeth with the exception of R 7/ L 4-5-7. Differences were statistically, yet not clinically, significant as the mean differences were within 1SD (Table IV.8).

Table IV.8: Comparison between CN&N for crown width

Measurements Maxilla	Mean difference (CN-N)	SD	P
CW. MR1	0.15	0.32	0.386
CW. MR2	0.04	0.61	0.875
CW. MR3	0.53	0.51	0.025
CW. MR4	0.48	0.34	0.008
CW. MR5	0.17	0.42	0.016
CW. MR6	0.34	0.58	0.069
CW. MR7	0.39	0.43	0.045
CW. ML1	0.38	0.48	0.137
CW. ML2	0.17	0.63	0.401
CW. ML3	0.36	0.44	0.117
CW. ML4	0.55	0.41	0.016
CW. ML5	0.49	0.69	0.239
CW. ML6	0.44	0.44	0.055
CW. ML7	0.64	0.65	0.063
Mandible	**Mean difference (CN-N)**	**SD**	**P**
CW. mR1	0.40	0.35	0.042
CW. mR2	0.51	0.39	0.036
CW. mR3	0.55	0.41	0.006
CW. mR4	0.54	0.46	0.046
CW. mR5	0.54	0.39	0.006
CW. mR6	0.72	0.58	0.045
CW. mR7	0.19	0.51	0.194
CW. mL1	0.45	0.35	0.021
CW. mL2	0.50	0.32	0.018
CW. mL3	0.51	0.46	0.017
CW. mL4	0.37	0.54	0.157
CW. mL5	0.30	0.45	0.191
CW. mL6	0.75	0.46	0.002
CW. mL7	0.19	0.65	0.5

CW: crown width, M: maxilla, m: mandible, R: right, L: left

The only significance for crown length between group N and their controls was noted for maxillary L6 and mandibular R 6-7 (Table IV.9). Although not statistically significant, group N presented a trend of decrease in crown length.

Table IV.9: Comparison between CN&N for crown length

Measurements Maxilla	Mean difference (CN-N)	SD	P
CL. MR1	0.70	0.87	0.246
CL. MR2	0.43	0.97	0.421
CL. MR3	0.50	0.90	0.21
CL. MR4	0.31	0.86	0.472
CL. MR5	-0.14	0.92	0.773
CL. MR6	0.01	0.76	0.965
CL. MR7	-0.43	0.72	0.319
CL. ML1	0.69	0.85	0.153
CL. ML2	0.05	0.86	0.908
CL. ML3	0.61	1.00	0.186
CL. ML4	0.12	0.93	0.954
CL. ML5	-0.41	0.74	0.276
CL. ML6	-0.77	0.61	0.047
CL. ML7	-0.66	0.89	0.153
Mandible	**Mean difference (CN-N)**	**SD**	**P**
CL. mR1	0.28	0.87	0.532
CL. mR2	0.10	0.88	0.793
CL. mR3	0.09	1.18	0.866
CL. mR4	-0.04	0.84	0.929
CL. mR5	-0.19	0.89	0.69
CL. mR6	0.35	0.46	0.046
CL. mR7	0.66	0.55	0.024
CL. mL1	0.26	0.81	0.546
CL. mL2	0.40	0.70	0.233
CL. mL3	0.92	0.81	0.939
CL. mL4	-0.09	0.82	0.821
CL. mL5	-0.28	1.03	0.624
CL. mL6	-0.44	0.65	0.262
CL. mL7	-0.78	0.70	0.063

CL: crown length, M: maxilla, m: mandible, R: right, L: left

A trend was observed in the mandible, rather than the maxilla, where root lengths tended to be more elongated in group N compared to their corresponding controls.

Statistical differences existed for the maxillary R 2/ L 6 and mandibular R 3-4-5-6/ L 1-3-4-5-6 (Table IV.10).

Table IV.10: Comparison between CN&N for root length

Measurements Maxilla	Mean difference (CN-N)	SD	P
RL. MR1	-0.06	0.26	0.582
RL. MR2	0.19	0.18	0.05
RL. MR3	0.06	0.27	0.605
RL. MR4	0.02	0.12	0.769
RL. MR5	0.09	0.21	0.442
RL. MR6	-0.11	0.14	0.102
RL. MR7	-0.06	0.14	0.365
RL. ML1	-0.01	0.19	0.92
RL. ML2	0.29	0.30	0.06
RL. ML3	0.08	0.19	0.326
RL. ML4	0.19	0.26	0.224
RL. ML5	0.18	0.56	0.225
RL. ML6	-0.10	0.11	0.023
RL. ML7	-0.07	0.13	0.354
Mandible	**Mean difference (CN-N)**	**SD**	**P**
RL. mR1	0.14	0.14	0.087
RL. mR2	0.06	0.15	0.467
RL. mR3	0.16	0.12	0.02
RL. mR4	0.24	0.13	0.002
RL. mR5	0.42	0.17	0.001
RL. mR6	0.27	0.20	0.035
RL. mR7	0.14	0.21	0.189
RL. mL1	0.20	0.10	0.004
RL. mL2	0.11	0.14	0.096
RL. mL3	0.23	0.15	0.004
RL. mL4	0.27	0.11	0.001
RL. mL5	0.52	0.24	0.003
RL. mL6	0.31	0.17	0.004
RL. mL7	-0.03	0.19	0.698

RL: root length, M: maxilla, m: mandible, R: right, L: left

1. Group N versus A

The comparison between the affected individuals (A) with the non-affected relatives (N) exposed a reduction in all parameters related to arch dimension, which manifested in constricted maxillary and mandibular arches. Less crowding was also observed (Table IV.11).

Table IV.11: Comparison between N&A for arch parameters

Measurements **Maxilla**	**Mean difference (N-A)**	**SD**	**P**
ACD	-3.02	1.51	0.006
ACA	6.03	3.02	0.02
ACR	9.05	4.53	0.002
Inter-molar width	6.27	3.14	<0.001
Inter-canine width	7.20	3.60	<0.001
Arch length	7.23	3.62	<0.001
Mandible	**Mean difference (N-A)**	**SD**	**P**
ACD	-3.02	1.51	0.003
ACA	3.35	1.68	0.013
ACR	2.37	1.19	0.002
Inter-molar width	4.98	2.49	<0.001
Inter-canine width	7.28	4.01	0.002
Arch length	4.61	2.30	0.002

Differences for crown width between affected and their non-affected relatives was only statistically significant for maxillary R2 (Table IV.12).

Table IV.12: Comparison between N&A for crown width

Measurements Maxilla	Mean difference (N-A)	SD	P
CW. MR1	0.19	0.43	0.262
CW. MR2	0.59	0.50	0.016
CW. MR3	0.02	0.40	0.894
CW. MR4	0.15	0.42	0.408
CW. MR5	0.56	0.49	0.588
CW. MR6	0.03	0.65	0.926
CW. MR7	-0.18	0.59	0.502
CW. ML1	0.11	0.42	0.515
CW. ML2	0.61	0.68	0.058
CW. ML3	0.04	0.44	0.848
CW. ML4	-0.09	0.37	0.536
CW. ML5	0.01	0.61	0.966
CW. ML6	0.36	1.24	0.629
CW. ML7	-0.45	0.70	0.115
Mandible	**Mean difference (N-A)**	**SD**	**P**
CW. mR1	-0.03	0.34	0.853
CW. mR2	0.05	0.46	0.778
CW. mR3	-0.15	0.47	0.473
CW. mR4	0.02	0.47	0.928
CW. mR5	0.12	0.40	0.489
CW. mR6	-0.11	0.51	0.613
CW. mR7	0.31	0.56	0.225
CW. mL1	0.01	0.33	0.946
CW. mL2	-0.01	0.31	0.95
CW. mL3	0.11	0.37	0.425
CW. mL4	-0.04	0.51	0.831
CW. mL5	0.18	0.46	0.34
CW. mL6	-0.07	0.50	0.748
CW. mL7	0.31	0.52	0.158

CW: crown width, M: maxilla, m: mandible, R: right, L: left

Statistically significant differences were noted for crown lengths were reduced in group A for maxillary R 5-7/ L 2-5-6 and mandibular R 3-4-6-7/ L 4-6. While not clinically significant, group A present a trend of decrease in crown length (Table IV.13).

Table IV.13: Comparison between N&A for crown length

Measurements Maxilla	Mean difference (N-A)	SD	P
CL. MR1	0.47	1.07	0.276
CL. MR2	0.83	0.86	0.064
CL. MR3	0.58	0.80	0.076
CL. MR4	0.53	0.94	0.16
CL. MR5	0.84	0.83	0.02
CL. MR6	0.46	0.82	0.179
CL. MR7	0.69	0.70	0.017
CL. ML1	0.57	0.96	0.134
CL. ML2	1.04	0.83	0.012
CL. ML3	0.68	0.87	0.084
CL. ML4	0.42	0.97	0.276
CL. ML5	1.05	0.75	0.003
CL. ML6	1.05	0.68	0.001
CL. ML7	0.46	0.87	0.267
Mandible	**Mean difference (N-A)**	**SD**	**P**
CL. mR1	0.29	0.94	0.444
CL. mR2	0.45	0.85	0.185
CL. mR3	0.88	0.74	0.022
CL. mR4	0.75	0.72	0.034
CL. mR5	0.81	0.95	0.054
CL. mR6	0.26	0.59	0.023
CL. mR7	-0.04	0.77	0.05
CL. mL1	0.21	0.83	0.569
CL. mL2	0.17	0.83	0.609
CL. mL3	-0.31	0.64	0.078
CL. mL4	0.86	0.65	0.011
CL. mL5	0.78	0.94	0.053
CL. mL6	0.61	0.62	0.023
CL. mL7	0.70	0.92	0.059

CL: crown length, M: maxilla, m: mandible, R: right, L: left

As for root length, Group A had significantly shorter roots for the majority of the teeth when compared with their relatives. Differences existed for maxillary R 1-2-3-6/ L 1-2-3-4-6 and mandibular R 1-2-3-6/ L 1-2-3-5 (Table IV.14).

Table IV.14: Comparison between N&A for root length

Measurements Maxilla	Mean difference (N-A)	SD	P
RL. MR1	0.22	0.22	0.023
RL. MR2	0.40	0.23	0.005
RL. MR3	0.29	0.24	0.007
RL. MR4	0.07	0.11	0.095
RL. MR5	0.09	0.13	0.175
RL. MR6	0.29	0.18	0.002
RL. MR7	-0.09	0.15	0.138
RL. ML1	0.27	0.24	0.011
RL. ML2	0.51	0.22	<0.001
RL. ML3	0.24	0.24	0.026
RL. ML4	0.11	0.11	0.03
RL. ML5	0.11	0.44	0.089
RL. ML6	0.27	0.16	0.004
RL. ML7	0.00	0.07	0.391
Mandible	**Mean difference (N-A)**	**SD**	**P**
RL. mR1	0.15	0.08	<0.001
RL. mR2	0.37	0.16	<0.001
RL. mR3	0.21	0.12	<0.001
RL. mR4	-0.07	0.13	0.216
RL. mR5	-0.12	0.19	0.158
RL. mR6	0.14	0.17	0.049
RL. mR7	0.01	0.21	0.926
RL. mL1	0.18	0.09	<0.001
RL. mL2	0.40	0.17	<0.001
RL. mL3	0.17	0.12	0.001
RL. mL4	-0.06	0.12	0.257
RL. mL5	-0.16	0.18	0.04
RL. mL6	0.13	0.16	0.076
RL. mL7	0.04	0.20	0.606

RL: root length, M: maxilla, m: mandible, R: right, L: left

C. Genetic analysis

The average characteristics of all selected family in terms of number of reads, quality scores, read length, total number of variants are displayed in Table IV.15. The 9

Families have an average total read bases of 10,399,532,574, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 95% respectively, indicating that 98% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more. The total number of variants equal to 154,616.

Table IV.15: Summary of the average characteristics of the 9 selected families.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	10,399,532,574	906,454,924	9,499,680,592	13,636,610,680
Total number of reads	68,871,077	6,003,013	62,099,310	94,401,106
GC-content (%)	52	0.145335	51.35	52.05
AT-content (%)	48	0.145335	47.95	48.65
Q20 (%)	98	0.086362	97.82	98.23
Q30 (%)	95	0.209444	94	94.98
Total number of variants	154,616	25,842	15,089	176,390

1. Family characteristics

The average characteristics of each selected family in terms of number of reads, quality scores, read length, total number of variants are presented in this section (Tables IV.16-24).

<u>Family A:</u>

Family A has an average total read bases of 11,220,856,206, 51% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 94% respectively, indicating that 98% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more. The total number of variants was equal to 111,181 (Table IV.16).

Table IV.16: Summary of the average characteristics of family A

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	11,220,856,206	1031632447	10,289,062,386	12,658,976,582
Total number of reads	74,310,306	6832002.96	68,139,486	83,834,282
GC-content (%)	51	0.136707	51.35	51.64
AT-content (%)	49	0.136707	48.36	48.65
Q20 (%)	98	0.114407	97.82	98.1
Q30 (%)	94	0.249043	94	94.61
Total number of variants	111,181	68,140	15,089	165,505

Family B:

Family B has an average total read bases of 11,832,428,177, 51.43% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 97.93% and 94.3% respectively, indicating that 97.93% of the fragments have 20 copies read and more and 94.3% of the fragments have 30 copies read and more. The total number of variants was equal to 155,736 (Table IV.17).

Table IV.17: Summary of the average characteristics of family B.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	11,832,428,177	781,018,396	10,823,206,766	12,942,278,856
Total number of reads	78,360,452	5,172,307	71,676,866	85,710,456
GC-content (%)	51.43	0.062849	51.35	51.52
AT-content (%)	48.57	0.062849	48.48	48.65
Q20 (%)	97.93	0.027386	97.9	97.97
Q30 (%)	94.3025	0.054025	94.24	94.37
Total number of variants	155,736	11,571	139,842	171879

Family C:

Family C has an average total read bases of 11,221,746,206, 51% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 94% respectively, indicating that 98% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more. The addition total number of variants was equal to 156,562 (Table IV.18).

Table IV.18: Summary of the average characteristics of family C.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	11,221,746,206	1011642447	10,278,025,386	12,618,976,582
Total number of reads	74,310,306	6832002.96	68,139,486	83,834,282
GC-content (%)	51	0.136707	51.35	51.64
AT-content (%)	49	0.136707	48.36	48.65
Q20 (%)	98	0.114407	97.82	98.1
Q30 (%)	94	0.249043	94	94.61
Total number of variants	156,562	5,779	146,805	161,027

Family D:

Family D has an average total read bases of 11,821,887,169, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 94% respectively, indicating that 98% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more. The total number of variants was equal to 158,415 (Table IV.19).

Table IV.19: Summary of the average characteristics of family D.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	11,821,887,169	908,474,852	10,348,040,570	12,622,456,024
Total number of reads	78,290,644	6,016,389	68,530,070	83,592,424
GC-content (%)	52	0.129904	51.37	51.73
AT-content (%)	48	0.129904	48.27	48.63
Q20 (%)	98	0.025495	97.91	97.98
Q30 (%)	94	0.081662	94.22	94.44
Total number of variants	158,415	6,808	150,514	167,639

Family E:

Family E has an average total read bases of 13,767,955,086, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 94% respectively, indicating that 98% of the fragments have 20 copies read and more and 94%

of the fragments have 30 copies read and more. The total number of variants was equal to 168,265 (Table IV.20).

Table IV.20: Summary of the average characteristics of family E.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	13,767,955,086	577,782,516	13,067,475,070	13,520,513,122
Total number of reads	91,178,511	3,826,374	86,539,570	94,401,106
GC-content (%)	52	0.209205	51.53	52.02
AT-content (%)	48	0.209205	47.98	48.47
Q20 (%)	98	0.095743	97.96	98.14
Q30 (%)	94	0.237627	94.21	94.76
Total number of variants	168,265	2,832	164,306	172,314

Family F:

Family F has an average total read bases of 11,689,109,698, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 94% respectively, indicating that 98% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more. The total number of variants was equal to 161,678 (Table IV.21).

Table IV.21: Summary of the average characteristics of family F.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	11,689,109,698	1,391,846,628	9,915,496,238	13,636,610,680
Total number of reads	77,411,323	9,217,527	65,665,538	90,308,680
GC-content (%)	52	0.174428	51.38	51.87
AT-content (%)	48	0.174428	48.13	48.62
Q20 (%)	98	0.023452	97.95	98.01
Q30 (%)	94	0.047434	94.33	94.45
Total number of variants	161,678	11,168	145,797	176,390

Family G:

Family G has an average total read bases of 13,767,955,086, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 94% respectively, indicating that 98% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more. The total number of variants was equal to 156,654 (Table IV.22).

Table IV.22: Summary of the average characteristics of family G.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	13,767,955,086	577,782,516	13,067,475,070	13,520,513,122
Total number of reads	91,178,511	3,826,374	86,539,570	94,401,106
GC-content (%)	52	0.209205	51.53	52.02
AT-content (%)	48	0.209205	47.98	48.47
Q20 (%)	98	0.095743	97.96	98.14
Q30 (%)	94	0.237627	94.21	94.76
Total number of variants	156,654	6,768	147,102	161,964

Family H:

Family H has an average total read bases of 11,821,887,169, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 94% respectively, indicating that 98% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more. The total number of variants was equal to 161,573 (Table IV.23).

Table IV.23: Summary of the average characteristics of family H.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	11,821,887,169	908,474,852	10,348,040,570	12,622,456,024
Total number of reads	78,290,644	6,016,389	68,530,070	83,592,424
GC-content (%)	52	0.129904	51.37	51.73
AT-content (%)	48	0.129904	48.27	48.63

Q20 (%)	98	0.025495	97.91	97.98
Q30 (%)	94	0.081662	94.22	94.44
Total number of variants	161,573	3,941	156,023	164,788

Family I:

Family I has an average total read bases of 10,062,817,697, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 98% and 95% respectively, indicating that 98% of the fragments have 20 copies read and more and 95% of the fragments have 30 copies read and more. The total number of variants was equal to 153,218 (Table IV.24).

Table IV.24: Summary of the average characteristics of family I.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	10,062,817,697	618,194,128	9,376,995,810	10,859,611,960
Total number of reads	66,641,177	4,094,001	62,099,310	71,917,960
GC-content (%)	52	0.141704	51.69	52.05
AT-content (%)	48	0.141704	47.95	48.31
Q20 (%)	98	0.044721	98.11	98.23
Q30 (%)	95	0.070106	94.79	94.98
Total number of variants	153,218	8,453	139,842	161,027

2. Comparison with previous studies

A comparison was performed, prior to the filtering steps, between the results of the present study and those from previous publications in which the following candidate genes were discovered: *DSPP, MSX1, PAX9, AXIN2, WNT10A and EDAR* for hypodontia and *CDH26, APC* for hyperdontia.

Variations in EDAR, CDH26 and APC were located in the genome of affected members of families E, F respectively in the present study, while WNT10A was found in both affected and non-affected members of family G.

3. Filtering results

As previously described, we started our 1st stringent filtering analysis by keeping only the variants that have a PASS filter status, a coverage read >40-50 and a high putative impact (clinically pathogenic variants) on protein structure and function. The number of variants was reduced to an average of 40000 in all families. Further reduction of variants was done by filtering out the non-affected subjects.

The common variants in specific genes among affected individuals in each family came as follow, while no shared variants were found in families C, E and G (Tables IV.25-26):

- 2 in family A: CRACR2A and RMDN1
- 2 in family B: ADCK2 and PER3
- 1 in family D: NOV CCN3
- 1 in family F: NME8
- 2 in family H: MUC4 and OR10A6
- 2 in family I: PDE4A and PICK1

Table IV.25: Summary of the common variants in specific genes between affected individuals of each family following the 1st filtering (high putative impact)

	A	B	C	D	E	F	G	H	I
Gene 1	CRACR2A	ADCK2	--	NOV -CCN3	--	NME8	--	MUC4	PDE4A
Gene 2	RMDN1	PER3	--	--	--	--	--	OR10A6	PICK1

Table IV.26: Summary of the characteristics of the shared variant in specific genes between affected individuals following the 1st filtering (high putative impact)

Family	Gene Name	Chrom	Position	HGVSp	dbSNP	Zygosity	Effect
A	***CRACR2A***	12	3747334	p.Thr520HisfsTer15	--	HET	Frameshift variant
	RMDN1	8	87519253	p.Gln73GlyfsTer11	--	HET	Frameshift variant
B	***ADCK2***	7	140374064	--	rs140400082	HET	Splice donor variant
	PER3	1	7902731	--	rs772840416	HET	Frameshift variant
D	***NOV-CCN3***	8	120431446	p.Trp213Ter	--	HET	Stop gained
F	***NME8***	7	37936670	p.Phe582Ter	--	HET	Frameshift variant
H	***MUC4***	3	195505766	p.Ser4229GlufsTer85	rs751479306	HET	Frameshift variant
	OR10A6	11	7949334	p.Leu292CysfsTer6	--	HET	Frameshift variant
I	***PDE4A***	19	10572358	--	--	HET	Frameshift variant
	PICK1	22	38470313	--	--	HET	Frameshift variant

As part of the 2nd stringent filtering, the analysis was then repeated by selecting only the variants having a PASS filter status a Minor Allele Frequency (MAF) ≤ 1% and a moderate putative impact, while ensuring that these variants affect protein function and were classified as “damaging” according to the Polyphen2 and SIFT prediction software. The number of variants was subsequently reduced to 29000 in all families.

Common gene(s) among affected individuals were found in all families except for families B, C and D (Tables IV.27-28):

- 4 in family A: GYS1; HOXA3; AGK and AKR1C4
- 1 in family E: EDAR
- 4 in family F: APCDD1; CDH26; DDX11 and LAMC2

- 3 in family G: LIMD1; DCAF6 and WNT10A. It should be noted that the gene WNT10A was expressed in non-affected individual.
- 2 in family H: FGFBP1 and DFFA
- 3 in family I: ABCA10, DYRK1A, PKN3

Table IV.27: Summary of the common variants in specific genes between affected individuals of each family following the 2nd filtering (moderate putative impact and MAF<0.001)

	Family A	Family B	Family C	Family D	Family E	Family F	Family G	Family H	Family I
Gene 1	GYS1	--	--	--	EDAR	APCDD1	LIMD1	FGFBP1	ABCA10
Gene 2	HOXA3	--	--	--	--	CDH26	DCAF6	DFFA	DYRK1A
Gene 3	AGK	--	--	--	--	DDX11	WNT10A	--	PKN3
Gene 4	AKR1C4	--	--	--	--	LAMC2	--	--	--

Table IV.28: Summary of the characteristics of the shared variant in specific genes between affected individuals (that segregate with the phenotype) following the 2nd stringent (moderate putative impact and MAF<0.001)

Family	Gene Name	Chrom	Position	HGVSp	dbSNP	Zygosity	Effect
A	***GYS1***	19	49477975	p.Pro442Thr	rs142951866	HET	Missense variant
	HOXA3	7	27147763	p.Gly368Ala	rs747577258	HET	Missense variant
	AGK	7	141321601	p.Lys196Asn	--	HET	Missense variant
	AKR1C4	10	5242311	p.Lys84Asn	--	HET	Missense variant
E	***EDAR***	2	109545744	p.Arg89His	rs121908450	HET	Missense variant
F	***APCDD1***	18	10485732	p.Gly350Ser	rs376017098	HET. HOM	Missense variant
	CDH26	20	58545197	p.Leu63Met	rs144755899	HET. HOM	Missense variant
	DDX11	12	31242081	p.Arg263Gln	rs201968272	HET. HOM	Missense variant
	LAMC2	1	183196682	p.Ile440Val	rs147889360	HOM	Missense variant

G	***LIMD1***	3	45707144	p.Arg505Trp	--	HET. HOM	Missense variant
	DCAF6	1	168014447	p.Arg747Gln	rs145189179	HET. HOM	Missense variant
	WNT10A	2	219755011	p.Phe228Ile	rs121908120	HET	Missense variant
H	***FGFBP1***	4	15937904	p.Arg118Trp	rs374462127	HET. HOM	Missense variant
	DFFA	1	10529326	p.Ile69Thr	rs138842024	HOM	Missense variant
I	***ABCA10***	17	67146204	p.Arg1466Ser	rs137945891	HET	Missense variant
	DYRK1A	15	90344377	p.Phe326Val	--	HET	Missense variant
	PKN3	9	131479036	p.Glu607Lys	rs549258373	HET	Missense variant

The 3rd step of stringent filtering consisted of function check to the 14 potential family candidate genes (***CRACR2A***, ***PER3***, ***NOV CCN3***, ***EDAR***, ***APCDD1***, ***CDH26***, ***LAMC2***, ***NME8***, ***LIMD1***, ***WNT10A***, ***FGFBP1***, ***DFFA***, ***OR10A6*** *and* ***DRK1A***) that may segregate with the investigated phenotypes (hypo/hyperdontia) based on either genotype/phenotype correlation or their functions were described to be related to development and differentiation.

The genes characteristics as well as the phenotype/genotype distribution are displayed in Tables IV.29-30.

Table IV.29: Summary of the characteristics of the 14 potential novel genes and their variants

	Gene	Chrom	Locus	Position	HGVSp	dbSNP	Zygosity	Effect
A	***CRACR2A***	12	12p13.32	3747334	p.Thr520HisfsTer15	--	HET	Frameshift
B	***PER3***	1	1p36.23	7902731	chr1:7902731:G:A	rs772840416	HET	Frameshift
D	***NOV CCN3***	8	8q24.12	120431446	p.Trp213Ter	--	HET	Stop gained
E	***EDAR***	2	2q13	109545744	p.Arg89His	rs121908450	HET	Missense
F	***APCDD1***	18	18p11.22	10485732	p.Gly350Ser	rs376017098	HET, HOM	Missense
	NME8	7	7p14.1	37936670	p.Phe582Ter	--	HET	Frameshift
	CDH26	20	20q13.33	58545197	p.Leu63Met	rs144755899	HET, HOM	Missense
	LAMC2	1	1q25.3	183196682	p.Ile440Val	rs147889360	HOM	Missense
G	***LIMD1***	3	3p21.31	45707144	p.Arg505Trp	--	HET, HOM	Missense
	WNT10A	2	2q35	219755011	p.Phe228Ile	rs121908120	HET	Missense
H	***FGFBP1***	4	4p15.32	15937904	p.Arg118Trp	rs374462127	HET, HOM	Missense
	OR10A6	11	11p15.4	7949334	p.Leu292CysfsTer6	--	HOM	Frameshift
	DFFA	1	1p36.22	10529326	p.Ile69Thr	rs138842024	HOM	Missense
I	***DYRK1A***	21	21q22.13	90344377	p.Phe326Val	--	HET	Missense

Bhbjkk Moderate putative impact
Bhbjkk High putative impact

Table IV.30: Genotype and phenotype distribution in the 9 families

	Genes			AFFECTED		NON-AFFECTED
Families	**HYPO**	**HYPER**	**Non-Affected**	**HYPO**	**HYPER**	
A	***CRACR2A***			2		1
B	***PER3***			2		2
C	**--**			2		2
D	***NOV/CCN3***			3		1
E	***EDAR***			3		1
H	***FGFBP1***	***DFFA***		2 *(1 oligo)*	1	0
	5	**1**		**14**		**7**
F	***CDH26, APCDD1, NME8***	***LAMC2, CDH26, NME8, APCDD1***		2	1	1
G	***WNT10A, LIMD1***	***LIMD1***	***WNT10A***	3	1	2
	5	**5**	**1**	**5**	**3**	**3**
I		***DYRK1A***			3	2
						5
Total	**10**	**7**	**1**	**19**	**6**	**10 (12)**
TOTAL	**13**			**37**		

individuals having hypodontia

Vvjhb kj individuals having hyperdontia

4. Family findings

Below are displayed the pedigrees of each family with their corresponding candidate genes.

Family A

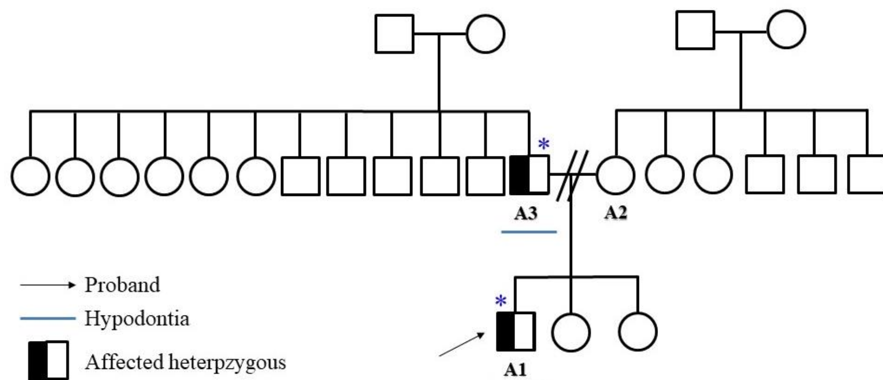


Figure IV.1: Phenotype and genotype distribution in family A

CRACR2A (Figure IV.1) segregates in a heterozygous pattern in subjects A1 and A3.

Family B

PER3 (Figure IV.2) segregates in a heterozygous pattern in subjects B2 and B4.

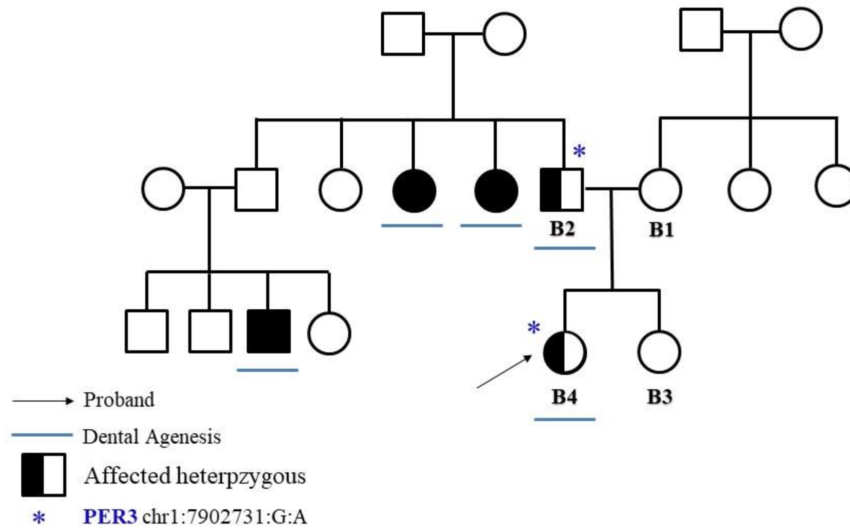


Figure IV.2: Phenotype and genotype distribution in family B

Family D

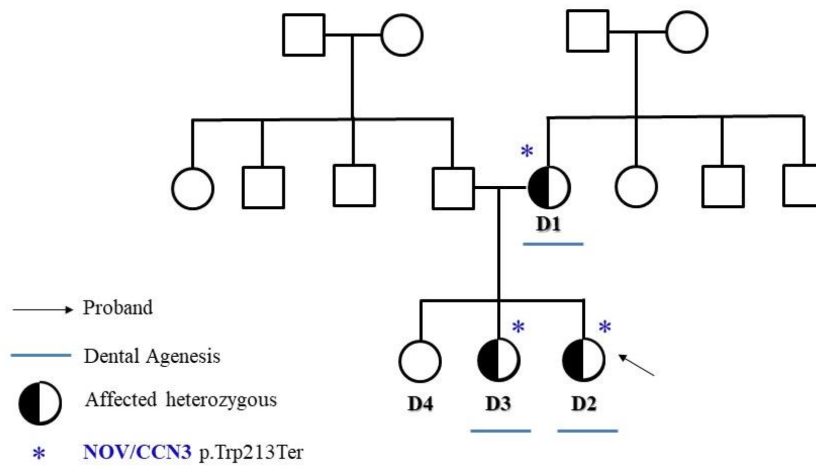


Figure IV.3: Phenotype and genotype distribution in family D

NOV/CCN3 (Figure IV.3) segregates in a heterozygous pattern in subjects D1-2-3.

Family E

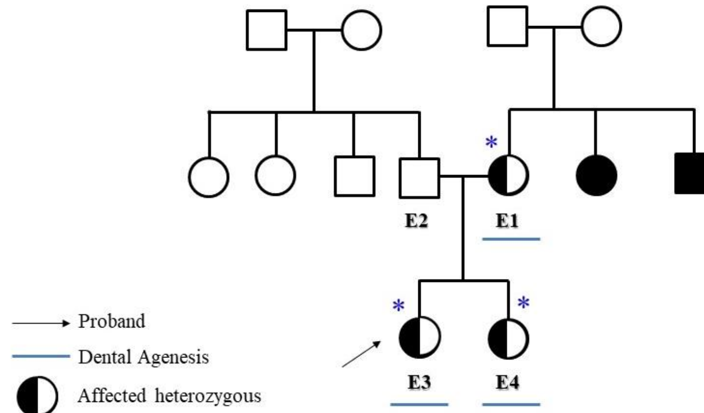


Figure IV.4: Phenotype and genotype distribution in family E

EDAR (Figure IV.4) segregates in a heterozygous pattern in subjects E1-E3 and E4.

Family F

CDH26, APCDD1, NME8, and LAMC2 were identified in family F (Figure IV.5).

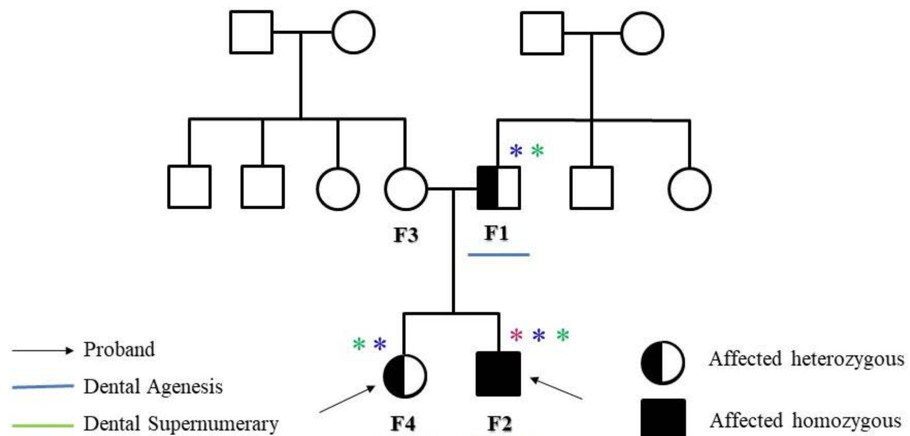


Figure IV.5: Phenotype and genotype distribution in family F

• *NME8:*

NME8 gene segregates in a heterozygous pattern in subjects F1 and F4 and homozygous in subject F2.

• *LAMC2:*

LAMC2 gene segregates in a homozygous pattern in subject F2.

• *CDH26:*

CDH26 gene segregates in a heterozygous pattern in subjects F1 and F4 and homozygous in subject F2.

• *APCDD1:*

APCDD1 gene segregates in a heterozygous pattern in subjects F1 and F4 and homozygous in subject F2.

Family G

WNT10A and LIMD1 were identified in family G (Figure IV.6).

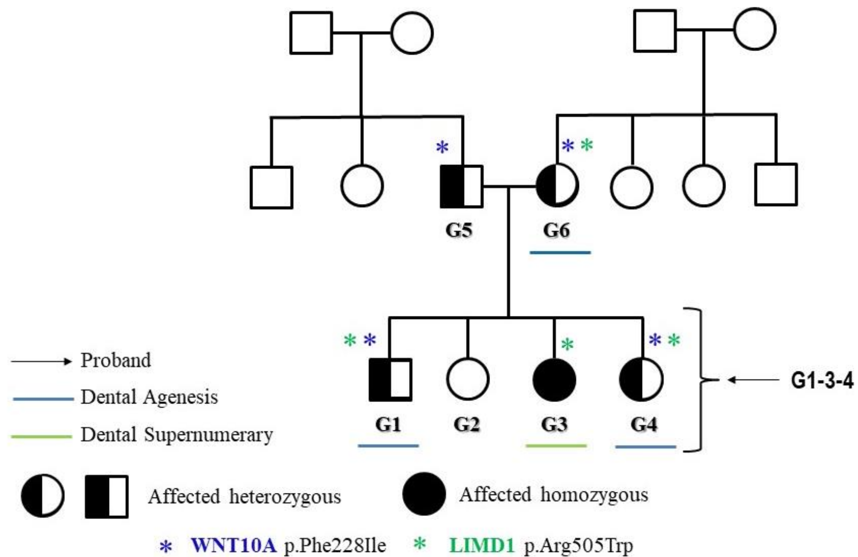


Figure IV.6: Phenotype and genotype distribution in family G

• <u>*WNT10A:*</u>

WNT10A gene segregates in a heterozygous pattern in subjects G1-4-5-6. Its expression in G5 (non-affected control) negates its direct role in dental agenesis.

• <u>*LIMD1:*</u>

LIMD1 gene segregates in a heterozygous pattern in subjects G1-4-6 and homozygous in subject G3.

Family H

FGFBP1, OR10A6 and DFFA were identified in family H (Figure IV.7).

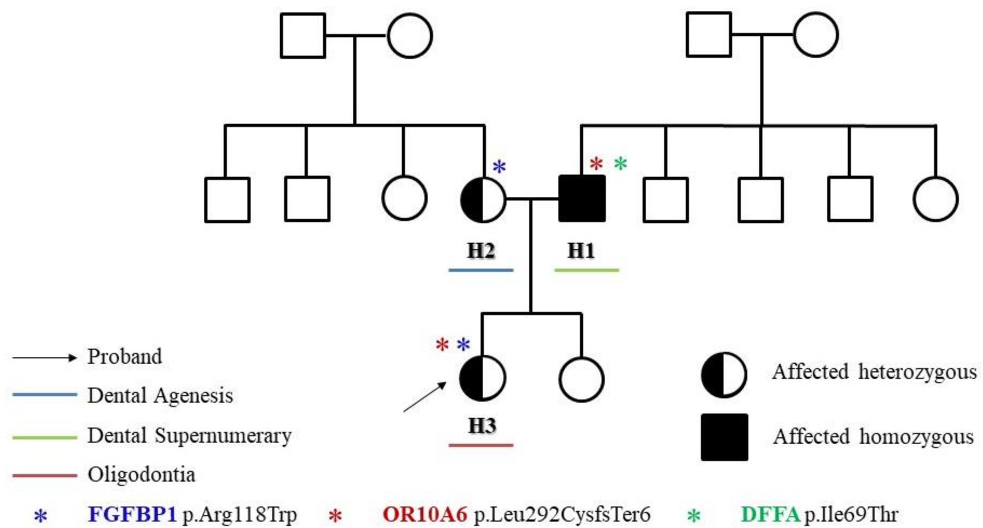


Figure IV.7: Phenotype and genotype distribution in family H

• <u>*FGFBP1:*</u>

FGFBP1 gene segregates in a heterozygous pattern in subjects H2 and H3.

• *OR10A6:*

OR10A6 gene segregates in a heterozygous pattern in subjects H3 and homozygous in subject H1.

• *DFFA:*

DFFA gene segregates in a homozygous in subject H1.

Family I

DYRK1A gene segregates in a heterozygous pattern in subjects I2-3-5 (Figure IV.8).

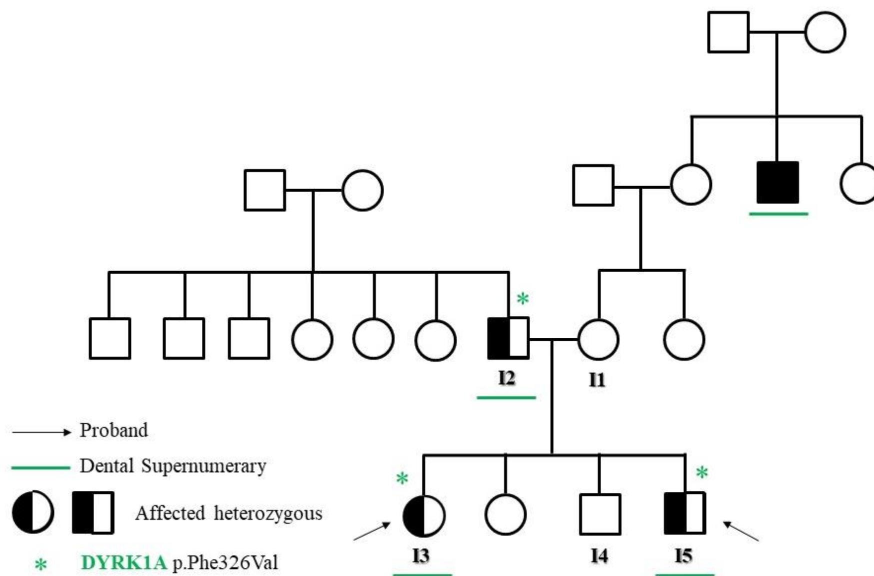


Figure IV.8: Phenotype and genotype distribution in family I

CHAPTER V

DISCUSSION

A. Introduction

The genetic component plays a substantial role in the etiology of hypo/hyperdontia, as supported by the observation of familial segregation. Different studies on human genome sequencing have been completed and through which, researchers documented the genetic basis of many common human traits and diseases.

The use of Genome Wide Association Studies (GWAS) enabled the association of specific genes loci with particular traits and diseases. Accordingly, genetic association data currently provide new routes to understanding the etiology of conditions, predicting a patient's risk to the trait, or treatment response, and improving personalized prevention and treatment. The technology and statistical methods for completing whole genome tagging of variants and GWAS has developed rapidly over the last decade.

This recent progress has allowed the investigations of susceptible genes that are responsible of the development of hypo/hyperdontia through genetic mapping studies that were performed in different ethnic populations: European, Italian, Spanish, Australian, Japanese, Chinese, African-American. Those studies identified several chromosomal regions or loci that inhouse susceptible genes for hypo/hyperdontia. In the present study, Whole Exome Sequencing (WES) was performed on 9 Mediterranean families including 37

individuals (affected and non-affected) of different generations. The objectives of this research project were met.

B. Genetic findings

The Pedigree analysis indicated that, all pedigrees suggest a Mendelian inheritance pattern and segregate in an autosomal dominant manner. This finding supports several previous genetic studies (Ahmad et al., 1998; Arte, 2001; Bergstrom, 1977; Coster et al., 2009; Das et al., 2002; Davis, 1987; Järvinen and Lehtinen, 1981; Polder et al., 2004; Rajab and Hamdan, 2002; Tanaka, 1998; Thesleff, 2000; Vastardis, 2000; Vastardis et al., 1996; Wang et al., 2018) .

The analysis further revealed equal number of reported generations per family (n=3) in most of the families, average number of reported affected males and females (n=1-2) per family, and more families with female predominance for hypodontia and male predominance for hyperdontia.

Considering the number of affected males and females, we can hypothesize that in the Middle Eastern families with female predominance, the number of affected females is greater for hypodontia and the number of affected males is greater for hyperdontia.

The results of this study support previous reports of no significant gender differences related to the prevalence of DA although female predominance was noted (Polder et al., 2004; Schalk-Van Der Weide and Bosman, 1996; Vastardis, 2000). No conclusive findings regarding gender predisposition to hypo/hyperdontia was elaborated possibly because of sampling or population differences.

Our genetic analysis revealed four common genes, associated with hypodontia and/or hyperdontia, between the present study and the results of previous studies done in different ethnic population:

- CDH26 and APCDD1 expressed in family F where both phenotypes are present:

CDH26 was previously described as being responsible for the development of isolated supernumerary teeth, on the contrary, our study findings revealed that CDH26 alone does not develop supernumerary teeth.

APCDD1 was previously described as being responsible for syndromic supernumerary teeth, whereas, our study findings revealed that APCDD1 was expressed in individuals with isolated supernumerary teeth.

- EDAR and WNT10A were expressed in family E and G respectively where dental agenesis phenotype was present:

EDAR gene was previously described in the literature as being responsible for syndromic dental agenesis, on the contrary, our study findings revealed its association with isolated dental agenesis.

WNT10A was previously described as being responsible for syndromic and non-syndromic (isolated) dental agenesis. In our study, we found that, WNT10A alone does not cause dental agenesis which was confirmed by the expression of WNT10A in one of the non-affected (control) individuals of the family G.

Therefore, the development of hypo/hyperdontia is polygenic. And since different genes are expressed in each family, we cannot search for one universal gene in all affected individuals.

C. Family's findings

1. Family A: "CRACR2A"

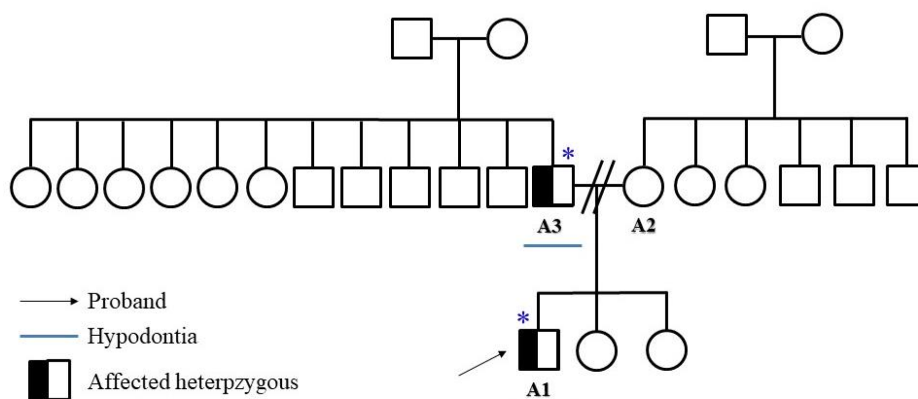


Figure V.1: Phenotype and genotype distribution in family A

The CRACR2A (Figure V.1) gene was previously reported as being involved in cell differentiation (ameloblast and odontoblasts) and the mutation of the gene plays a key role in the development of amelogenesis imperfecta in humans causing the teeth to be unusually small, discolored, pitted or grooved, and prone to rapid wear. (Robinson et al., 2012)

No clear relation with dental agenesis was previously reported.

2. *Family B: "PER3"*

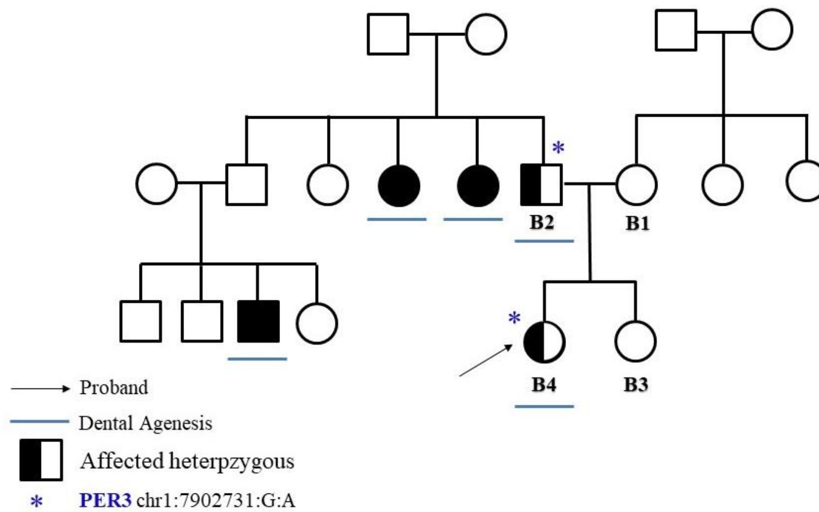


Figure V.2: Phenotype and genotype distribution in family B

• *Dental relation:*

The PER3 (Figure V.2) (among the family of the clock genes) was previously reported to be highly expressed in periodontal and gingival cells and its level is modulated by hypoxia mimetic agents (master regulators of the hypoxia response).

Previous studies reported that the mutation of the PER3 gene will affect the periodontal health (Janjić et al., 2017; Mortola, 2007).

• *Non-dental relation*:

Clock genes family encode components of the circadian rhythms of locomotor activity, metabolism, and behavior. Polymorphisms in those genes (including the PER3) have been linked to sleep disorders and Major Depressive Disorders (Xu et al., 2019). Downregulation of the PER3 gene is associated with shortened circadian period length (Leocadio-Miguel et al., 2018).

• <u>*Subject health report:*</u>

Subject B4 reported migraine issues and episodes of insomnia.

Subject B2 reported some sleep disorders including: snoring, coughing and waking arousals caused by overweight and reported afternoon fatigue and sleepiness which may be a diagnosis for sleep apnea.

The health report confirms the effect of hypoxia on the PER3 expression and its effect on the patient's health.

3. Family D: "NOV/CCN3"

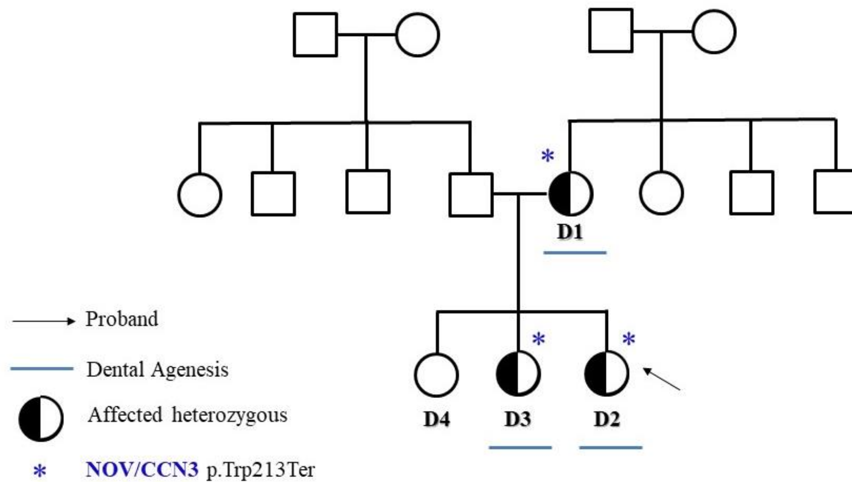


Figure V.3: Phenotype and genotype distribution in family D

NOV (Figure V.3) gene was published in the literature as being involved in promoting dentin regeneration through proliferation and differentiation of DPSCs (dental pulp stem cells) by modulating Notch and BMP2 signaling pathways. (Wang et al., 2014)

After dentin injury, CCN3 expression increases activating the Notch signaling pathway which will activate the cell proliferation (DPSCs). The cell proliferation will then

decrease the expression of the CCN3 which will activate the BMP signaling pathway and induce the cell odontoblastic differentiation. (Wang et al., 2014)

4. Family E: "EDAR"

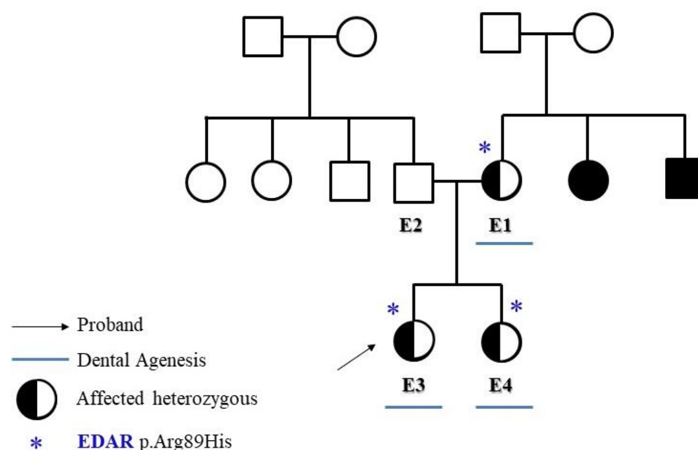


Figure V.4: Phenotype and genotype distribution in family E

EDAR (Figure V.4) gene plays a role in the embryonic development (Parveen et al., 2019; Zeng et al., 2017).

It has been previously reported that the EDAR gene is associated with syndromic dental agenesis causing hypohidrotic and anhidrotic ectodermal dysplasia (HED). The HED features comprise of hypodontia/oligodontia, along with hypohidrosis/anhidrosis, and hypotrichosis (Cluzeau et al., 2011; Parveen et al., 2019)

Non syndromic tooth agenesis associated with EDAR gene mutation has not been previously reported.

5. Family F:

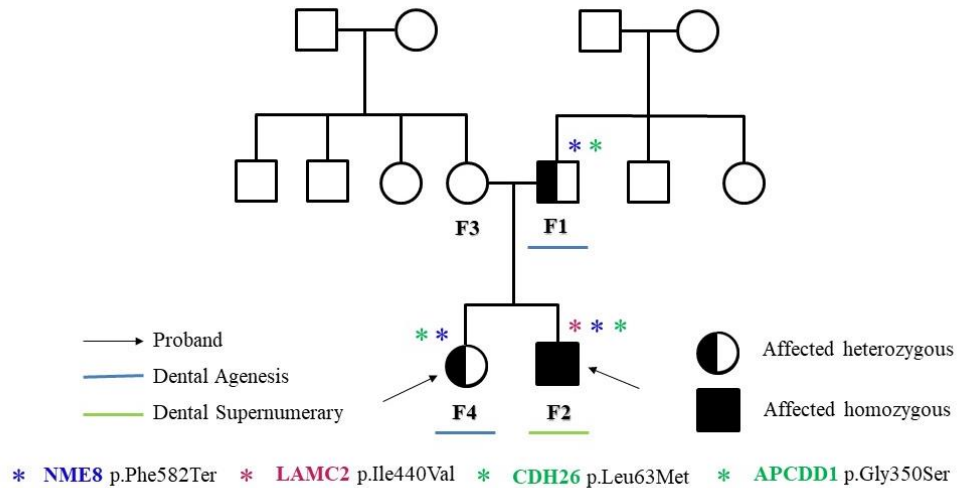


Figure V.5: Phenotype and genotype distribution in family F

• *NME8:*

The study conducted by Shimizu et. al in 2015 on Asian population highlighted genes causing predisposition for periodontal disease (Shimizu et al., 2015) and two suggestive were reported: KCNQ5 on chromosome 6q13 and GPR141-NME8 at chromosome 7p14.1

This study concluded that GPR141-NME8 locus had a strong genetic effect on the susceptibility to generalized periodontitis in Japanese individuals with a history of smoking. (Shimizu et al., 2015)

• *LAMC2:*

A study was conducted by Song et al. in 2013 to determine the molecular, functional and histological differences between human deciduous and permanent periodontal ligament (PDL) tissues by comparing their gene expression patterns.

LAMC2 was among the genes up-regulated in deciduous PDL tissues and involved in the formation of the extracellular matrix.(Song et al., 2013)

A second study was conducted Kim et al. in 2016 to determine the gene expression of the human coronal pulp (CP) and apical pulp complex (APC) aiming to explain differences in their function. LAMC2 was among the genes strongly expressed the APC.(Kim et al., 2016)

Conclusion of both studies:

- LAMC2 gene is an active gene in the early stages of dental development.
- Down regulation can be related to dental anomaly whether in number or in structure.

• *CDH26:*

A study was conducted using whole-exome sequencing of non-syndromic Japanese individuals possessing supernumerary teeth to identify genes and/or loci involved in the pathogenesis of the condition (Takahashi et al., 2017a).

An autosomal-dominant transmission pattern of non-syndromic multiple supernumerary teeth has been reported by: (Batra et al., 2005; Takahashi et al., 2017b)

• APCDD1:

APCDD1 is a negative regulator of Wnt signaling which plays a role in tooth development. It is expressed in the condensed mesenchyme at the bud stage, and in the inner enamel epithelium (IEE), including enamel knot (EK) at the cap stage. It is involved in tooth cusp patterning by modulating the epithelial rearrangement in the IEE (Neupane et al., 2015).

Mutation in APC gene was described causing syndromic supernumerary teeth: "Gardner syndrome" (Lu et al., 2017).

• <u>Summary of Family F:</u>

NME8, CDH26, and APCDD1 are common among all affected members of this family. Subject F2 having supernumerary tooth have an additional gene LAMC2 not expressed within the parents thus, it is called “denovo gene”.

This gene distribution indicates that polygenicity of NME8, CDH26 and APCDD1 cause dental agenesis, which means that those three genes are potentially individually or in combination causing the hypodontia phenotype.

When LAMC2 is expressed, it downregulates the NME8 and masks its effect thus inducing supernumerary teeth.

The hypothesis is: the difference in phenotypes expression is a gene dosage effect made by the addition of LAMC2.

6. Family G:

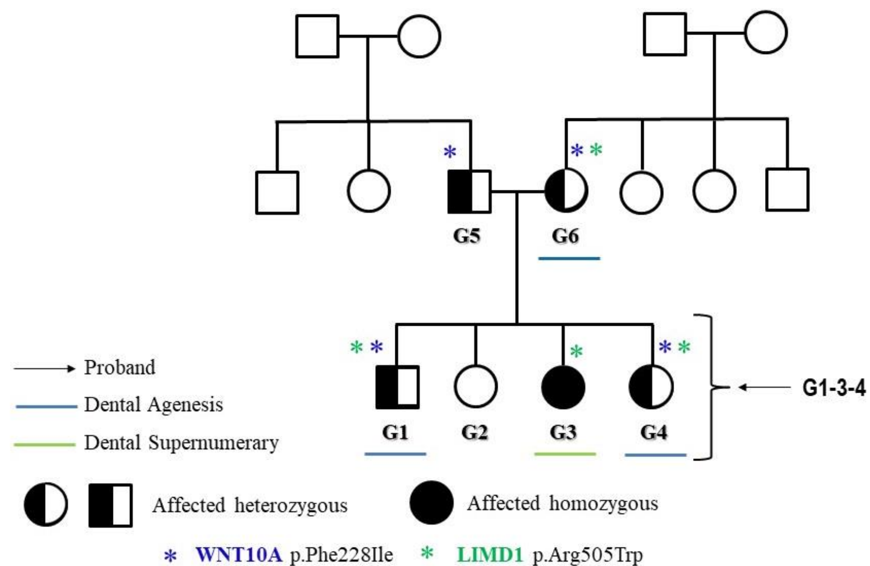


Figure V.6: Phenotype and genotype distribution in family G

• *LIMD1:*

LIMD1 is highly expressed in PDLCs: mesenchymal cells which can differentiate into osteoblastic and cementoblastic cells that will be involved in the alveolar bone and root cementum remodeling process during the orthodontic tooth treatment. (Huang et al., 2016; Tsuge et al., 2016)

Results indicate that the osteoblastic/cementoblastic differentiation of PDLCs was increased when mechanical force was imposed, and higher expression of osteoblastic/cementoblastic genes, among which: "LIMD1" (Wu et al., 2019)

• *WNT10A:*

WNT10A plays an important role in the formation and shaping of both the primary and permanent dentition.

WNT10A was previously described to be associated with syndromic and non-syndromic dental agenesis. (Abdalla et al., 2014; Dinckan et al., 2018; Du et al., 2018; Martínez-Romero et al., 2019; Parveen et al., 2019; Zeng et al., 2017, 2016)

Non-dental relation:

Mutation of WNT10A was published in the literature as being pathogenic and disease causing regardless of the disease type although few articles including (Abdalla et al., 2014; Parveen et al., 2019) described its presence without a disease linked and refuted its pathogenic effect.

The hypothesis of the pathogenicity of this gene may be explained in the current family since subjects G5, G6, G1 and G2 reported hypertension and Diabetes, subjects G3 and G4 reported prediabetes history.

It is strongly expressed in the cell lines of promyelocytic leukemia and Burkitt's lymphoma. In addition, WNT10A and WNT6 gene, are strongly co-expressed in colorectal cancer cell lines (Cluzeau et al., 2011).

WNT10A Gene has a role in the embryonic development of many parts of the body including: skin, hair, nails, teeth and sweat glands (Ectodermal tissues) (Cluzeau et al., 2011).

- Summary of Family G:

WNT10A and LIMD1 are common genes among affected members with hypodontia. Subject G3 having supernumerary tooth expressed only the LIMD1 gene. Subject G5 (control) expressed the WNT10A gene.

Therefore, it can be assumed that WNT10A alone does not cause dental agenesis. WNT10A works in digenic mode with the LIMD1 gene to cause dental agenesis. LIMD1 alone cause dental supernumerary.

The same hypothesis can be added in this case: difference in phenotype expression, is a gene dosage effect made by the LIMD1 gene in this case.

*7. **Family H:***

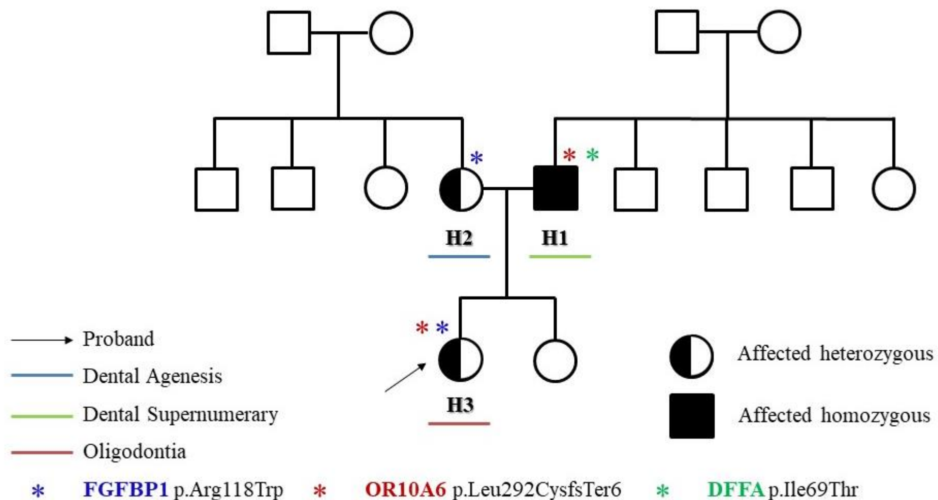


Figure V.7: Phenotype and genotype distribution in family H

• *FGFBP1:*

A study done by Kim et al., in 2012, described the embryologic stages of dental development and the role of the FGF proteins in it.

FGF-binding proteins such as FGFBP1 and the epidermal growth factor receptor (EGFR) were highly expressed in the epithelium at the initiation and bell stages of dental development (Kim et al., 2012).

A study done on mice reported that FGFBP1 is expressed in dental germ, dental pulp, external enamel epithelium and apical side of the ameloblasts, which indicates that the later may play roles in ameloblast and odontoblast differentiation (Aigner et al., 2002).

• OR10A6:

Although no dental relation was previously described whether in human or animal studies, this gene was considered as potential gene because the mutation reported in the present study is frameshift with a high putative impact. The gene variant was only

expressed in those two family members and not present in other families or previous database.

Non-dental relation:

The human odorant receptor OR10A6 is part of the olfactory receptors gene family (OR). Each individual is found to harbor as many as 636 OR allelic variants, this has a direct effect on smell perception diversity between individuals. Mutation at the level of the OR genes affect the smell perception. (Malnic et al., 2004; Olender et al., 2012)

- *DFFA:*

The gene function was not yet described in humans.

A study conducted by Gonzalez et. Al in 2013 using a nonhuman primate model reported detailed gene expression in apoptotic pathways that occur in oral mucosal tissues (Gonzalez et al., 2013)

DFFA was among the upregulated genes which have a function as block points for apoptosis thus decreasing the apoptotic potential of cells in aging gingiva. The mutation of the gene will cause gingival problems (Gonzalez et al., 2013).

- Summary of Family H:

FGFBP1 was common between H2 (hypodontia) and H3 (oligodontia). The two individuals have dental agenesis but with different severity, but having the same gene variant, this can be explained by the different degree of penetrance.

The digenic work of OR10A6 with the DFFA causes dental supernumerary but when OR10A6 is present in addition to the FGFBP1, this digenic work causes oligodontia.

Therefore, the presence of OR10A6 can explain the phenotype/genotype difference between the individuals H2 and H3.

8. Family I "DYRK1A":

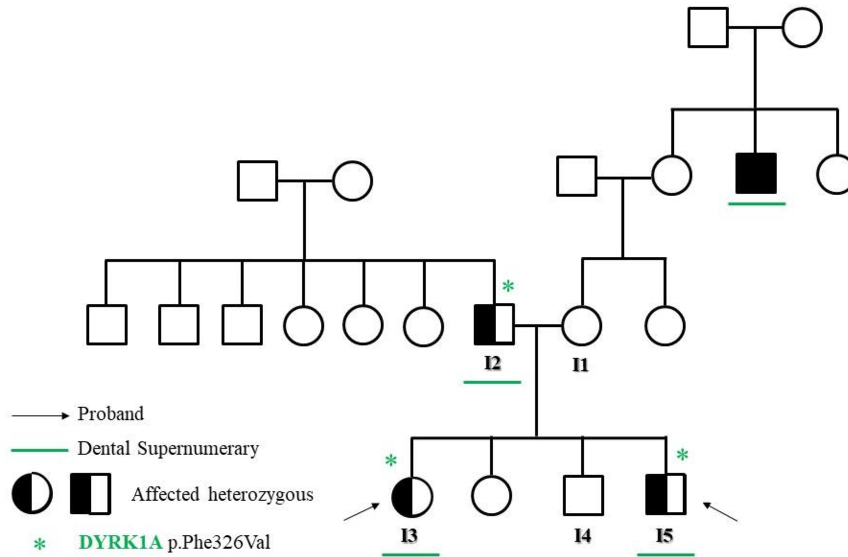


Figure V.8: Phenotype and genotype distribution in family I

The DYRK1A (Figure V.8) mutation was reported in patients having DYRK1A syndrome "intellectual disability syndrome" and supernumerary teeth (Bwm et al., 2015; van Bon et al., 1993).

Among the Syndrome features: extreme dental calculus, delayed primary dentition, neonatal teeth, and supernumerary teeth.

<u>*Non-dental relation:*</u>

DYRK1A gene is overexpressed in down syndrome. It is the most studied member of DYRK kinases, because it is located on the chromosome 21 within the Down syndrome critical region. This gene has a pro-oncogenic activity (Guimera et al., 1999; Laham et al., 2021).

DYRK1A gene mutation is related to neurodegenerative disorders causing Alzheimers and Parkinson’s disease (Laham et al., 2021).

D. Dental findings

1. Comparison between group A and Control:

Group A has significantly decreased arch dimensions thus, constricted arches and significantly less crowding (ACD>0).

This can be explained by the fact that group A have missing teeth and have smaller teeth (crown width) thus, more space in the arch.

Although not significant for all teeth, but hypodontia population have shorter teeth crowns and shorter roots.

2. Comparison between group N and Control:

Arch dimension differences between both groups are not significant. This can be explained by the absence of dental agenesis and therefore, presence of crowding.

When we compare the crown width between both groups, the non-affected group have smaller teeth although they don’t have missing teeth. Therefore, the non-affected group is affected in a way having small teeth.

When we compare the crown length and root length, group N have shorter crowns and roots although not significant for all teeth.

3. Comparison between group A and N:

Group A have significant smaller arch dimensions (constricted arches) and significantly less crowding. This is explained by the fact that group A have dental agenesis and the remaining teeth are smaller thus, this group have extra spaces in the arches.

Both groups have smaller teeth compared to control but group N have larger values compared to group A.

When we compare the crown and root length, the same previous pattern exists between both groups: group A have shorter crowns and shorter roots compared to their relatives although not significant for all teeth.

E. Strengths and limitations

1. Strengths

Very few human genetic studies to identify genes conferring susceptibility to hyperdontia have been performed. More genetic studies have been conducted for hypodontia because it is more common and more readily diagnosed within families. Most genetic studies of hypodontia and hyperdontia have been associated with syndromes and craniofacial anomalies such as: anhidrotic ectodermal dysplasia syndrome and Van der Woude syndrome (Kondo et al., 2002; Parveen et al., 2019; Tao et al., 2006; Zeng et al., 2016) were associated with hypodontia. Cleidocranial dysplasia and Gardner syndrome were associated with hyperdontia (Suda et al., 2007; Yu et al., 2018).

Moreover, studies have not been conducted in the Mediterranean population, indicating that the genetic determinants of hypo/hyperdontia in this ethnic population were

completely unclear. Therefore, identifying the candidate genes in this population is a new contribution, especially that many families with several affected individuals over many generations were observed.

The most important strengths of this study are related to: 1- its novelty as the first genetic study of hypodontia conducted in 5 families, the greater number to date. 2- the first study to be conducted not only in families with hypodontia but also in families with combined hypodontia and hyperdontia (3 families) and a family with DE. 3- New theories about genetic influences could be formulated.

The present study contributed to better comprehend the genetic determinants and the variation in the risk for hypo/hyperdontia in this population.

The results of this study were possible through the application of the "Whole Exome Sequencing (WES)" technique, which is part of the Next Generation Sequencing (NGS) technology. WES was preferred over the Whole Genome sequencing (WGS), even though WES convers only 2% (around 180000 genes) of the entire genome, WES focuses on the protein coding sequences (exomes) which contain high portions of the functional variants and around 85% of known-disease related variants, making this method a cost-effective alternative to Whole-Genome sequencing.

WES also have the capability to expand targeted content to include untranslated regions (UTRs) and microRNA for a more comprehensive view of gene regulation. Majority of previous studies did not include WES using NGS while exploring hypo/hyperdontia. In addition, previous studies applied linkage analysis and did not explore all the genes of each individual. This linkage analysis was based on a search for specific

genes or loci that were found to be associated in the literature with the phenotype tested or with dental development.

The genetic screening did not show any aberration in the reported genes linked to hypo/hyperdontia that were revealed in the earlier genetic studies except for the WNT10A which was determined in the present study as not being individually a causing gene for hypodotnia.

Therefore, 14 potentially novel genes (CRACR2A, PER3, NOV CCN3, EDAR, APCDD1, CDH26, LAMC2, NME8, LIMD1, WNT10A, FGFBP1, DFFA, OR10A6 and DRK1A) were recognized that segregate with the phenotypes and could be implicated in the development of hypo/hyperdontia. Accordingly, if those genes are present in the genotype of an individual, the later may express the trait.

A significant contribution of this study is also in its design, including subjects and families with hypo/hyperdontia, thus focusing on the heritability characteristic of the phenotypes.

Such phenotypic characterization will represent data infrastructure to future large-scale genetic studies that should allow in-depth analysis of the etiology of hypo/hyperdontia.

The early diagnosis through the identification of genetic influences would aid in preventing the development of malocclusion or at least reduce its severity, and improve treatment planning.

The sample used in this study constitutes by itself a strength because it includes several families (N=9) with affected individuals over 2-3 generations, demonstrating clearly the segregation of the phenotype across generations, and allowing comparisons to

find common genes across individuals of the same family and across different families. Other studies, (Arikan et al., 2018; Bergendal et al., 2011; Klein et al., 2005; Liu et al., 2014; Ockeloen et al., 2016; Pegelow et al., 2008; Shokeh, 2014) had included non-related individuals which means non-familial studies, or limited number of families (1-2 families) or the sequencing technique was not a WES but rather it was targeting a specific gene or loci thus called "linkage analysis", in other words, analysis did not explore all the genes of each individuals.

The present study involved also non-affected individuals of each family that served as controls when comparing the genes and variants across individuals and families, as well as in panoramic and intra-oral scanner analysis.

2. Limitations

Some limitations were related to the fact that previous database results: did not exist in Lebanon or the Mediterranean region regarding WES analysis in normal individuals.

Therefore, we could not verify if the 14 candidate genes were present or not in the normal populations of this region. We compared our results to the normal database of the whole globe, which includes 6000 healthy individuals for WES and found that the 14 genes also are not present in these databases.

Other limitations related to specific families or the number of families in the study: No potential candidate gene for hypodontia could be noted in the family C following the 1st and 2nd filtering process.

Although the number of families having dental agenesis exceeded the number included in previous studies and some were distinguished with numbers having hypodontia and hyperdontia, only one family presented exclusively with hyperdontia.

This limitation was due to the COVID-19 situation that restrained initially accepting families from enrolling in the study.

Finally, some limitations were related to the sample included in the dental analysis: The small sample size of the non-affected members limited the generalization of the dental findings.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

A. Conclusion

In both the developmental and clinical contexts, this study represents a significant advance in the fields of orthodontics and dentofacial orthopedics and in prosthodontics and periodontics. It is the first genetic study on large families with hypo/hyperdontia worldwide using NGS/WES to better understand the variations and risks for this condition.

The key conclusions are summarized:

- A visual inspection of the pedigrees corresponding to the 16 Mediterranean families approached during this study suggests an autosomal dominant mode of inheritance of hypo/hyperdontia with incomplete penetrance. Analysis indicates an equal number of reported generations per family (n=3-4), number of families with female predominance (n=11) and male predominance (n= 4), an equal number of reported affected males and females per family (n=1).
- Through WES, 14 genetic variants were identified in exonic regions of the 37 subjects (25 affected and 12 non-affected) that are part of the 9 enrolled families. These variants were classified as family-candidate genes. CRACR2A, PER3, NOV/CCN3, EDAR were present in affected members of families with hypodontia. CDH26, APCDD1 and NME8 were common between all affected members (with hypo and hyperdontia) of family F. LAMC2 was found in one affected member (with hyperdontia) of family F.

WNT10A was observed in affected members (with hypodontia) of family G and one non-affected member of the same family. LIMD1 was present in all affected members (hypo and hyperdontia) of the family G. FGFBP1 was found in affected members (hypo and oligodontia) of the family H. OR10A6 was present in affected members (oligo and hyperdontia) of the family H. DFFA was observed in only one affected member (hyperdontia) of the family H. DYRK1A was present in all affected members (hyperdontia) of the family I.

- No specific chromosome was found to be suggestive of linkage to familial hypodontia/hyperdontia in the Eastern Mediterranean population as the 14 genes were located on different chromosomes, except for the PER3, LAMC2 and DFFA which were present on the chromosome 1 and EDAR and WNT10A on chromosome 2.
- The presence of families with combined hypodontia and hyperdontia helped to confirm the evolutionary hypothesis:
  - ✓ Hypodontia and hyperdontia are genetically accordionized and synchronized and genes mutation would drive the phenotype one way or another.
  - ✓ An "accordion pattern" exists between the two phenotypes in other words, the data suggest that hypodontia is an evolutionary effect whereas hyperdontia is a compensatory effect.
  - ✓ The difference in phenotypes expression from hypodontia to hyperdontia is a gene dosage effect made by the "3 stars genes": LAMC2, LIMD1 and OR10A6.
- Dental analysis revealed a "continuum pattern" which exists between all sample groups for all variables: teeth crowns tend to get thinner and shorter, and root length tend to get

shorter in a progression from control to non-affected to agenesis group. The control group have the highest values for all variables.

B. Recommendations and future research

Following the recent advances in molecular genetic studies investigating the candidate genes associated with hypodontia/hyperdontia, it would be beneficial in families with a history of affection to have a blood test and assess the individuals' genes for early forecasting of the condition, if present. This identification would allow the anticipation of hypo or hyperdontia, thus early approach to manage space within the affected clinical arch.

With further genetic and clinical association in future studies, the genetic test (involving blood collection, DNA extraction and NGS) might become a diagnostic tool in candidate families.

Future research should also focus on a larger number of pedigrees for more conclusive findings especially for the families with distributed hyperdontia. Therefore, a larger sample of non-affected individuals (relatives) for dental analysis should allow for better result generalization.

The etiology behind hypo/hyperdontia resides not only in genetics but also the contribution of environmental developments that in consequence lead to the ultimate phenotype.

A well-designed twin studies (mono and dizygotic twins) would contribute to quantifying the relative impact of genetic and environment factors on the development of hypo/hyperdontia.

APPENDIX

A. Teeth dimensions norms

Table III.3: Normal values of Dental Measurements

Crown width and length of Maxillary teeth				
Tooth	**Crown Width**	**SD**	**Crown Length**	**SD**
Central	**9**	0.3	**11**	0.4
Lateral	**7**	0.3	**10.1**	0.4
Canine	**8.4**	0.39	**11.4**	0.7
1st Premolar	**7.5**	0.3	**9.3**	0.8
2nd Premolar	**7.2**	0.6	**8.8**	0.9
1st Molar	**11.3**	0.7	**8**	0.5
2nd Molar	**10**	0.4	**7.8**	0.6

Crown width and length of Mandibular teeth				
Tooth	**Crown Width**	**SD**	**Crown Length**	**SD**
Central	**6**	0.37	**10**	0.4
Lateral	**6.5**	0.24	**10.6**	0.5
Canine	**7.3**	0.28	**11.5**	0.7
1st Premolar	**7.8**	0.43	**9.5**	0.8
2nd Premolar	**7.8**	0.47	**9**	0.8
1st Molar	**11.9**	0.58	**8.2**	0.6
2nd Molar	**11**	0.5	**8**	0.5

Root Length								
Maxilla								
Tooth	**Crown Width**	**SD**	**Crown Length**	**SD**	**Root Length**	**SD**	**Total Length**	**SD**
2nd Molar	**10.09**	0.52	**7.8**	0.47	**12.7**	0.5	**20.5**	0.5
1st Molar	**10.16**	0.53	**8**	0.75	**13.5**	0.70	**21.5**	0.7
2nd Premolar	**6.49**	0.41	**8.8**	0.70	**13.4**	0.6	**22.2**	0.7
1st Premolar	**6.93**	0.48	**9.3**	0.65	**13.2**	0.45	**22.5**	0.95
Canine	**7.79**	0.43	**11.4**	0.5	**17.6**	0.5	**29**	1.25
Lateral incisor	**6.60**	0.51	**10.1**	0.5	**12.4**	0.6	**22.5**	0.5
Central incisor	**8.44**	0.56	**11.3**	0.5	**14.2**	0.6	**25.5**	1.25
Mandible								
2nd Molar	**10.50**	0.66	**8**	0.47	**13**	0.5	**21**	1.2
1st Molar	**10.67**	0.57	**8.2**	0.75	**13.8**	0.70	**22**	1
2nd Premolar	**6.84**	0.56	**9**	0.70	**15**	0.6	**24**	1.7
1st Premolar	**6.86**	0.43	**9.5**	0.65	**14.5**	0.45	**24**	1.2
Canine	**6.68**	0.44	**11.5**	0.5	**17.5**	0.5	**29**	1.7
Lateral incisor	**5.77**	0.42	**10.6**	0.5	**13.4**	0.6	**24**	1.45
Central incisor	**5.21**	0.31	**10**	0.5	**12**	0.6	**22**	1.3

B. Descriptive statistics

Table IV.3: Arch Dimensions

Measurements	Groups											
	Group A (N=18)			Group CA (N=18)			Group N (N=10)			Group CN (N=10)		
Maxilla	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
ACD	18	1.222	3.8472	18	-0.722	1.5739	10	-1.8	1.3166	10	-2.7	1.0593
ACA	18	60.55	6.76	18	71.49	2.18554	10	66.58	5.28	10	70.903	4.148
ACR	18	59.33	7.26	18	72.22	2.863	10	68.38	5.43	10	73.603	4.197
Inter-molar width	18	21.5506	1.95288	18	27.7039	3.17229	10	27.822	1.92186	10	28.032	2.57924
Inter-canine width	18	43.3039	3.05566	18	50.1133	4.35705	10	50.502	4.03183	10	50.728	4.00376
Arch length	18	26.6567	1.30439	18	32.8889	3.64312	10	33.887	2.05586	10	35.578	5.3504
Mandible	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
ACD	18	1.222	2.6359	18	-0.889	1.7112	10	-1.8	1.8135	10	-1.65	0.8182
ACA	18	54.59	6.26	18	62.74	3.30	10	57.94	3.41	10	62.756	3.159
ACR	18	57.37	7.92	18	63.62	3.16	10	59.74	2.56	10	64.406	3.282
Inter-molar width	18	19.1561	1.22814	18	24.3044	2.00224	10	24.137	2.25695	10	24.873	1.58261
Inter-canine width	18	38.7444	2.66082	18	44.4533	3.1166	10	46.758	5.88323	10	46.027	1.72134
Arch length	18	21.9339	2.00745	18	26.6344	2.06029	10	26.541	3.38034	10	27.788	2.31354

Table IV.4: Crown width

Measurements	Groups											
	Group A (N=18)			Group CA (N=18)			Group N (N=10)			Group CN (N=10)		
Maxilla	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
CW. MR1	18	8.2294	0.59947	18	8.5672	0.60101	10	8.4189	0.2558	10	8.564	0.38088
CW. MR2	10	6.2	0.54171	18	6.6111	0.44588	10	6.792	0.45301	10	6.829	0.76693
CW. MR3	18	7.3194	0.42073	18	7.6378	0.37951	10	7.341	0.37403	10	7.875	0.65554
CW. MR4	18	6.2928	0.4843	18	6.7778	0.37203	10	6.44	0.35587	10	6.915	0.33009
CW. MR5	15	6.0513	0.51972	18	6.5583	0.38442	10	6.613	0.46246	10	6.785	0.37227
CW. MR6	17	9.9812	0.75885	18	10.2017	0.59327	10	10.007	0.54999	10	10.351	0.60128
CW. MR7	18	8.9456	0.74596	18	9.2361	0.69472	10	8.77	0.42862	10	9.16	0.43074
CW. ML1	18	8.2267	0.44541	18	8.5117	0.64945	10	8.339	0.40308	10	8.714	0.56506
CW. ML2	11	5.98364	0.770808	18	6.63111	0.500563	10	6.592	0.585051	10	6.7603	0.684658
CW. ML3	17	7.2265	0.50223	18	7.6411	0.3888	10	7.262	0.37213	10	7.617	0.50669
CW. ML4	17	6.4024	0.35024	18	6.5394	0.3358	10	6.3078	0.3942	10	6.861	0.41627
CW. ML5	13	6.1838	0.44581	18	6.7456	0.95413	10	6.195	0.77695	10	6.683	0.60986
CW. ML6	18	9.33294	2.177112	18	10.04778	0.456322	10	9.69333	0.31277	10	10.134	0.557439
CW. ML7	18	8.7439	0.70072	18	8.9778	0.72594	10	8.294	0.6988	10	8.931	0.59263
Mandible	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
CW. mR1	16	5.0944	0.37238	18	5.4206	0.3657	10	5.068	0.3036	10	5.469	0.3906
CW. mR2	17	5.4088	0.43731	18	5.8033	0.459	10	5.46	0.47326	10	5.97	0.31009
CW. mR3	18	6.27	0.62047	18	6.5306	0.28196	10	6.116	0.3198	10	6.669	0.49321
CW. mR4	17	6.3929	0.47356	18	6.8872	0.46295	10	6.41	0.46774	10	6.95	0.45497
CW. mR5	13	6.5515	0.48742	18	7.1689	0.75259	10	6.676	0.30714	10	7.213	0.47689
CW. mR6	16	10.1863	0.57083	18	10.6933	0.6215	10	10.0722	0.45656	10	10.792	0.69504
CW. mR7	17	9.1341	0.7319	18	9.7117	0.65159	10	9.446	0.38306	10	9.64	0.63254
CW. mL1	16	5.0031	0.31155	18	5.4256	0.29993	10	5.012	0.34159	10	5.458	0.35615
CW. mL2	17	5.5141	0.35002	18	5.8494	0.46895	10	5.506	0.26044	10	6.007	0.37038
CW. mL3	18	6.0683	0.30301	18	6.5717	0.33456	10	6.183	0.44502	10	6.691	0.46715
CW. mL4	16	6.5875	0.50027	18	6.9322	0.47932	10	6.543	0.52924	10	6.917	0.54626
CW. mL5	14	6.58	0.55394	18	7.0394	0.40068	10	6.764	0.36927	10	7.062	0.52873
CW. mL6	15	10.1073	0.60953	18	10.5978	0.47985	10	10.0333	0.38623	10	10.783	0.53323
CW. mL7	18	9.1017	0.55669	18	9.7161	0.77083	10	9.409	0.49285	10	9.6	0.80474

Table IV.5: Crown Length

Measurements	Groups											
	Group A (N=18)			Group CA (N=18)			Group N (N=10)			Group CN (N=10)		
Maxilla	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
CL. MR1	18	8.3839	0.95583	18	9.2578	0.84919	10	8.8533	1.17829	10	9.552	0.56413
CL. MR2	10	6.804	0.5578	18	7.5883	0.66666	10	7.632	1.16482	10	8.059	0.77548
CL. MR3	18	7.7844	0.79832	18	8.6728	0.80211	10	8.366	0.79847	10	8.864	0.99565
CL. MR4	18	6.4128	0.90111	18	6.8467	0.60418	10	6.941	0.97228	10	7.247	0.74627
CL. MR5	15	5.486	0.79303	18	6.0667	0.68665	10	6.326	0.85879	10	6.183	0.98748
CL. MR6	17	5.3171	0.88302	18	5.5644	0.7467	10	5.78	0.75818	10	5.795	0.75338
CL. MR7	18	5.0756	0.6577	18	5.1956	0.69663	10	5.768	0.75098	10	5.34	0.69009
CL. ML1	18	8.3906	0.81326	18	9.3106	0.78153	10	8.957	1.11515	10	9.652	0.58518
CL. ML2	11	6.8955	0.59618	18	7.4989	0.51492	10	7.932	1.06024	10	7.986	0.66667
CL. ML3	17	7.7471	0.7171	18	8.4078	0.68514	10	8.424	1.01566	10	9.035	0.99266
CL. ML4	17	6.5218	0.69231	18	6.7628	0.58607	10	6.9444	1.25542	10	7.064	0.5958
CL. ML5	13	5.4362	0.63071	18	5.8672	0.6209	10	6.482	0.86723	10	6.068	0.6113
CL. ML6	18	5.3317	0.62672	18	5.7322	0.67118	10	6.3767	0.73352	10	5.604	0.49541
CL. ML7	18	5.2194	0.5822	18	5.1739	0.70932	10	5.675	1.15769	10	5.017	0.62716
Mandible	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
CL. mR1	16	7.0487	0.74785	18	7.615	0.64437	10	7.334	1.12714	10	7.609	0.6073
CL. mR2	17	6.9535	0.72194	18	7.765	0.56493	10	7.402	0.98334	10	7.501	0.78277
CL. mR3	18	7.72	0.48422	18	8.6061	0.71169	10	8.597	0.98773	10	8.686	1.37814
CL. mR4	17	6.6188	0.51887	18	7.3256	0.58053	10	7.368	0.91298	10	7.327	0.75906
CL. mR5	13	5.9931	0.8025	18	6.3922	0.57498	10	6.802	1.10447	10	6.61	0.68443
CL. mR6	16	5.6044	0.62681	18	5.8028	0.53311	10	5.863	0.55329	10	6.2144	0.3643
CL. mR7	17	5.2376	0.9232	18	5.2911	0.53191	10	5.199	0.6264	10	5.854	0.47428
CL. mL1	16	7.0563	0.63919	18	7.655	0.5585	10	7.267	1.02401	10	7.522	0.60347
CL. mL2	17	6.95	0.8419	18	7.64	0.56442	10	7.122	0.81677	10	7.524	0.57579
CL. mL3	18	7.835	0.70211	18	8.6411	0.70523	10	7.524	0.57579	10	8.439	1.04293
CL. mL4	16	6.6244	0.47118	18	7.2294	0.71945	10	7.484	0.83613	10	7.393	0.79765
CL. mL5	14	5.8993	0.79433	18	6.1933	0.57595	10	6.677	1.07679	10	6.4	0.98084
CL. mL6	15	5.404	0.45922	18	5.6872	0.55244	10	6.0144	0.77634	10	5.579	0.52784
CL. mL7	18	5.0683	0.84808	18	5.1489	0.73365	10	5.77	0.98811	10	4.994	0.41234

Table IV.6: Root Length (4 groups)

Measurements	Groups											
	Group A (N=18)			Group CA (N=18)			Group N (N=10)			Group CN (N=10)		
Maxilla	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
RL. MR1	18	13.7667	0.3675	18	13.7806	0.53357	10	13.99	0.0817	10	13.926	0.44247
RL. MR2	10	11.637	0.34734	18	12.7561	0.49024	10	12.039	0.10999	10	12.233	0.24042
RL. MR3	18	16.9367	0.27819	18	17.0817	0.67321	10	17.228	0.20137	10	17.293	0.33556
RL. MR4	18	12.9572	0.11385	18	13.4583	0.65716	10	13.032	0.10097	10	13.051	0.12974
RL. MR5	15	12.978	0.17933	18	13.425	0.49721	10	13.064	0.08922	10	13.152	0.32907
RL. MR6	17	12.9888	0.2551	18	13.2039	0.34799	10	13.278	0.09807	10	13.166	0.18781
RL. MR7	18	12.6128	0.22759	18	12.4844	0.1645	10	12.526	0.07304	10	12.463	0.20543
RL. ML1	18	13.74	0.38543	18	13.9656	0.26796	10	14.01	0.10066	10	14	0.28437
RL. ML2	11	11.5136	0.31945	18	12.6989	0.75207	10	12.021	0.11532	10	12.31	0.48279
RL. ML3	17	16.9718	0.27476	18	17.0972	0.60615	10	17.209	0.20058	10	17.287	0.17082
RL. ML4	17	12.9012	0.11968	18	13.3911	0.58817	10	13.0111	0.10671	10	13.205	0.42201
RL. ML5	13	12.9446	0.17567	18	13.5028	0.63993	10	13.05	0.7071	10	13.228	0.41614
RL. ML6	18	12.9922	0.25082	18	13.1939	0.28934	10	13.2656	0.07367	10	13.169	0.14418
RL. ML7	18	12.525	0.07122	18	12.455	0.18763	10	12.525	0.07122	10	12.455	0.18763
Mandible	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
RL. mR1	16	11.8113	0.11815	18	12.1406	0.53506	10	11.959	0.04701	10	12.101	0.23881
RL. mR2	17	12.6447	0.2677	18	12.9928	0.61276	10	13.011	0.05705	10	13.071	0.24655
RL. mR3	18	16.9683	0.13049	18	17.0872	0.55234	10	17.177	0.11804	10	17.334	0.12122
RL. mR4	17	14.1247	0.19109	18	14.4156	0.23405	10	14.059	0.07203	10	14.295	0.19597
RL. mR5	13	14.6169	0.19168	18	14.7928	0.10395	10	14.501	0.18406	10	14.918	0.14793
RL. mR6	16	13.095	0.18744	18	13.6333	0.2142	10	13.2378	0.14898	10	13.507	0.25065
RL. mR7	17	12.7312	0.20331	18	13.0433	0.34707	10	12.739	0.22168	10	12.882	0.19697
RL. mL1	16	11.7638	0.12457	18	12.24	0.69132	10	11.943	0.04785	10	12.145	0.15813
RL. mL2	17	12.6047	0.27929	18	13.0244	0.28945	10	13.005	0.06948	10	13.12	0.20221
RL. mL3	18	16.9722	0.11563	18	17.0106	0.38597	10	17.142	0.12665	10	17.374	0.16453
RL. mL4	16	14.1044	0.17565	18	14.2194	0.51263	10	14.048	0.06408	10	14.315	0.15443
RL. mL5	14	14.5493	0.17843	18	14.5183	0.62885	10	14.387	0.18148	10	14.904	0.29102
RL. mL6	15	13.1053	0.17715	18	13.71	0.31546	10	13.2344	0.13848	10	13.546	0.20321
RL. mL7	18	12.7011	0.20719	18	12.7733	0.31671	10	12.743	0.19613	10	12.712	0.17612

C. **FLYERS**

Invitation for participation in a research study

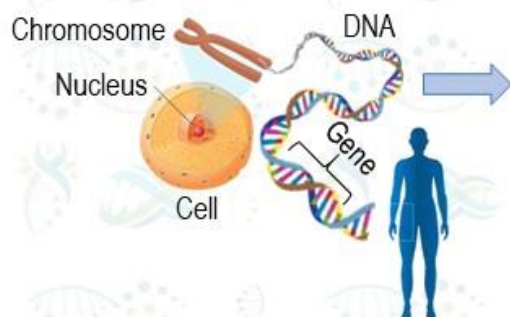
You are invited to participate in a research study entitled "**Genetic basis of familial oligo-hypo-hyperdontia in eastern Mediterranean families** ", conducted by Dr. Joseph Ghafari (Faculty of Medicine, Division of Orthodontics and Dentofacial Orthopedics at the American University of Beirut Medical Center). The conduct of this study will adhere to the IRB approved conditions and terms.

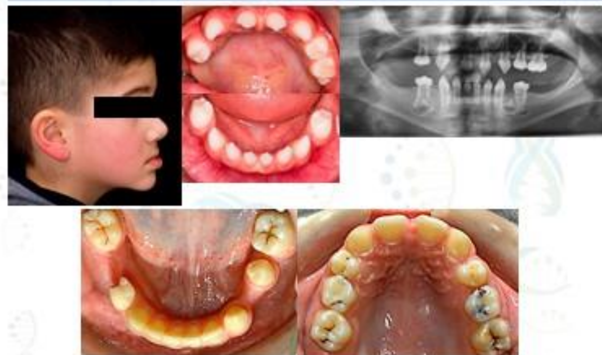
Purpose

- To gain more insight into the prevalence of dental agenesis.
- Clarify the inheritance pattern of dental agenesis.
- Highlight the concomitance of hypodontia/hyperdontia in one family.

Inclusion

- Families known to have subjects affected by dental agenesis/excess (because of previous or ongoing treatment of some of them in the Division of Orthodontics and Dentofacial Orthopedics at AUBMC)
- Affected and non-affected members will be enrolled in the study





Potential Benefits

- This study has benefits to the society and science as a whole because it will allow us to better understand the specific genes contributing to variation in the risk of dental agenesis in our population and the genetic mechanisms that may influence dental morphogenesis.
- Early prediction of the condition would also lead also to variation in the approach of treatment
- You will not receive payment for participation in this study.

Agreement of Research Subject

If after reading the present document, you are interested to participate in the study and/or you would like to have further information, please don't hesitate to contact Dr. Joseph Ghafari at (01)350 000 ext 5707.

Commitment Required

This will require you to pass by AUBMC for one (45mins) or two (30mins + 15mins) visits: the first one to explain the project in detail, sign this consent form, have a clinical examination and a panoramic x-ray, and the second visit to have 5cc of your blood collected.

Location of Research

Division of Orthodontics and Dentofacial Orthopedics/ American University of Beirut Medical Center (AUBMC) (6th floor)/ Hamra Street / Beirut, Lebanon

دعوة للمشاركة في دراسة

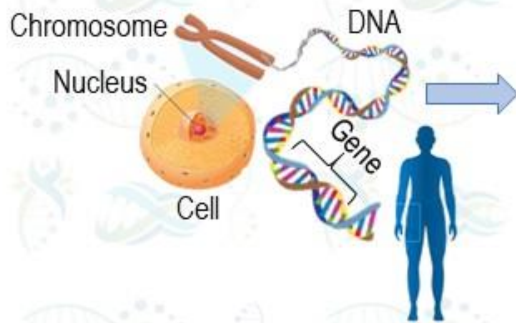
أنت مدعو للمشاركة في دراسة بحثية سريرية عنوانها **الارتباط بين الجينات المسببة بخلل في عدد الاسنان سواء نقصان او زيادة لدى سكان منطقة شرق البحر المتوسط**. يجريها الدكتور جوزيف غفري. ستجرى الدراسة في الجامعة الأميركية في بيروت (قسم تقويم الأسنان وتأهيل الفكين). تسير مجريات الدراسة وفقا" للشروط والأحكام الموافق عليها من قبل لجنة الأخلاقيات.

التضمين:

الأسر المعروف أن لديها أفراد مصابين خلل تكويني في عدد الأسنان (بسبب علاج سابق أو جاري لأحد منهم في قسم تقويم الأسنان وتأهيل الفكّين في الجامعة الأميركية في بيروت). سيتم ادراج الأعضاء المصابين وغير المصابين في الدراسة.

أهداف الدراسة :

للحصول على مزيد من المعلومات حول انتشار حالة نقصان عدد الأسنان.
استكشاف ما إذا كان يتم توريث حالة نقصان أو زيادة عدد الأسنان.
تسليط الضوء على تواجد حالتي نقصان و زيادة عدد الأسنان ضمن عائلة واحدة.



الفوائد الناتجة عن المشاركة في الدراسة

انها تقدم بعض الفوائد للمجتمع والطب ككل لأن هذه الدراسة ستسمح لنا بأن نفهم أكثر الجينات المعنية بتكوين و نمو الأسنان لدى شعبنا والأليات الوراثية التي تؤثر على نوعيّة و توقية العلاج لدى الأجيال القادمة.
الكشف المبكر للحالة قد يؤدي إلى إختلاف في منهج العلاج.
لن تحصل على أي مردود مالي مقابل مشاركتك في الدراسة.

الموافقة على موضوع البحث

اذا, بعد قراءتك لهذه الوثيقة, ترغب في المشاركة في الدراسة و/أو ترغب في الحصول على المزيد من المعلومات, لا تتردد في الاتصال بالدكتور جوزيف غفري على الرقم 01350000 مقسم 5707.

الالتزام المطلوب:

سيتطلب هذا الأمر منك أن تقوم بزيارة AUBMC مرّة(45 دقيقة) أو مرّتين (30 دقيقة +15 دقيقة): الزيارة الأول لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء استمارة الموافقة هذه، ولمعاينة أسنانك وأخذ صورة بالاشعة السينية أمامية لدراسة عدد الأسنان. الزيارة الثانية لنأخذ 5 سنتيمتر مكّعّب من دمك.

موقع الدراسة :

قسم تقويم الأسنان وتأهيل الفكين, المركز الطبي في الجامعة الأميركية, شارع الحمرا/بيروت, لبنان.

D. **CHILD ASSENT FORM**

Minor's Initials ____

Institutional Review Board
American University of Beirut
Faculty of Medicine
Bliss Street
Beirut, Lebanon
Tel: (01) 350-000 ext. 5445

CHILD PARTICIPANT ASSENT FORM

(approximate ages 7-12)

Project Title: Genetic basis of familial oligo-hypo-hyperdontia in eastern Mediterranean families

Protocol Number: IRB.ID (BIO-2019-0464)

Principal Investigator(s): Dr Joseph Ghafari

Co- Investigators: Dr Anthony Macari
Dr Josephine Boueiri

Address: American University of Beirut Medical Center
Bliss Street
Beirut – Lebanon

Site where the study will be conducted: Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

Phone: (01) 350 000 ext: 5707

We want to tell you about a research study we are doing. A research study is a special way to find out about something. We are trying to find out more about genes associated with your teeth condition. You are being asked to join the study because you have either more or less teeth than normal people.

If you decide that you want to be in this study among the 30 people that we are planning to enroll, this is what will happen.

- **We will take some blood from you. We will use a clean needle. We will take the blood from your arm. We will take about a quarter to half tablespoon. This is done to find out what caused the decreased/increased number of your teeth. And even if you don't want to do the research study, you have the right. You will also be given flyers to distribute amongst your close relatives whom you think could benefit from this project.**
- **A picture of your teeth will be taken to count them and check their position.**
- **We will collect data from your medical file.**
- **We need you to pass by AUBMC for one (45mins) or two (30mins + 15mins) visits: the first one to explain the study in detail, sign this paper, have a clinical exam and a picture for your teeth, and the second visit to take the blood.**

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Minor's Initials ____

Can anything bad happen to me?

We want to tell you about some things that might hurt or upset you if you are in this study. The needle we use to take the blood may hurt. There is also radiation risk from the image, however there are no proven harmful effects from irradiation levels that you will be exposed to during the study.

Can anything good happen to me?

We don't know if being in this research study will help you feel better or get well. But we hope to learn something that will help other people someday.

Do I have other choices?

You can choose not to be in this study

Will anyone know I am in the study?

Only your parents and those of us running the study. We won't tell anyone you took part in this study. When we are done with the study, we will write a report about what we found out. We won't use your name in the report.

What happens if I get hurt?

Your parents/legal guardians have been given information on what to do if you are injured during the study

You will not receive any payment for being in this research study.

Before you say yes to be in this study; be sure to ask Dr. Josephine Boueri to tell you more about anything that you don't understand.

What if I do not want to do this?

You don't have to be in this study. It's up to you. If you say yes now, but you change your mind later, that's okay too. All you have to do is tell us.

If you want to be in this study, please sign or print your name.

□ Yes, I will be in this research study. □ No, I don't want to do this.

____________________	____________________	____________
Child's name	signature of the child	Date & Time
____________________	____________________	____________
Person obtaining Assent	signature	Date & Time

Institutional Review Board
Version IRB.ID (BIO-2019-0464)

Institutional Review Board
American University of Beirut
10 JUL 2020
APPROVED

Protocol Date: January 2020

أحرف القاصر:

لجنة الأخلاقيات
الجامعة الأميركية في بيروت
كلية الطب
شارع بلس
بيروت، لبنان
هاتف: 350000 -(01)

الموافقة للاشتراك في البحث العلمي للمشترك القاصر

الأطفال ذو العمر الذي يتراوح بين ٧-١٢

العنوان: الارتباط بين الجينات المسببّة بخلل في عدد الأسنان سواء نقصان او زيادة لدى سكّان منطقة شرق البحر المتوسّط

محضر رقم:	IRB.ID (BIO-2019-0464)
إسم الباحث:	د. جوزف غفري
الباحثون المساعدون:	د. انطوني مكاري د. جوزفين بواري
مكان إجراء البحث:	الجامعة الاميريكية في بيروت شارع بلس بيروت – لبنان قسم تقويم الأسنان و الفكين
هاتف:	000 350-01- مقسم : 5707

نريد أن نعلمك عن دراسة بحثية نقوم بها. الدراسة البحثية هي طريقة خاصة لمعرفة شيء ما. نحن نحاول معرفة المزيد عن الجينات المرتبطة بحالة أسنانك. يُطلب منك الانضمام إلى الدراسة لأن لديك أسنانًا أكثر أو أقل من الأشخاص العاديين.

إذا قررت أنك تريد أن تكون في هذه الدراسة من بين الأشخاص الثلاثين الذين نخطط تسجيلهم ، فهذا ما سيحدث.

- سنأخذ منك بعض الدم. سوف نستخدم إبرة نظيفة. سنأخذ الدم من ذراعك. سنأخذ حوالي ربع إلى نصف ملعقة كبيرة. يتم ذلك لمعرفة سبب انخفاض/ زيادة عدد أسنانك. وحتى إذا كنت لا ترغب في إجراء الدراسة البحثية ، فلديك الحق. سيتم منحك أيضًا منشورات لتوزيعها بين أقاربك الذين تعتقد أنهم قد يستفيدون من هذا المشروع.
- سيتم التقاط صورة لأسنانك لتحديد عدها والتحقق من موضعها.
- سنقوم بجمع البيانات من ملفك الطبي.
- نحتاج منك أن تزور المركز الطبي في الجامعة الأمريكية في بيروت زيارة واحدة (45 دقيقة) أو زيارتين (30 دقيقة + 15 دقيقة): الزيارة الأولى لتوضيح الدراسة بالتفصيل ، والتوقيع على هذه الورقة ، وإجراء فحص سريري وصورة لأسنانك ، و الزيارة الثانية لأخذ الدم.

هل يمكن أن يلحق بي الأذى من خلالها؟

نريد أن نخبرك عن بعض الأمور التي قد تؤذيك أو تزعجك إذا كنت في هذه الدراسة. قد تؤذي الإبرة التي نستخدمها لأخذ الدم. وهناك أيضنا خطر الإشعاع من الصورة ، ولكن لا توجد آثار ضارّة مثبّتة من مستويات الإشعاع التي ستتعرّض لها أثناء الدّراسة.

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أحرف القاصر:

هل يمكن الاستفادة منها؟
لا نعرف ما إذا كان التواجد في هذه الدّراسة البحثية سيساعدك على الشعور بالتحسّن. لكنّنا نأمل من خلالها أن نتعلّم شيئًا ما سيساعد الآخرين في المستقبل.

هل لدي خيارات أخرى؟
يمكنك اختيار عدم الاشتراك في هذه الدّراسة.

هل سيعلم أحد أنني شاركت في الدّراسة؟
فقط والديك والفريق الذي يدير الدّراسة. لن يعرف أي شخص بمشاركتك في هذه الدّراسة. عندما ننتهي من الدّراسة ، سنقوم بكتابة تقرير حول ما اكتشفناه ولن نستخدم اسمك في التقرير.

ماذا يحدث إذا تعرّضت للأذى؟
تم تزويد والديك / الأوصياء القانونيين بمعلومات حول ما يجب فعله في حالة إصابتك أثناء الدراسة
لن تتلقى أي مدفوعات مقابل التواجد في هذه الدراسة البحثية.

قبل أن توافق على المشاركة في هذه الدّراسة ؛ يمكنك أن تستوضح من الدكتورة جوزيفين بواري عن أيّ أمر لم تفهمه.

ماذا لو لم أرغب في المشاركة؟
لا يجب أن تكون في هذه الدراسة. الأمر يعود لك. إذا قلت نعم الآن ، ولكن غيرت رأيك لاحقًا ، فلا بأس أيضًا. كل ما عليك فعله هو إخبارنا.

إذا كنت تريد المشاركة في هذه الدراسة ، يرجى التوقيع أو طباعة اسمك.

☐ نعم أريد الإشتراك ☐ لا أريد الإشتراك

اسم المشترك(ة) القاصر توقيع المشترك(ة) القاصر

التاريخ و السّاعة

اسم الشخص الحاصل على الموافقة التوقيع

التاريخ و السّاعة

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E. **ADOLESCENT ASSENT FORM**

Minor's Initials: ____

Institutional Review Board
American University of Beirut
Faculty of Medicine
Bliss Street
Beirut, Lebanon
Tel: (01) 350-000 ext. 5445

ADOLESCENT PARTICIPANT ASSENT FORM

(approximate ages 13-17)

Protocol Number: IRB.ID (BIO-2019-0464)

Principal Investigator(s): Dr Joseph Ghafari

Co- Investigators: Dr Anthony Macari
Dr Josephine Boueiri

Project Title: Genetic basis of familial oligo-hypo-hyperdontia in eastern Mediterranean families

Address: American University of Beirut Medical Center
Bliss Street
Beirut – Lebanon

Site where the study will be conducted: Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

Phone: (01) 350 000 ext: 5707

You are being asked to join the study because:

1- If you are a patient treated in our division: during your orthodontic appointment, you will be approached first by your primary physician. He/she will explain in detail and ask you if you are willing to participate in the research project. If you agree, you will be then introduced to the research coordinator (Dr. Josephine Boueri) to sign this consent form. After signing it, you will be asked questions about your family histories, including affection status of other individuals in your family. You will also be given flyers to distribute amongst your close relatives whom you think could benefit from this project.

2- Or if you are a relative of a patient treated in our division who read flyer handed to you by him/her.

Therefore, in order to determine the responsible genes and the familial transmission some further tests will be needed.

Please take time to read the following information carefully before you decide whether you want to take part in this study or not. Feel free to ask the doctor if you need more information or clarification about what is stated in this form and the study as a whole.

A- Purpose of the research study and overview of participation:

The aim of the study:

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We are trying to find out more about the genes involved in the development of the teeth and the if the alteration of those genes will lead to missing (decreased number) and/or supernumerary teeth (increased number).

1. Clarify the Inheritance pattern of missing teeth (decreased number)/supernumerary (increased number) in Mediterranean population especially Arab population.
2. Explore the occurrence of missing teeth/supernumerary in one family in Mediterranean population.
3. Compare between the expression of the genes in families having only missing teeth and families having same time occurrence of missing/supernumerary of teeth.

What is involved in the study?

1. The study will include families who have members with missing teeth/ dental excess. Your enrolment is not obligatory.
2. If you would like to participates, you will commit to go through the following steps:

Steps	Procedures
1	- Medical history will be filled out: demographics (gender, date of birth, age, and family origin), health status. - The orthodontist will look at your teeth and will take one panoramic x-ray. - You will be positioned in the x-ray machine, which will move next to your face, for about 1 minute. - In case you had a previous x-ray taken within a period of 1 year, no further x-ray will be required. - An image of the lower part of your face will appear on the screen, showing your teeth and surrounding structures. - This is done to count all you teeth (erupted or not yet) and assess their position. - Even if you don't want to participate in the research study, this x-ray would be needed if you decide to undergo an orthodontic evaluation and treatment. In case an abnormal finding was present on the X-ray, further investigation will be performed accordingly. You will benefit from free diagnosis and treatment will be suggested. If you were a patient in our department, and we have the above records, we will not repeat them unless long time has passed which requires updating them. - "You may not participate in this study if you are pregnant. If you are capable of becoming pregnant, a pregnancy test will be performed before you are exposed to any radiation. You must tell us if you may have become pregnant within the previous 14 days because the pregnancy test is unreliable during that time" - After the x-ray analysis, a family pedigree will be drawn to evaluate the affected members.
2	- Blood withdrawal (5cc) will be done at the hospital (AUBMC), by a specialized nurse or physician. The AUBMC Laboratory Medicine rules and regulations will be followed. - Your DNA will be stored in encoded tubes at 80°C at key facilities at the American University of Beirut, where the next generation of DNA sequencing technologies will be implemented. The data will be collected at the American University of Beirut and analyzed after that.

This will require you to pass by AUBMC for one (45mins) or two (30mins + 15mins) visits: the first one to explain the project in detail, sign this consent form, have a clinical examination and a panoramic x-ray, and the second visit to have 5cc of your blood collected.

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B-Number of subjects to be enrolled

An approximate number of at least 30 individuals will be enrolled in the study.

C-WHAT ARE THE RISKS OF THE STUDY?

Risks	Procedures
Radiation risk	Although there are no proven harmful effects from irradiation levels that you will be exposed to during this study, long-term effects on your health cannot be ruled out with certainty. The effective dose of a single panoramic x-ray is only 1.1mrem, which is minor compared to the average annual dose that a person receives from environmental radiation (~300 mrem).
Associated with blood withdrawal	Such risks may include bruising, local pain, hematoma, slight possibility of infection or fainting. Blood will be withdrawn by a specialized nurse or physician at the hospital (AUBMC), using a clean needle; thus, this risk is minimal.

For more information about these risks and side effects, ask your study doctor and please note that there may be unforeseeable risks.

D- Are there benefits from participating in this study?

There is no direct benefit to you from this study. However, it has some benefits to the class to which you belong and to the society and science/ medicine as a whole.

This study will allow us to better understand the specific genes contributing to variation in the risk for dental agenesis and dental excess. This knowledge can lead to a better estimation of the susceptibility to this condition and a better comprehension of the genetic mechanisms that may influence the process of dental development. Early prediction of the condition would lead to:

- Earlier treatment in case of dental agenesis will preserve the bone volume and density thus reducing the later need for bone graft and implants in the affected area. In case of dental excess, early treatment will help in the extraction decision.
- More importantly, earlier interventions may eliminate or at least reduce the severity of a later orthognathic surgery when mandibular or maxillary micrognathism is due to lack of bone development which is dictated by dental development.

Please note that refusal of participation will not lead to a loss of benefits.

E- What other options are there?

This is not a treatment study so the only alternative is not to participate in the study.

F- Can you be removed from the study without your consent?

You will not be removed from this study by the study team without your consent. However, the investigator may end your participation at any time.

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G- Confidentiality

If you agree to participate in this study, your name will remain confidential. No person, unless the law provides for this, will have the right to see your medical file except for the doctor responsible for the study and his assistants, independent professional ethics committees, and inspectors from the organized government agencies.
The radiograph will be taken in the division of Orthodontics and Dentofacial Orthopedics, AUBMC and stored in the bank of radiographs generated and housed in the corresponding radiologic software (CLINIVIEW). It will be placed in a separate digital folder on a computer in our division and codes will be applied so that the folder can be accessed only by the research group members.
The blood samples will be numbered, coded and stored in a safe place until the end of the study. All blood samples and their derivatives will be kept if you opt to participate in further genetic studies. Otherwise, they will be trashed at the end of the study.
We will keep your records confidential unless we are required by law to share any information.

Depending on your request, your individual results will be disclosed to you and to your family members as soon as the study is completed (approximate date: April 2021).
□ I want to be informed of the results of the study.
□ I don't want to be informed of the results of the study.

I do not wish to know the results of the study or test done, and I am made aware of the following:

- I was well informed through this consent and I fully understand that some of these genetic tests may yield information or results that can affect me or any of my family members or progenies in the future.
- I was well informed that I can change my mind, and contact the Principal Investigator or research team to update them on my decision at any time up to 3 years of study closure.

Only results of genes related to the condition will be relayed back for research purposes.

H- Use of your coded samples for future research:

I permit coded use of my genetic materials (blood, DNA) for the proposed study. I specify the use of the samples in the following manner (please check only one of the following):

a. I permit further contact to seek permission to do further studies on the samples.
b. I do not allow the use of my biological samples for further studies.
c. I permit anonymized (samples cannot be linked to subject) the use of my biological materials for other studies without contact.

I- What if you are injured in the study?

AUBMC will cover the cost of treating, on its premises, medical adverse events resulting directly from the medication and/or medical procedures of this research study. Otherwise, it will not cover for the costs of medical care for any medical condition or issue. But you should know the risk is minimal.

J- What are the costs?

There are no costs associated with participation. The study will cover the costs of the procedures done for its purpose which includes: blood withdrawal and genetic analysis and the X-ray that is taken for you and

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for every individual who will be enrolled in the study, along with an initial consultation for the need of an orthodontic treatment. All other costs will not be covered.

K- Will you get paid to be in this study?

You will not be paid to participate in this study.

L-What are your rights as a research subject?

Being in this study is voluntary. You don't have to be in this study if you don't want to or you can stop being in the study at any time. Your decision will not result in any penalty or loss of benefits that you have now. If you have questions about your rights you may call:
Institutional Review Board on 01-350000 ext. 5445
You will be told about any new information that may affect your health, welfare, or willingness to stay in this study.
I understand that I am free to withdraw this consent and discontinue participation in this project at any time without loss of benefits, even after signing this form, and it will not affect my care. You are allowed to ask questions related to the study at any time.

M- Agreement on being contacted in future research

- □ I agree to be contacted in future research studies
- □ I do not agree to be contacted in future research studies

N- Signature of the investigator

I have reviewed, in detail, the informed consent document for this research study with

____
(name of patient)
the purpose of the study and its risks and benefits.
I have answered all the patient's questions clearly. I will inform the participant in case of any changes to the research

______________________ ______________________
Name of Investigator or designee **Signature**

____
Date & Time

O- Signature of the participant:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. Joseph Ghafari at (01) 350 000 ext. 5707 or any of his/her designee involved in the study in case of any questions.
If I felt that my questions have not been answered, I contact the Institutional Review Board for human rights at (01) 350 000 ext. 5445. I understand that I am free to withdraw this consent and discontinue

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participation in this project at any time without loss of benefits, even after signing this form, and it will not affect my care. I know that I will receive a copy of this signed informed consent.

_______________________________	______________
NAME OF SUBJECT	AGE (13 YRS - 17 YRS)
_______________________________	______________
SIGNATURE OF SUBJECT	DATE & Time

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أحرف القاصر:

Institutional Review Board
American University of Beirut
Faculty of Medicine
Bliss Street
Beirut, Lebanon
Tel: (01) 350-000 ext. 5445

موافقة للاشتراك في البحث العلمي

المراهقين ذو العمر يتراوح بين ١٣-١٧

محضر رقم: IRB.ID (BIO-2019-0464)

إسم الباحث: د. جوزف غفري

الباحثون المساعدون: د. انطوني مكاري
د. جوزفين بواري

عنوان البحث: الارتباط بين الجينات المسببّة بخلل في عدد الأسنان سواء نقصان او زيادة لدى سكّان منطقة شرق البحر المتوسّط

مكان إجراء البحث: الجامعة الأميريكيّة في بيروت
شارع بلس
بيروت – لبنان
قسم تقويم الأسنان و الفكين

هاتف: 000 350-01- مقسم : 5707

أنت مدعو للانضمام الى الدّراسة:

1. إذا كنت مريضاً تتلقّى العلاج في قسمنا: خلال موعدك الطبّي سيشرح لك طبيبك المعالج أهداف الدّراسة، اجراءاتها، و فوائدها/ مخاطرها، وستسألك/ يسألك إذا كنت ترغب في المشاركة فيها. إذا وافقت، سيعرّفك طبيبك على منسّقة الدّراسة (د. جوزفين بواري) و سيُطلبُ منك الإمضاء على إستمارة الموافقة هذه. بعد إمضائها، ستُسأل عن تاريخ عائلتك، بما في ذلك حالة نقصان او زيادة عدد الأسنان لأفراد آخرين من عائلتك. كما و سيتمّ اعطاؤك نشرات لتُوزعها على أقربائك المقرّبين الذين قد يستفدون، بحسب اعتقادك، من هذه الدّراسة.
2. إذا كنت من أقرباء لمريض يتلقّى العلاج في قسمنا، و كنت قد أخذت النّشرة منه/ها و قرأتها.

لذلك ، من أجل تحديد الجينات المسؤولة والعامل الوراثي ، ستكون هناك حاجة إلى مزيد من الاختبارات.
نرجو منك قراءة المعلومات الواردة بدقّةٍ واستشر أهلك قبل أخذ القرار بالمشاركة، كما أنّه بامكانك طلب إيضاحات او معلومات إضافيّة عن أيّ شيء مذكور في هذه الاستمارة أو عن هذه الدّراسة ككلّ من طبيبك.

أ. وصف البحث العلمي و هدفه و تفسير مجرياته:

أهداف الدّراسة:

إنّنا نحاول معرفة المزيد حول خلل الجينات المعنيّة في تكوين و نموّ الأسنان الخاصّة بك ممّا قد يؤثّر على نموّ الفكّ لديك.

1. توضيح أساليب الوراثة العائليّة لحالة نقصان أو زيادة في عدد الأسنان لدى سكّان منطقة شرق البحر المتوسّط و بالأخصّ السكّان العرب.
2. استكشاف تلازم حالتي نقصان و زيادة في عدد الأسنان لدى العائلة الواحدة.
3. مقارنة التكوين الجينيّ بين العائلات التي تشمل افراد ذو نقصان عدد الأسنان فقط والعائلات التي تشمل الحالتين

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أحرف القاصر:

ضمن أفرادها.

ماذا تشمل هذه الدّراسة:

1. ستشمل الدّراسة الأسر التي لديها خلل في عدد الأسنان لدى أفرادها. تسجيلك ليس إلزاميًا.
2. إذا قرّرت الإشتراك في الدّراسة، ستلتزم بالقيام بالخطوات التالية:

الخطوة	الإجراءات
1	- سيتم ملء التاريخ الطبي: التركيبة السكانية (الجنس ، تاريخ الميلاد ، العمر ، والأصل العائلي) ، الحالة الصحية. - سيعاين طبيب تقويم الأسنان أسنانك وسيأخذ صورة بالأشعة السينيّة. - سيتمّ وضعك في جهاز الفحص بالأشعة السينيّة الذي سيتحرّك حول وجهك لمدّة دقيقة. - في حال كان لديك صورة أشعة سابقة مأخوذة في غضون سنة واحدة ، فلن تكون هناك حاجة إلى صورة أشعة أخرى. - ستظهر صورة للجزء السفلي من وجهك على الشاشة ، تظهر أسنانك والتراكيب المحيطة بها. - يتم ذلك لحساب جميع أسنانك (المبزوغة أو غير المبزوغة بعد) وتقييم موقعها. - حتّى إذا كنت لا ترغب في المشاركة في الدّراسة البحثيّة، إلاّ أنّ الأشعة السينيّة هذه قد تلزمك في حال قررت الخضوع لعلاج تقويم الأسنان. في حال وجود اكتشاف غير طبيعيّ على الأشعة السينيّة، سيتمّ إجراء المزيد من التحقيقات وفقًا لذلك. ستستفيد من تشخيص مجانيّ وسيتمّ اقتراح العلاج المناسب. إذا كنت (مريض/ ة) في قسمنا، فلدينا السجلاّت المذكورة أعلاه، لن نقوم بتكرارها إلا إذا قد مضى وقت طويل ممّا يتطلّب تحديثها. - لا يمكنكِ المشاركة في هذه الدّراسة إذا كنت حاملاً . إذا كنت قادرة على الحمل، سيتمّ إجراء اختبار الحمل قبل أن تتعرضي لأيّ إشعاع. ينبغي أن تخبرينا إذا ثمّة احتمال أن تكوني قد حملت في غضون الأيّام الأربعة عشرة الماضية، وذلك لأنّه لا يمكن الاعتماد على اختبار الحمل خلال تلك الفترة. - عقب تحليل الأشعة السينيّة، سيتمّ رسم شجرة النسب (شكل من أشكال شجرة العائلة) لمعرفة من هم الأفراد في الأسرة المصابين بحالة نقصان أو زيادة في عدد الأسنان.
2	-ستسحب ممرضة أو طبيب ٥ سنتيمتر مكّعب من الدّم من ذراعك باستخدام إبرة نظيفة. سيتمّ ذلك في مستشفى الجامعة الأميركيّة. سيتم اتباع قواعد ولوائح الطب المخبري في الجامعة الأمركية في بيروت. - سيتمّ تخزين الحمض النووي الخاص بك في أنابيب مشفرة بدرجة حرارة منوية ٨٠ تحت الصّفر في المنشآت الأساسيّة في الجامعة الأميركيّة في بيروت، حيث سيتمّ تنفيذ الجيل القادم من تكنولوجيّات ترتيب الحمض النوويّ. سيتمّ جمع البيانات في الجامعة الأميركيّة في بيروت وتحليلها بعد ذلك.

سيتطلب منك أن تقوم بزيارة AUBMC مرّة واحدة (٤٥دقيقة) أو مرّتين (٣٠دقيقة + ١٥ دقيقة)، الزيارة الأولى لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء إستمارة الموافقة هذه ولمعاينة أسنانك وأخذ صورة بالأشعة السينيّة الأماميّة لتحديد عدد الأسنان. الزيارة الثّانية لنأخُذ ٥ سنتيمتر مُكعب من دمك.

ب. عدد الأشخاص الذين سيتمّ تسجيلهم في الدّراسة:

سيتمّ تسجيل عدد تقريبي من ٣٠ فردٍ متأثر بحالة نقصان أو زيادة في عدد الأسنان.

ت. ما هي التأثيرات السلبيّة لهذه الدّراسة ؟

المخاطر	الإجراءات
خطرالإشعاع	على الرّغم من أنّه ليس هناك أيّ آثار ضارّة مُثبتة تنتج عن مستويات الإشعاع التي ستتعرّض لها أثناء هذه الدّراسة، إلاّ أنّه لا يمكن استبعاد آثار طويلة الأمد على صحّتك. الجرعة الفعّالة لصورة واحدة هي١.١ ميلي ريم فقط ،وهي نسبة ضئيلة مقارنةً بمعدّل الجرعة السنويّة التي يتلقّاها الشخص من الإشعاع البيئي ما يقارب ٣٠٠ ميلي ريم.
المرتبطة	قد تشمل هذه المخاطر الكدمات، ألم موضعي، ورم دمويّ، واحتمال ضئيل للإلتهاب أو الإغماء. سيتمّ

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أحرف القاصر:

يسحب الدم	سحب ذلك باستخدام إبرة نظيفة من قبل ممرّضة متخصّصة أو طبيب في المستشفى وبالتّالي، هذا الخطر ضئيل.

للمزيد من المعلومات حول هذه المخاطر، إسأل الطّبيب المسؤول عن الدّراسة. يرجى ملاحظة أنه قد تكون هناك مخاطر غير متوقّعة.

ث. ما هي الفوائد التي قد تنتج عن هذه الدّراسة؟

ليس ثمّة أي فوائد مباشرة لك من هذه الدّراسة . إلاّ أنّها تقدّم بعض الفوائد للفئة التي تنتمي إليها وكذلك للمجتمع والطبّ والعلم ككلّ.

ستسمح لنا هذه الدراسة بفهم أفضل للجينات المحددة التي تساهم في الاختلاف في خطر الإصابة بنقصان أو زيادة عدد الأسنان. ويمكن أن تؤدّي هذه المعرفة إلى تقدير أفضل لقابليّة حصول هذه الحالة وكذلك إلى فهم أفضل للآليّات الوراثيّة التي تؤثّر على نمو الأسنان. قد يؤدّي التنبّؤ المبكر للحالة إلى :

- العلاج المبكر سيحافظ على حجم العظم وكثافته وبالتالي تقليل الحاجة لاحقاً إلى زرع العظم وزرعات الأسنان في المنطقة المصابة. في حالة زيادة الأسنان ، فإن العلاج المبكر سيساعد في قرار خلع الأسنان.
- الأهم من ذلك ، قد تؤدي التدخلات المبكرة إلى القضاء على أو على الأقل تخفيف شدة جراحة تقويم الفكين اللاحقة عندما يكون صغر الفك العلوي أو السفلي بسبب نقص نمو العظام الذي يمليه نمو الأسنان.

يرجى ملاحظة أن رفض المشاركة لن يؤدّي إلى خسارة الفوائد.

ج. هل هناك طرق بديلة للوصول إلى الهدف المرجو؟

هذه ليست دراسة مخصصّة للعلاج، فالوسيلة الوحيدة البديلة هي عدم مشاركتك في هذه الدّراسة.

ح. هل يمكن اخراجك من الدّراسة بدون موافقتك؟

لن يتمّ اخراجك من الدّراسة من قبل الفريق المختصّ بالدّراسة من دون موافقتك. غير أنّه يحقّ للباحث الرئيسيّ إنهاء مشاركتكم فالدّراسة في أيّ وقت.

خ. السرّية

فـي حـال وافقـت علـى المشـاركة فـي هـذه الدّراسـة، سـيبقى اسـمك طـي الكتمـان . لـن يسـمح لأيّ شـخص، مـا لـم يـنصّ القـانون علـى ذلـك، حـقّ الإطـلاع علـى ملفّـك الطّبـي باسـتثناء الطبيـب المسـؤول عـن الدّراسـة ومعاونيـه، ولجـان الأخـلاق المهنيّـة المسـتقلّة، و مفتشـين مـن الإدارات الحكوميّـة المنظمّة.

سـتؤخذ الصّـورة بالأشـعّة فـي قسـم تقـويم الأسـنان وتأهيـل الفكّـين فـي المركـز الطبـيّ فـي الجامعـة الأميركيـة فـي بيـروت وتحفـظ فـي بنـك الصـور الشـعاعيّة (CLINIVIEW)الـذي تـمّ احـداثها وضـمها فـي البرنـامج الشـعاعي المطـابق و سـيتمّ وضـعها فـي ملـف رقمـيّ منفصـل علـى كومبيـوتر فـي قسمنا، وسيتمّ تطبيق رموز بحثيّة لا يمكن لأحد اللّجوء إلى الملف إلا أعضاء فريق البحث وحسب.

سـيتمّ تـرقيم عينـات الـدمّ وترميزهـا وتخزينهـا فـي مكـان أمـن حتّـى نهايـة الدّراسـة. سـيتمّ الاحتفـاظ بجميـع عينـات الـدمّ ومشـتقّاتها إذا أردت المشـاركة فـي دراسـات جينيّـة أخـرى وإلأ، سـيتم الـتخلص منهـا فـي نهاية الدّراسة.

بنـاءً علـى طلبـك، سـيتمّ الكشـف عـن النتـائج الشخصـيّة لـك ولأفـراد عائلتـك بمجـرد الانتهاء من الدّراسة التاريخ المتوقع: (نيسان 2021)

- ☐ **أريد أن أبلّغ بنتائج الدّراسة.**
- ☐ **لا أريد أن أبلّغ بنتائج الدّراسة.**

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أحرف القاصر:

لا أرغب في معرفة نتائج الدّراسة أو الاختبار الذي تمّ ، وأنا على يقين بما يلي:

- لقد تمّ إطلاعي جيّدًا من خلال هذه الموافقة وأدرك تمامًا أنّ بعض هذه الاختبارات الجينيّة قد تسفر عن معلومات أو نتائج يمكن أن تؤثّر على أو على أيّ من أفراد أسرتي أو نسلي في المستقبل.
- لقد تبلّغت أنّه بإمكاني تغيير رأيي ، والاتصال بالمحقّق الرئيسيّ أو بفريق البحث لتحديثهم بقراري في أيّ وقت و لغاية ٣ سنوات من إغلاق الدّراسة.

سيتمّ إعادة نتائج الجينات المتعلّقة بالحالة فقط لأغراض البحث.

د. <u>أبحاث مستقبليّة:</u>

أسمح باستخدام موادي الجينيّة (الدمّ، اللّعاب، الحمض النوويّ) للدّراسة المقترحة. أحدّد استخدام العينات بالطّريقة التّالية (يرجى التحقّق من واحد فقط ممّا يلي):

أ. أسمح بالاتّصال للحصول على إذن لإجراء المزيد من الدّراسات على العيّنات.
أو
ب. لا أسمح باستخدام عيّناتي البيولوجيّة لإجراء المزيد من الدّراسات.
أو
ت. أسمح باستخدام موادي البيولوجيّة من دون امكانيّة ربط العيّنات بالفرد في دراسات أخرى دون اتصال.

ذ. <u>ماذا لو حصل لي أي عارض سلبي؟</u>

إن المركز الطبيّ في الجامعة الأمريكيّة في بيروت سوف يغطّي تكاليف العلاج في المركز للعوارض الطبيّة السلبيّة الناجمة مباشرة عن الأدوية و/أو الإجراءات الطبّيّة الخاصّة بهذه الدّراسة البحثيّة. فيما عدا ذلك، لن يقوم المركز الطبيّ بتغطية تكاليف العناية الطبيّة لأيّة حالة أو مشكلة مرضيّة. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جدًا .

ر. <u>هل هناك تكاليف من خلال المشاركة في هذه الدّراسة؟</u>

ليس ثمّة أيّ تكاليف مرتبطة بالمشاركة. ستغطي الدّراسة تكاليف الاجراءات المطلوبة لتحقيق أهدافها ممّا تتضمن: سحب الدمّ، التحليل الجيني والأشعة السينيّة التي ستؤخذ لك و لأيّ فرد قد ينضمّ للدراسة بالإضافة إلى كشف سريري من أجل التحقّق إن كان علاج تقويم الأسنان لازمًا. لن تتمّ تغطية أيّ تكاليف أخرى.

ز. <u>هل ستتقاضى أي مردود مالي مقابل المشاركة في الدّراسة؟</u>

لن تحصل على أيّ مردود مالي مقابل مشاركتك في الدّراسة.

س. <u>ما هي حقوقك كموضوع للبحث؟</u>

مشاركتك في الدّراسة أمرٌ طوعي. لست مجبرًا على المشاركة في الدّراسة إذا لم ترغب في ذلك، كما أنّه يمكنك الإنسحاب من الدّراسة في أيّ وقت. قرارك هذا لن يؤدّي إلى أيّ عقوبة أو خسارة المزايا التي تملكها الآن. إذا كان لديك أسئلة تتعلّق بحقوقك، يمكنك الاتصال ب:
لجنة الأخلاقيّات على ٠١/٣٥٠٠٠٠ مقسم ٥٤٤٥
سيتمّ اعلامك عن أيّ معلومات جديدة قد تؤثّر على صحّتك أو رغبتك في البقاء في هذه الدّراسة .
يمكنك تغيير رأيك في أيّ وقت و هذا حتمًا لن يؤثّر على اهتمام الأطباء بك. بامكانك طرح الأسئلة المتعلّقة بالدّراسة متى شئت.

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أحرف القاصر:

ش. الإتفاق على التّواصل من أجل أبحاث أخرى مستقبلية:

☐ أوافق على أن يتمّ الاتصال بي من أجل الدّراسات البحثيّة المستقبليّة.

☐ أنا لا أوافق على أن يتمّ الاتصال بي من أجل الدّراسات البحثيّة المستقبليّة.

ص. موافقة الباحث:

لقد استعرضت، بالتفصيل، وثيقة الموافقة المسبقة لهذه الدّراسة البحثيّة مع

____

(اسم: _المريض، _الممثل القانوني، _أو والد، والدة / وصي)، الغرض من الدّراسة، مخاطرها وفوائدها.
لقد أجبت على كل أسئلة المريض بوضوح. سأبلغ المشارك في حال طرأت أي تغييرات على البحث.

______________	______________
اسم المحقق أو من ينوب عنه	التوقيع

____

التاريخ و السّاعة

ض. موافقة المشترك:

لقد قرأت وفهمت كل جوانب الدّراسة البحثية وقد تمّت الإجابة على جميع أسئلتي. وأنا أوافق طوعًا على المشاركة في هذه الدراسة البحثية وأعلم أنه بإمكاني الاتصال بالدكتور جوزف غفري على الرقم 01350000 مقسم 5707 أو بمن ينوب عنه في الدّراسة في حال لدينا أي سؤال.
إذا شعرت أنهّ لم يتم الاجابة على أسئلتي، يمكنني الاتصال بلجنة الأخلاقيات على الرقم 01350000 مقسم5445 وأنا أفهم أنني حر في سحب هذه الموافقة والتوقف عن المشاركة في هذا المشروع في أي وقت، حتى بعد التوقيع على هذا النموذج، وأن ذلك لن يؤثّر على إهتمام الأطباء بي .كما وأنه يحق للباحث الرئيسي إنهاء مشاركتي فالدّراسة في أي وقت. أعلم أنني سأحصل على نسخة من هذه الموافقة الموقعّة.

______________	______________
اسم المشترك(ة)	السن (١٣-١٧سنة)
______________	______________
توقيع المشترك(ة)	التّاريخ و السّاعة

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F. **ADULT CONSENT FORM**

Institutional Review Board
American University of Beirut
Faculty of Medicine
Bliss Street
Beirut, Lebanon
Tel: (01) 350-000 ext. 5445

ADULT PARTICIPANT CONSENT FORM

(age above 18 years old)

Protocol Number: IRB.ID (BIO-2019-0464)

Principal Investigator(s): Dr Joseph Ghafari

Co- Investigators: Dr Anthony Macari
Dr Josephine Boueiri

Project Title: Genetic basis of familial oligo-hypo-hyperdontia in eastern Mediterranean families

Address: American University of Beirut Medical Center
Bliss Street
Beirut - Lebanon

Site where the study will be conducted: Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

Phone: (01) 350 000 ext: 5707

You are being asked to join the study because:

1- If you are a patient treated in our division: during your orthodontic appointment, you will be approached first by your primary physician. He/she will explain in detail and ask you if you are willing to participate in the research project. If you agree, you will be then introduced to the research coordinator (Dr. Josephine Boueri) to sign this consent form. After signing it, you will be asked questions about your family histories, including affection status of other individuals in your family. You will also be given flyers to distribute amongst your close relatives whom you think could benefit from this project.

2- Or if you are a relative of a patient treated in our division who read flyer handed to you by him/her.

3- Or if you are the parent of a patient and the research coordinator told you about the study.

Therefore, in order to determine the responsible genes and the familial transmission some further tests will be needed.

Please take time to read the following information carefully before you decide whether you want to take part in this study or not. Feel free to ask the doctor if you need more information or clarification about what is stated in this form and the study as a whole.

A- Purpose of the research study and overview of participation:

The aim of the study:

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We are trying to find out more about the genes involved in the development of the teeth and the if the alteration of those genes will lead to missing (decreased number) and/or supernumerary teeth (increased number).

1. Clarify the Inheritance pattern of missing teeth (decreased number)/supernumerary (increased number) in Mediterranean population especially Arab population.
2. Explore the occurrence of missing teeth/supernumerary in one family in Mediterranean population.
3. Compare between the expression of the genes in families having only missing teeth and families having same time occurrence of missing/supernumerary of teeth.

What is involved in the study?

1. The study will include families who have members with missing teeth/ dental excess. Your enrolment is not obligatory.
2. If you would like to participates, you will commit to go through the following steps:

Steps	Procedures
1	- Medical history will be filled out: demographics (gender, date of birth, age, and family origin), health status. - The orthodontist will look at your teeth and will take one panoramic x-ray. - You will be positioned in the x-ray machine, which will move next to your face, for about 1 minute. - In case you had a previous x-ray taken within a period of 1 year, no further x-ray will be required. - An image of the lower part of your face will appear on the screen, showing your teeth and surrounding structures. - This is done to assess the number and position of your teeth (erupted or not yet) - Even if you don't want to participate in the research study, this x-ray would be needed if you decide to undergo an orthodontic evaluation and treatment. In case an abnormal finding was present on the X-ray, further investigation will be performed accordingly. You will benefit from free diagnosis and treatment will be suggested. If you were a patient in our department, and we have the above records, we will not repeat them unless long time has passed which requires updating them. - "You may not participate in this study if you are pregnant. If you are capable of becoming pregnant, a pregnancy test will be performed before you are exposed to any radiation. You must tell us if you may have become pregnant within the previous 14 days because the pregnancy test is unreliable during that time" - After the x-ray analysis, a family pedigree will be drawn to evaluate the affected members.
2	- Blood withdrawal (5cc) will be done at the hospital (AUBMC), by a specialized nurse or physician. The AUBMC Laboratory Medicine rules and regulations will be followed. - Your DNA will be stored in encoded tubes at 80°C at key facilities at the American University of Beirut, where the next generation of DNA sequencing technologies will be implemented. The data will be collected at the American University of Beirut and analyzed after that.

This will require you to pass by AUBMC for one (45mins) or two (30mins + 15mins) visits: the first one to explain the project in detail, sign this consent form, have a clinical examination and a panoramic x-ray, and the second visit to have 5cc of your blood collected.

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B- Number of subjects to be enrolled

An approximate number of at least 30 individuals will be enrolled in the study.

C- What are the risks of the study?

Risks	Procedures
Radiation risk	Although there are no proven harmful effects from irradiation levels that you will be exposed to during this study, long-term effects on your health cannot be ruled out with certainty. The effective dose of a single panoramic x-ray is only 1.1mrem, which is minor compared to the average annual dose that a person receives from environmental radiation (~300 mrem).
Associated with blood withdrawal	Such risks may include bruising, local pain, hematoma, slight possibility of infection or fainting. Blood will be withdrawn by a specialized nurse or physician at the hospital (AUBMC), using a clean needle; thus, this risk is minimal.

For more information about these risks and side effects, ask your study doctor and please note that there may be unforeseeable risks.

D- Are there benefits to taking part in the study?

There is no direct benefit to you from this study. However, it has some benefits to the class to which you belong and to the society and science/ medicine as a whole.

This study will allow us to better understand the specific genes contributing to variation in the risk for dental agenesis and dental excess. This knowledge can lead to a better estimation of the susceptibility to this condition and a better comprehension of the genetic mechanisms that may influence the process of dental development. Early prediction of the condition would lead to:

1. Earlier treatment in case of dental agenesis will preserve the bone volume and density thus reducing the later need for bone graft and implants in the affected area. In case of dental excess, early treatment will help in the extraction decision.

2. More importantly, earlier interventions may eliminate or at least reduce the severity of a later orthognathic surgery when mandibular or maxillary micrognathism is due to lack of bone development which is dictated by dental development.

Please note that refusal of participation will not lead to a loss of benefits.

E- What other options are there?

This is not a treatment study so the only alternative is not to participate in the study.

F- Can you be removed from the study without your consent?

You will not be removed from this study by the study team without your consent. However, the investigator may end your participation at any time.

G- Confidentiality

If you agree to participate in this study, your name will remain confidential. No person, unless the law provides for this, will have the right to see your medical file except for the doctor responsible for the study and his assistants, independent professional ethics committees, and inspectors from the organized government agencies.

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The radiograph will be taken in the division of Orthodontics and Dentofacial Orthopedics, AUBMC and stored in the bank of radiographs generated and housed in the corresponding radiologic software (CLINIVIEW). It will be placed in a separate digital folder on a computer in our division and codes will be applied so that the folder can be accessed only by the research group members.
The blood samples will be numbered, coded and stored in a safe place until the end of the study. All blood samples and their derivatives will be kept if you opt to participate in further genetic studies. Otherwise, they will be trashed at the end of the study.

Depending on your request, your individual results will be disclosed to you and to your family members as soon as the study is completed (approximate date: April 2021).
☐ I want to be informed of the results of the study
☐ I don't want to be informed of the results of the study

I do not wish to know the results of the study or test done, and I am made aware of the following:

- I was well informed through this consent and I fully understand that some of these genetic tests may yield information or results that can affect me or any of my family members or progenies in the future.
- I was well informed that I can change my mind, and contact the Principal Investigator or research team to update them on my decision at any time up to 3 years of study closure.

Only results of genes related to the condition will be relayed back for research purposes.

H- Use of your coded samples for future research:

I permit coded use of my genetic materials (blood, DNA) for the proposed study. I specify the use of the samples in the following manner (please check only one of the following):

a. I permit further contact to seek permission to do further studies on the samples.
b. I do not allow the use of my biological samples for further studies.
c. I permit anonymized (samples cannot be linked to subject) the use of my biological materials for other studies without contact.

I- What if you are injured in the study?

AUBMC will cover the cost of treating, on its premises, medical adverse events resulting directly from the medication and/or medical procedures of this research study. Otherwise, it will not cover for the costs of medical care for any medical condition or issue. But you should know the risk is minimal.

J- What are the costs?

There are no costs associated with participation. The study will cover the costs of the procedures done for its purpose which includes: blood withdrawal and genetic analysis and the X-ray that is taken for you and for every individual who will be enrolled in the study, along with an initial consultation for the need of an orthodontic treatment. All other costs will not be covered.

K- Will you get paid to be in this study?

You will not be paid to participate in this study.

L- What are your rights as a research subject?

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Being in this study is voluntary. You don't have to be in this study if you don't want to or you can stop being in the study at any time. Your decision will not result in any penalty or loss of benefits that you have now. If you have questions about your rights you may call:
Institutional Review Board on 01-350000 ext. 5445
You will be told about any new information that may affect your health, welfare, or willingness to stay in this study.
I understand that I am free to withdraw this consent and discontinue participation in this project at any time without loss of benefits, even after signing this form, and it will not affect my care. You are allowed to ask questions related to the study at any time.

M- Agreement on being contacted in future research

- ☐ I agree to be contacted in future research studies
- ☐ I do not agree to be contacted in future research studies

N- Signature of the investigator

I have reviewed, in detail, the informed consent document for this research study with

____
(name of patient)
the purpose of the study and its risks and benefits.
I have answered all the patient's questions clearly. I will inform the participant in case of any changes to the research

____________________ ____________________
Name of Investigator or designee **Signature**

____
Date & Time

O- Signature of the participant:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. Joseph Ghafari at (01) 350 000 ext. 5707 or any of his/her designee involved in the study in case of any questions.
If I felt that my questions have not been answered, I contact the Institutional Review Board for human rights at (01) 350 000 ext. 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time without loss of benefits, even after signing this form, and it will not affect my care. I know that I will receive a copy of this signed informed consent.

____________________ ____________________
Name of participant **Age (Above 18 years)**

____________ ____________________
Signature **Date & Time**

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Name of the legal representative **Relation**

Signature **Date & Time**

Name of the witness

Signature **Date & Time**

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أحرف القاصر:

Institutional Review Board
American University of Beirut
Faculty of Medicine
Bliss Street
Beirut, Lebanon
Tel: (01) 350-000 ext. 5445

الموافقة للاشتراك في البحث العلمي

الراشدين

محضر رقم: IRB.ID (BIO-2019-0464)

إسم الباحث: د. جوزف غفري

الباحثون المساعدون: د. انطوني مكاري
د. جوزفين بواري

عنوان البحث: الارتباط بين الجينات المسبّبة بخلل في عدد الأسنان سواء نقصان او زيادة لدى سكان منطقة شرق البحر المتوسط.

مكان إجراء البحث: الجامعة الاميريكية في بيروت
شارع بلس
بيروت – لبنان
قسم تقويم الأسنان و الفكين

هاتف: 000 350-01- مقسم : 5707

أنت مدعو للانضمام الى الدّراسة:

1. إذا كنت مريضاً تتلقى العلاج في قسمنا: خلال موعدك الطبّي سيشرح لك طبيبك المعالج أهداف الدراسة، اجرأتها، و فوائدها/ مخاطرها، و ستسألك/ يسألك إذا كنت ترغب في المشاركة فيها. إذا وافقت، سيعرفك طبيبك على منسّقة الدراسة (د. جوزفين بواري) و سيُطلَبُ منك الإمضاء على إستمارة الموافقة هذه. بعد إمضائها، ستُسأل عن تاريخ عائلتك، بما في ذلك حالة نقصان أو زيادة في عدد الأسنان لأفراد آخرين من عائلتك. كما و سيتمّ اعطاؤك نشرات لتُوزعها على أقربائك المقرّبين الذين قد يستفدون، بحسب اعتقادك، من هذه الدّراسة.
2. إذا كنت من أقرباء المريض الذي يتلقّى العلاج في قسمنا، و كنت قد أخذت النشرة منه/ها و قرأتها.
3. إذا كنت والد/والدة المريض لدينا و قد شرحت لك/لكِ المنسّقة عن الدّراسة ككلّ.

لذلك ، من أجل تحديد الجينات المسؤولة والعامل الوراثي ، ستكون هناك حاجة إلى مزيد من الاختبارات.
نرجو منك قراءة المعلومات الواردة بدقّةٍ و بإمكانك طلب إيضاحات او معلومات إضافية عن أي شيء مذكور في هذه الاستمارة أو عن هذه الدّراسة ككلّ من طبيبك.

أ. وصف البحث العلمي و هدفه و تفسير مجرياته:

أهداف الدّراسة:

إننا نحاول معرفة المزيد حول خلل الجينات المعينة في تكوين و نموّ الاسنان الخاص بك ممّا قد يؤثّر على نموّ الفك لديك.

1. توضيح أساليب الوراثة العائليّة لحالة نقصان أو زيادة في عدد الأسنان لدى سكّان منطقة شرق البحر المتوسط و بالأخصّ سكّان العرب.
2. استكشاف تلازم حالتي نقصان و زيادة فيعدد الأسنان لدى العائلة الواحدة.

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3. مقارنة التكوين الجيني بين العائلات التي تشمل أفراد ذو نقصان في عدد الأسنان فقط و العائلات التي تشمل الحالتين ضمن أفرادها.

ماذا تشمل هذه الدّراسة؟

1. ستشمل الدراسة الأسر المعروف أن لديها أفراد لديهم خلل في عدد الأسنان. تسجيلك ليس إلزاميًا.
2. إذا قمت بالمشاركة، ستلتزم في اتّباع الخطوات التالية:

الخطوة	الإجراءات
1	- سيتم ملء التاريخ الطبي: التركيبة السكانية (الجنس ، تاريخ الميلاد ، العمر ، والأصل العائلي) ، الحالة الصحية. - سيعاين طبيب تقويم الأسنان أسنانك وسيأخذ صورة بالأشعة السينيّة. - سيتمّ وضعك في جهاز الفحص بالأشعة السينيّة الذي سيتحرّك حول وجهك لمدّة دقيقة. - في حال كان لديك صورة أشعة سابقة مأخوذة في غضون سنة واحدة ، فلن تكون هناك حاجة إلى صورة أشعة أخرى. - ستظهر صورة للجزء السفلي من وجهك على الشاشة ، تظهر أسنانك والتراكيب المحيطة بها. - يتم ذلك لحساب جميع أسنانك (المبزوغة أو غير المبزوغة بعد) وتقييم موقعها. - حتّى إذا كنت لا ترغب في المشاركة في الدّراسة البحثيّة، إلاّ أنّ الأشعة السينيّة هذه قد تلزمك في حال قررت الخضوع لعلاج تقويم الأسنان. في حال وجود اكتشاف غير طبيعيّ على الأشعة السينيّة، سيتمّ إجراء المزيد من التحقيقات وفقًا لذلك. ستستفيد من تشخيص مجانيّ وسيتمّ اقتراح العلاج المناسب. إذا كنت (مريض/ ة) في قسمنا، فلدينا السجلاّت المذكورة أعلاه، لن نقوم بتكرارها إلا إذا قد مضى وقت طويل ممّا يتطلّب تحديثها. - لا يمكنكِ المشاركة في هذه الدّراسة إذا كنت حاملاً . إذا كنت قادرة على الحمل، سيتمّ إجراء اختبار الحمل قبل أن تتعرضي لأيّ إشعاع. ينبغي أن تخبرينا إذا ثمّة احتمال أن تكوني قد حملت في غضون الأيّام الأربعة عشرة الماضية، وذلك لأنّه لا يمكن الاعتماد على اختبار الحمل خلال تلك الفترة. - عقب تحليل الأشعة السينيّة، سيتمّ رسم شجرة النسب (شكل من أشكال شجرة العائلة) لمعرفة من هم الأفراد في الأسرة المصابين بحالة نقصان أو زيادة في عدد الأسنان.
2	-ستسحب ممرضة أو طبيب ٥ سنتيمتر مكّعب من الدّم من ذراعك باستخدام إبرة نظيفة. سيتمّ ذلك في مستشفى الجامعة الأميريكيّة. سيتم اتباع قواعد ولوائح الطب المخبري في الجامعة الأمريكية في بيروت. - سيتمّ تخزين الحمض النووي الخاص بك في أنابيب مشفرة بدرجة حرارة مئوية ٨٠ تحت الصّفر في المنشآت الأساسيّة في الجامعة الأميريكيّة في بيروت، حيث سيتمّ تنفيذ الجيل القادم من تكنولوجيّات ترتيب الحمض النوويّ. سيتمّ جمع البيانات في الجامعة الأميريكيّة في بيروت وتحليلها بعد ذلك.

سيتطلّب منك أن تقوم بزيارة AUBMC مرّة واحدة (٤٥ دقيقة) أو مرّتين (٣٠ دقيقة +١٥ دقيقة)، الزيارة الأولى لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء إستمارة الموافقة هذه، ولمعاينة أسنانك و أخذ صورة بالأشعة السينيةُ الأماميةُ لتحديد عدد الأسنان. الزيارة الثانية لنأخُذ ٥ سنتيمتر مُكعب من دمك.

ب. عدد الأشخاص الذين سيتمذ تسجيلهم في الدّراسة:

سيتمّ تسجيل عدد تقريبي من 30 فرد متأثربحالة نقصان أو زيادة فيعدد الأسنان.

ت. ما هي التأثيرات السلبيّة لهذه الدّراسة ؟

المخاطر	الإجراءات
خطرالإشعاع	على الرّغم من أنّه ليس هناك أيّ آثار ضارّة مُثبتة تنتج عن مستويات الإشعاع التي ستتعرّض لها أثناء هذه الدّراسة، إلاّ أنّه لا يمكن استبعاد آثار طويلة الأمد على صحّتك. الجرعة الفعّالة لصورة واحدة هي١.١ ميلي ريم فقط ،وهي نسبة ضئيلة مقارنةً بمعدّل الجرعة السنويّة التي يتلقّاها الشخص من الإشعاع البيئي ما يقارب ٣٠٠ ميلي ريم.
المرتبطة	قد تشمل هذه المخاطر الكدمات، ألم موضعي، ورم دمويّ، واحتمال ضئيل للإلتهاب أو الإغماء. سيتمّ

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بسحب الدم	سحب ذلك باستخدام إبرة نظيفة من قبل ممرّضة متخصّصة أو طبيب في المستشفى وبالتّالي، هذا الخطر ضئيل.

للمزيد من المعلومات حول هذه المخاطر، إسأل الطبيب المسؤول عن الدّراسة. يرجى ملاحظة أنّه قد تكون هناك مخاطر غير متوقّعة.

ث. ما هي الفوائد التي قد تنتج عن هذه الدّراسة ؟

ليس ثمّة أي فوائد مباشرة لك من هذه الدّراسة. إلاّ أنّها تقدّم بعض الفوائد للفئة التي تنتمي إليها وكذلك للمجتمع والطبّ والعلم ككلّ.
ستسمح لنا هذه الدراسة بفهم أفضل للجينات المحددة التي تساهم في الاختلاف في خطر الإصابة بنقصان أو زيادة عدد الأسنان. ويمكن أن تؤدّي هذه المعرفة إلى تقدير أفضل لقابليّة حصول هذه الحالة وكذلك إلى فهم أفضل للآليّات الوراثيّة التي تؤثّر على نمو الأسنان. قد يؤدّي التنبّؤ المبكر للحالة إلى :

- العلاج المبكر سيحافظ على حجم العظم وكثافته وبالتالي تقليل الحاجة لاحقاً إلى زرع العظم وزرعات الأسنان في المنطقة المصابة. في حالة زيادة الأسنان ، فإن العلاج المبكر سيساعد في قرار خلع الأسنان.
- الأهم من ذلك ، قد تؤدي التدخلات المبكرة إلى القضاء على أو على الأقل تخفيف شدة جراحة تقويم الفكين اللاحقة عندما يكون صغر الفك العلوي أو السفلي بسبب نقص نمو العظام الذي يمليه نمو الأسنان.

يرجى ملاحظة أن رفض المشاركة لن يؤدّي إلى خسارة الفوائد.

ج. هل هناك طرق بديلة للوصول إلى الهدف المرجو؟

هذه ليست دراسة مخصصّة للعلاج، فالوسيلة الوحيدة البديلة هي عدم مشاركتك في هذه الدّراسة.

ح. هل يمكن اخراجك من الدّراسة بدون موافقتك؟

لن يتم اخراجك من الدّراسة من قبل الفريق المختص بالدّراسة من دون موافقتك. غير أنّه يحقّ للباحث الرئيسيّ إنهاء مشاركتكم فيالدراسة في أيّ وقت.

خ. السرّية

فـي حـال وافقـت علـى المشـاركة فـي هـذه الدّراسـة، سـيبقى اسـمك طـي الكتمـان . لـن يسـمح لأي شـخص، مـا لـم يـنصّ القـانون علـى ذلـك، حـقّ الإطـلاع علـى ملفـك الطبـي باسـتثناء الطبيـب المسـؤول عـن الدّراسـة ومعاونيـه، ولجـان الأخـلاق المهنيّـة المسـتقلة، و مفتشـين مـن الإدارات الحكوميّـة المنظمة.
سـتؤخذ الصـورة بالأشـعّة فـي قسـم تقـويم الأسـنان وتأهيـل الفكّـين فـي المركـز الطبّـي فـي الجامعـة الأميركيـة فـي بيـروت وتحفـظ فـي بنـك الصّـور الشـعاعيّة (CLINIVIEW)الـذي تـم احـداثها وضـمّها فـي البرنـامج الشّـعاعي المطـابق و سـيتمّ وضـعها فـي ملـف رقمـيّ منفصـل علـى كومبيـوتر فـي قسمنا، وسيتمّ تطبيق رموز بحثيّة لا يمكن لأحد اللّجوء إلى الملف إلا أعضاء فريق البحث وحسب.
سـيتمّ تـرقيم عيّنـات الـدم وترميزهـا وتخزينهـا فـي مكـان آمـن حتّـى نهايـة الدّراسـة. سـيتمّ الاحتفـاظ بجميـع عيّنـات الـدمّ ومشـتقّاتها إذا أردت المشـاركة فـي دراسـات جينيّـة أخـرى. وإلاّ، سـيتمّ الـتخلصّ منهـا فـي نهاية الدّراسة.

بنـاءً علـى طلبـك، سـيتم الكشـف عـن النتـائج الشخصـيّة لـك ولأفـراد عائلتـك بمجـرد الانتهاء من الدّراسة التاريخ المتوقع: (نيسان 2021)

- ☐ أريد أن أبلّغ بنتائج الدّراسة.
- ☐ لا أريد أن أبلّغ بنتائج الدّراسة.

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أحرف القاصر:

لا أرغب في معرفة نتائج الدّراسة أو الاختبار الذي تمّ ، وأنا على يقين بما يلي:

- لقد تمّ إطلاعي جيّدًا من خلال هذه الموافقة وأدرك تمامًا أن بعض هذه الاختبارات الجينيّة قد تسفر عن معلومات أو نتائج يمكن أن تؤثّر عليّ أو على أي من أفراد أسرتي أو نسلي في المستقبل.
- لقد تبلّغت أنّه بإمكاني تغيير رأيي ، والاتصال بالمحقّق الرئيسي أو بفريق البحث لتحديثهم بقراري في أي وقت و لغاية ٣ سنوات من إغلاق الدّراسة.

سيتمّ إعادة نتائج الجينات المتعلّقة بالحالة فقط لأغراض البحث.

د. أبحاث مستقبليّة:

أسمح باستخدام موادي الجينيّة (الدمّ، اللّعاب، الحمض النوويّ) للدّراسة المقترحة. أحدّد استخدام العيّنات بالطريقة التالية (يرجى التحقّق من واحد فقط مما يلي):

أ. أسمح بالاتصال للحصول على إذن لإجراء المزيد من الدّراسات على العيّنات.
أو
ب. لا أسمح باستخدام عيّناتي البيولوجيّة لإجراء المزيد من الدراسات.
أو
ت. أسمح باستخدام موادي البيولوجيّة من دون امكانيّة ربط العيّنات بالفرد في دراسات أخرى دون اتصال.

ذ. ماذا لو حصل لي أي عارض سلبي؟
إن المركز الطبّي في الجامعة الأميركيّة في بيروت سوف يغطّي تكاليف العلاج في المركز للعوارض الطبّية السلبيّة الناجمة مباشرة عن الأدوية و/أو الإجراءات الطبيّة الخاصّة بهذه الدّراسة البحثيّة. فيما عدا ذلك، لن يقوم المركز الطبّي بتغطية تكاليف العناية الطبيّة لأية حالة أو مشكلة مرضيّة. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جدًا .

ر. هل هناك تكاليف من خلال المشاركة في هذه الدّراسة؟

ليس ثمّة أيّ تكاليف مرتبطة بالمشاركة. ستغطي الدّراسة تكاليف الاجراءات المطلوبة لتحقيق أهدافها مما تتضمن: سحب الدمّ، التحليل الجيني والأشعّة السينيّة التي ستؤخذ لك و لأيّ فرد قد ينضمّ للدراسة بالإضافة إلى كشف سريري من أجل التحقّق إن كان علاج تقويم الأسنان لازمًا. لن تتمّ تغطية أيّ تكاليف أخرى.

ز. هل ستتقاضى أي مردود مالي مقابل المشاركة في الدّراسة؟
لن تحصل على أيّ مردود مالي مقابل مشاركتك في الدّراسة.

س. ما هي حقوقك كموضوع للبحث؟

مشاركتك في الدّراسة أمرٌ طوعي. لست مجبراً على المشاركة في الدّراسة إذا لم ترغب في ذلك، كما أنّه يمكنك الإنسحاب من الدّراسة في أيّ وقت. قرارك هذا لن يؤدّي إلى أي عقوبة أو خسارة المزايا التي تملكها الآن. إذا كان لديك أسئلة تتعلق بحقوقك، يمكنك الاتصال ب:
لجنة الأخلاقيّات على ٠١/٣٥٠٠٠٠ مقسم ٥٤٤٥
سيتمّ اعلامك عن أيّ معلومات جديدة قد تؤثّر على صحتّك أو على رغبتك في البقاء في هذه الدّراسة .

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يمكنك تغيير رأيك في أي وقت و هذا حتما لن يؤ ثر على اهتمام الأطباء بك. بامكانك طرح الأسئلة المتعلّقة بالدّراسة متى شئت.

ش. الإتفاق على التواصل من أجل أبحاث أخرى مستقبلية:

- ☐ أوافق على أن يتمّ الاتصال بي من أجل الدّراسات البحثية المستقبليّة.
- ☐ أنا لا أوافق على أن يتمّ الاتصال بي من أجل الدّراسات البحثية المستقبليّة.

ص. موافقة الباحث:

لقد استعرضت، بالتفصيل، وثيقة الموافقة المسبقة لهذه الدّراسة البحثيّة مع

____

(اسم: _المريض، _الممثل القانوني، _أو والد، والدة / وصي)، الغرض من الدّراسة، مخاطرها وفوائدها.
لقد أجبت على كل أسئلة المريض بوضوح. سأبلغ المشارك في حال طرأت أي تغييرات على البحث.

______________________ ______________________

اسم المحقق أو من ينوب عنه التوقيع

____

التاريخ و السّاعة

ض. موافقة المشترك:

لقد قرأت وفهمت كل جوانب الدّراسة البحثية وقد تمّت الإجابة على جميع أسئلتي. وأنا أوافق طوعًا على المشاركة في هذه الدراسة البحثية وأعلم أنه بإمكاني الاتصال بالدكتور جوزف غفري على الرقم 01350000 مقسم 5707 أو بمن ينوب عنه في الدّراسة في حال لدينا أي سؤال.
إذا شعرت أنهّ لم يتم الاجابة على أسئلتي، يمكنني الاتصال بلجنة الأخلاقيات على الرقم 01350000 مقسم5445 وأنا أفهم أنني حر في سحب هذه الموافقة والتوقف عن المشاركة في هذا المشروع في أي وقت، حتى بعد التوقيع على هذا النموذج، وأن ذلك لن يؤثّر على إهتمام الأطباء بي .كما وأنه يحق للباحث الرئيسي إنهاء مشاركتي فالدّراسة في أي وقت. أعلم أنني سأحصل على نسخة من هذه الموافقة الموقعة.

______________________ ______________________

اسم المشترك(ة) السن (راشد)

______________________ ______________________

توقيع المشترك(ة) التاريخ و الساعة

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أحرف القاصر:

اسم الممثل القانوني	القرابة
التوقيع	التاريخ و الساعة
اسم الشاهد	
التوقيع	التاريخ و الساعة

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VII. PARENTAL CONSENT FORM

Institutional Review Board
American University of Beirut
Faculty of Medicine
Bliss Street
Beirut, Lebanon
Tel: (01) 350-000 ext. 5445

PARENTAL ASSENT FORM

(Parents)

Protocol Number: IRB.ID (BIO-2019-0464)

Principal Investigator(s): Dr Joseph Ghafari

Co- Investigators: Dr Anthony Macari
Dr Josephine Boueiri

Project Title: Genetic basis of familial oligo-hypo-hyperdontia in eastern Mediterranean families

Address: American University of Beirut Medical Center
Bliss Street
Beirut – Lebanon

Site where the study will be conducted: Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

Phone: (01) 350 000 ext: 5707

Your child is being asked to join the study because:

1- He/She is a patient treated in our division: during his/her orthodontic appointment, you will be approached first by his/her primary physician who will explain in detail and ask you if you are willing to participate in the research project. If you agree, your child and yourself will be introduced to the research coordinator (Dr. Josephine Boueri) to sign this consent form. After signing it, you will be asked questions about your family histories, including affection status of other individuals in your family. You will also be given flyers to distribute amongst your close relatives whom you think could benefit from this project.

2- Or if you are a relative of a patient treated in our division who read flyer handed to you by him/her.

Therefore, in order to determine the responsible genes and the familial transmission some further tests will be needed.

Please take time to read the following information carefully before you decide whether you want to take part in this study or not. Feel free to ask the doctor if you need more information or clarification about what is stated in this form and the study as a whole.

A- Purpose of the research study and overview of participation:

The aim of the study:

We are trying to find out more about the genes involved in the development of the teeth and the if the alteration of those genes will lead to missing (decreased number) and/or supernumerary teeth (increased number).

1. Clarify the Inheritance pattern of missing teeth (decreased number)/supernumerary (increased number) in Mediterranean population especially Arab population.
2. Explore the occurrence of missing teeth/supernumerary in one family in Mediterranean population.
3. Compare between the expression of the genes in families having only missing teeth and families having same time occurrence of missing/supernumerary of teeth.

What is involved in the study?

1. The study will include families who have members with missing teeth/ dental excess. Your child's enrolment is not obligatory.
2. If your child participates, he/she will commit to go through the following steps:

Steps	Procedures
1	- Medical history will be filled out: demographics (gender, date of birth, age, and family origin), health status. - The orthodontist will look at your child's teeth and will take one panoramic x-ray. - Your child will be positioned in the x-ray machine, which will move next to his/her face, for about 1 minute. - In case your child had a previous x-ray taken within a period of 1 year, no further x-ray will be required. - An image of the lower part of your child's face will appear on the screen, showing his/her teeth and surrounding structures. - This is done to count all the teeth (erupted or not yet) and assess their position. - Even if your child doesn't want to participate in the research study, this x-ray would be needed if you decide for them to undergo an orthodontic evaluation and treatment. In case an abnormal finding was present on the X-ray, further investigation will be performed accordingly. Your child will benefit from free diagnosis and treatment will be suggested. If your child was a patient in our department, and we have the above records, we will not repeat them unless long time has passed which requires updating them. - After the x-ray analysis, a family pedigree will be drawn to evaluate the affected members.
2	- Blood withdrawal (5cc) will be done at the hospital (AUBMC), by a specialized nurse or physician. The AUBMC Laboratory Medicine rules and regulations will be followed. - Your child's DNA will be stored in encoded tubes at 80°C at key facilities at the American University of Beirut, where the next generation of DNA sequencing technologies will be implemented. The data will be collected at the American University of Beirut and analyzed after that.

This will require you and your child to pass by AUBMC for one (45mins) or two (30mins + 15mins) visits: the first one to explain the project in detail, sign this consent form, have a clinical examination and a panoramic x-ray, and the second visit to have 5cc of your child blood collected.

B- Number of subjects to be enrolled

An approximate number of at least 30 individuals will be enrolled in the study.

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C- What are the risks of the study?

Risks	Procedures
Radiation risk	Although there are no proven harmful effects from irradiation levels that your child will be exposed to during this study, long-term effects on your child's health cannot be ruled out with certainty. The effective dose of a single panoramic x-ray is only 1.1mrem, which is minor compared to the average annual dose that a person receives from environmental radiation (~300 mrem).
Associated with blood withdrawal	Such risks may include bruising, local pain, hematoma, slight possibility of infection or fainting. Blood will be withdrawn by a specialized nurse or physician at the hospital (AUBMC), using a clean needle; thus, this risk is minimal.

For more information about these risks and side effects, ask your study doctor and please note that there may be unforeseeable risks.

D- Are there benefits to taking part in the study?

There is no direct benefit to your child from this study. However, it has some benefits to the class to which your child belongs and to the society and science/ medicine as a whole.
This study will allow us to better understand the specific genes contributing to variation in the risk for dental agenesis and dental excess. This knowledge can lead to a better estimation of the susceptibility to this condition and a better comprehension of the genetic mechanisms that may influence the process of dental development. Early prediction of the condition would lead to:

- Earlier treatment in case of dental agenesis will preserve the bone volume and density thus reducing the later need for bone graft and implants in the affected area. In case of dental excess, early treatment will help in the extraction decision.
- More importantly, earlier interventions may eliminate or at least reduce the severity of a later orthognathic surgery when mandibular or maxillary micrognathism is due to lack of bone development which is dictated by dental development.

Please note that refusal of participation will not lead to a loss of benefits.

E- What other options are there?

This is not a treatment study so the only alternative is not to participate in the study.

F- Confidentiality

If you agree to participate in this study, your child's name will remain confidential. No person, unless the law provides for this, will have the right to see their medical files except for the doctor responsible for the study and his assistants, independent professional ethics committees, and inspectors from the organized government departments.
The radiograph will be taken in the division of Orhtodontics and Dentofacial Orthopedics, AUBMC and stored in the bank of radiographs generated and housed in the corresponding radiologic software

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(CLINIVIEW). It will be placed in a separate digital folder on a computer in our division and codes will be applied so that the folder can be accessed only by the research group members.
The blood samples will be numbered, coded and stored in a safe place until the end of the study. All blood samples and their derivatives will be kept if you opt to participate in further genetic studies. Otherwise, they will be trashed at the end of the study.

Depending on your request, your child's individual results will be disclosed to you and to your family members as soon as the study is completed (approximate date: April 2021).
☐ I want to be informed of the results of the study
☐ I don't want to be informed of the results of the study

I do not wish to know the results of the study or test done, and I am made aware of the following:

- I was well informed through this consent and I fully understand that some of these genetic tests may yield information or results that can affect my child or any of their family members or progenies in the future.
- I was well informed that my child can change their mind, and I can contact the Principal Investigator or research team to update them on our decision at any time up to 3 years of study closure.

Only results of genes related to the condition will be relayed back for research purposes.

G- Use of your child's coded samples for future research:

I permit coded use of my child's genetic materials (blood, DNA) for the proposed study. I specify the use of the samples in the following manner (please check only one of the following):

a. I permit further contact to seek permission to do further studies on the samples.
b. I do not allow the use of my child's biological samples for further studies.
c. I permit anonymized (samples cannot be linked to subject) the use of my child's biological materials for other studies without contact.

H- What if you child is injured in the study?

AUBMC will cover the cost of treating, on its premises, medical adverse events resulting directly from the medication and/or medical procedures of this research study. Otherwise, it will not cover for the costs of medical care for any medical condition or issue. But you should know the risk is minimal.

I- What are the costs?

There are no costs associated with participation. The study will cover the costs of the procedures done for its purpose which includes**:** blood withdrawal and genetic analysis and the X-ray that is taken for your child and for every individual who will be enrolled in the study, along with an initial consultation for the need of an orthodontic treatment. All other costs will not be covered.

J- Will your child get paid to be in this study?

Your child will not be paid to participate in this study.

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K- Agreement on being contacted in future research

- ☐ I agree to have my child contacted in future research studies
- ☐ I do not agree to have my child contacted in future research studies

L- Signature of the investigator:

I have reviewed, in detail, the informed consent document for this research study with

____

(name of patient, legal representative, or parent/guardian)
the purpose of the study and its risks and benefits.
I have answered all the patient's questions clearly. I will inform the participant in case of any changes to the research

___________________________ ___________________________
Name of Investigator or designee **Signature**

____
Date & Time

M-Signature of the participant

We have read and understood all aspects of the research study and all our questions have been answered. We voluntarily agree to have our child as part of this research study and we know that we can contact Dr. Joseph Ghafari at (01) 350 000 ext. 5707 or any of his designee involved in the study in case of any questions.
If we felt that our questions have not been answered, we can contact the Institutional Review Board for human rights at (01) 350 000 ext. 5445. We understand that we are free to withdraw this consent and discontinue participation in this project at any time without loss of our child's benefits, even after signing this form, and it will not affect our child's care. We know that we will receive a copy of this signed informed consent.

___________________________ ___________________________
Name of Dad **Signature**

____
Date & Time

___________________________ ___________________________
Name of Mother **Signature**

____
Date & Time

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Name of the witness

Signature **Date & Time**

Institutional Review Board
American University of Beirut

1 9 JUL 2020

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أحرف القاصر:

لجنة الأخلاقيات
الجامعة الأميركية في بيروت
كلية الطب
شارع بلس
بيروت، لبنان
هاتف: 350000 -(01)

الموافقة للإشتراك في البحث العلمي للمشترك القاصر
الأهل

محضر رقم: IRB.ID (BIO-2019-0464)

إسم الباحث: د. جوزف غفري

الباحثون المساعدون: د. انطوني مكاري
د. جوزفين بواري

عنوان البحث: الإرتباط بين الجينات المسببّة بخلل في عدد الأسنان سواء نقصان او زيادة لدى سكّان منطقة شرق البحر المتوسط

مكان إجراء البحث: الجامعة الاميركية في بيروت
شارع بلس
بيروت – لبنان
قسم تقويم الأسنان و الفكين

هاتف: 000 350-01- مقسم : 5707

طفلك مدعو للانضمام الى الدّراسة:

1- إذا كان طفلك يتلقى العلاج في قسمنا: خلال الموعد الطبّي سيشرح لكم طبيبه المعالج أهداف الدراسة، اجراءاها، و فوائدها/ مخاطرها، و ستسألكم إذا كنتم ترغبون في المشاركة فيها. إذا وافقتم، سيعرفكم طبيبه على منسّقة الدراسة (د. جوزيفين بواري) و سيُطلَبُ منكم الإمضاء على إستمارة الموافقة هذه. بعد إمضائها، ستُسألون عن تاريخ عائلتكم، بما في ذلك حالة نقصان او زيادة في عدد الأسنان لأفراد آخرين من عائلتكم. كما و سيتمّ اعطاؤكم نشرات لتوزيعها على أقربائكم المقرّبين الذين قد يستفيدون، بحسب اعتقادكم، من هذه الدّراسة.

2- إذا كنتم من أقرباء لمريض يتلقى العلاج في قسمنا، وكنتم قد أخذتم النشرة منه وقرأتوها.

لذلك ، من أجل تحديد الجينات المسؤولة والعامل الوراثي ، ستكون هناك حاجة إلى مزيد من الاختبارات.

نرجو منكم قراءة المعلومات الواردة بدقّةٍ قبل أن تقرّروا الموافقة على مشاركة طفلكم في هذه الدّراسة أم لا، كما بإمكانكم طلب إيضاحات او معلومات إضافيّة عن أي شيء مذكور في هذه الا ستمارة أو عن هذه الدّراسة ككلّ من طبيبه.

أ. وصف البحث العلمي و هدفه و تفسير مجرياته:

أهدف الدراسة:

إنّنا نحاول معرفة المزيد حول خلل الجينات المعينة في تكوين و نموّ الأسنان الخاصّ بطفلك ممّا قد يؤثّر على نموّ الفكّ لديه.

1- توضيح النمط الوراثي عند حالات نقصان أو زيادة في عدد الأسنان لدى سكّان البحر المتوسط و بالأخص السكّان العرب.

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2- استكشاف تداول حالتي نقصان و زيادة في عدد الأسنان ضمن العائلة الواحدة من سكان البحر المتوسط.
3- المقارنة بين التركيب الوراثي للعائلات اللّواتي لديهم حالة نقصان في الأسنان معزولةً أو العائلات اللّواتي لديهم الحالتين مندمجتين.

ماذا تشمل هذه الدّراسة؟

1- ستشمل الدّراسة الأسر المعروف أن لديها أفراد لديهم خلل في عدد الأسنان. تسجيل طفلكم ليس إلزاميا.
2- في حال موافقتكم، سيلتزم طفلكم باتباع الخطوات التّالية:

الخطوة	الإجراآت
1	- سيتم ملء التاريخ الطبي: التركيبة السكانية (الجنس ، تاريخ الميلاد ، العمر ، والأصل العائلي) ، الحالة الصحية. - سيعاين طبيب تقويم الأسنان أسنان طفلكم وسيأخذ صورة بالأشعة السينيّة - سيتم وضع طفلكم في جهاز الفحص بالأشعة السينيّة الذي سيتحّرك حول وجهه لمدّة دقيقة. - في حال كان لدى طفلكم صورة أشعة سابقة مأخوذة في غضون سنة واحدة ، فلن تكون هناك حاجة إلى صورة أشعة أخرى. - ستظهر صورة للجزء السفلي من وجهه على الشاشة ، تظهر أسنانه والتراكيب المحيطة بها. - يتم ذلك لحساب جميع أسنانه (المبزوغة أو غير المبزوغة بعد) وتقييم موقعها. - حتّى إذا كنتم لا ترغبون في المشاركة في الدّراسة البحثيّة، إلاّ أنّ الأشعة السينيّة هذه قد تلزم طفلكم في حال قررتم إخضاعه لعلاج تقويم الأسنان. في حال وجود اكتشاف أمر غير طبيعي على الأشعة السينيّة، سيتمّ إجراء المزيد من التحقيقات وفقًا لذلك. ستستفيدون من تشخيص مجاني وسيتم اقتراح العلاج المناسب. إذا كان طفلكم مريض في قسمنا، فلدينا السجلأت المذكورة أعلاه، لن نقوم بتكرارها إلا إذا قد مضى وقت طويل ممّا يتطلّب تحديثها. - عقب تحليل الأشعة السينيّة، سيتمّ رسم شجرة النسب (شكل من أشكال شجرة العائلة) لمعرفة من هم الأفراد في الأسرة المصابين بحالة نقصان أو زيادة في عدد الأسنان.
2	- ستسحب ممرضة أو طبيب ٥ سنتيمتر مكّعب من الدم من ذراع طفلكم باستخدام إبرة نظيفة. سيتمّ ذلك في مستشفى الجامعة الأميريكيّة. سيتم اتباع قواعد ولوائح الطب المخبري في الجامعة الأمريكية في بيروت. - سيتم تخزين الحمض النووي الخاص بطفلكم في أنابيب مشفرة بدرجة حرارة مئوية ٨٠ تحت الصّفر في المنشآت الأساسية في الجامعة الأميريكية في بيروت، حيث سيتمّ تنفيذ الجيل القادم من تكنولوجيّات ترتيب الحمض النووي. سيتمّ جمع البيانات في الجامعة الأميريكية في بيروت وتحليلها بعد ذلك.

سيتطلب منكم برفقة طفلكم أن تقوموا بزيارة AUBMC مرّة واحدة (٤٥دقيقة) أو مرّتين (٣٠دقيقة + ١٥ دقيقة)، الزيارة الأولى لنشرح لكم المشروع بالتفصيل، لتقوموا بإمضاء إستمارة الموافقة هذه ولمعاينة أسنان طفلكم وأخذ صورة بالأشعة السينيّة الأماميّة لتحديد عدد الأسنان. الزيارة الثّانية لنأخذ ٥ سنتيمتر مكّعب من دمه.

ب. عدد الأشخاص الذين سيتم تسجيلهم في الدراسة:

سيتمّ تسجيل عدد تقريبي من ٣٠ فردٍ متأثر بحالة نقصان أو زيادة في عدد الأسنان.

ت. ما هي مخاطر هذه الدراسة ؟

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أحرف القاصر:

المخاطر	الإجراءات
خطر الإشعاع	على الرّغم من أنّه ليس هناك أيّ آثار ضارّة مُثبتة تنتج عن مستويات الإشعاع التي سيتعرّض لها طفلكم أثناء هذه الدّراسة، إلاّ أنّه لا يمكن استبعاد آثار طويلة الأمد على صحّته. الجرعة الفعّالة لصورة واحدة هي١.١ ميلي ريم فقط ،وهي نسبة ضئيلة مقارنةً بمعدّل الجرعة السنويّة التي يتلقّاها الشخص من الإشعاع البيئي ما يقارب ٣٠٠ ميلي ريم.
المرتبطة بسحب الدم	قد تشمل هذه المخاطر الكدمات، ألم موضعي، ورم دمويّ، واحتمال ضئيل للإلتهاب أو الإغماء. سيتمّ سحب ذلك باستخدام إبرة نظيفة من قبل ممرّضة متخصّصة أو طبيب في المستشفى وبالتّالي، هذا الخطر ضئيل.

للمزيد من المعلومات حول هذه المخاطر، اسألوا الطّبيب المسؤول عن الدّراسة. يرجى ملاحظة أنّه قد تكون هناك مخاطر غير متوقّعة.

ث. هل هناك فوائد من المشاركة في الدراسة؟

ليس ثمّة أي فوائد مباشرة لطفلك من هذه الدّراسة . إلاّ أنّها تقدّم بعض الفوائد للفئة التي ينتمي إليها وكذلك للمجتمع والطبّ والعلم ككلّ.

هذه الدّراسة ستسمح لنا بأن نفهم أكثر الجينات المعنيّة التي تساهم في في الاختلاف في خطر الإصابة بنقصان أو زيادة عدد الأسنان. ويمكن أن تؤدّي هذه المعرفة إلى تقدير أفضل لقابليّة حصول هذه الحالة وكذلك إلى فهم أفضل للآليّات الوراثيّة التي تؤثّر على نمو الأسنان. قد يؤدّي التنبّؤ المبكر للحالة إلى :

- العلاج المبكر سيحافظ على حجم العظم وكثافته وبالتالي تقليل الحاجة لاحقاً إلى زرع العظم وزرعات الأسنان في المنطقة المصابة. في حالة زيادة الأسنان ، فإن العلاج المبكر سيساعد في قرار خلع الأسنان.
- الأهم من ذلك ، قد تؤدي التدخلات المبكرة إلى القضاء على أو على الأقل تخفيف شدة جراحة تقويم الفكين اللاحقة عندما يكون صغر الفك العلوي أو السفلي بسبب نقص نمو العظام الذي يمليه نمو الأسنان.

يرجى ملاحظة أن رفض المشاركة لن يؤدّي إلى خسارة الفوائد.

ج. هل هناك طرق بديلة للوصول إلى الهدف المرجو؟

هذه ليست دراسة مخصصّة للعلاج، فالوسيلة الوحيدة البديلة هي عدم مشاركة طفلك في هذه الدّراسة.

ح. السرّية

في حال وافقتم على المشاركة في هذه الدّراسة، سيبقى اسم طفلكم طي الكتمان . لن يسمح لأي شخص، ما لم ينصّ القانون على ذلك، حقّ الإطلاع على ملفه الطبّي باستثناء الطبيب المسؤول عن الدّراسة ومعاونيه، ولجان الأخلاق المهنية المستقلّة، و مفتشين من الإدارات الحكوميّة المنظّمة.

ستؤخذ الصورة بالأشعة في قسم تقويم الأسنان وتأهيل الفكّين في المركز الطبّي في الجامعة الأميركية في بيروت وتحفظ في بنك الصّور الشعاعية (CLINIVIEW)الذي تمّ احداثها وضعها في البرنامج الشّعاعي المطابق سيتمّ وضعها في ملفّ رقميّ منفصل على كومبيوتر في قسمنا، وسيتم تطبيق رموز بحيث لا يمكن لأحد اللّجوء إلى الملفّ إلا أعضاء فريق البحث وحسب.

سيتم ترقيم عينات الدم وترميزها وتخزينها في مكان آمن حتى نهاية الدراسة. سيتم الاحتفاظ بجميع عيّنات الدم ّومشتقّاتها إذا أردت المشاركة في دراسات جينية أخرى. وإلا، سيتم التخلصّ منها في نهاية الدّراسة.

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أحرف القاصر:

بنــاءً علــى طلــبكم، ســيتمّ الكشــف عــن النتــائج الشخصــيّة لكــم ولأفــراد عــائلتكم بمجــرد الانتهاء من الدّراسة في التّاريخ المتوقع: (نيسان 2021).

- ☐ **أريد أن أبلّغ بنتائج الدّراسة.**
- ☐ **لا أريد أن أبلّغ بنتائج الدّراسة.**

لا أرغب في معرفة نتائج الدّراسة أو الاختبار الذي تم ، وأنا على يقين بما يلي:

- لقــد تــمّ إطلاعنــا جيــدًا مــن خــلال هــذه الموافقــة وأدركنــا تمامًــا أن بعــض هــذه الاختبــارات الجينيــة قــد تســفر عــن معلومــات أو نتــائج يمكــن أن تــؤثر علــى طفلنــا أو علــى أي مــن أفــراد أسرتنا أو نسل طفلنا في المستقبل.
- لقــد تبلّغنــا أنّــه بإمكاننــا تغييــر رأينــا ، و بإمكانناالاتصــال بــالمحقق الرئيســي أو بفريــق البحــث لتحديثهم بقرارنا في أيّ وقت و لغاية ٣ سنوات من إغلاق الدّراسة.

سيتمّ إعادة نتائج الجينات المتعلّقة بالحالة فقط لأغراض البحث.

خ. أبحاث مستقبلية:

أسمح باستخدام مواد طفلي الجينية (الدمّ، اللّعاب، الحمض النووي) للدراسة المقترحة. أحدّد استخدام العّينات بالطّريقة التّالية (يرجى التحقّق من واحد فقط ممّا يلي):

أ. أسمح بالاتصال للحصول على إذن لإجراء المزيد من الدّراسات على عيّنات طفلي.

أو

ب. لا أسمح باستخدام عيّنات طفلي البيولوجيّة لإجراء المزيد من الدّراسات.

أو

ت. أسمح باستخدام مواد طفلي البيولوجيّة من دون امكانيةً ربط العيّنات بالفرد في دراسات أخرى دون اتصال.

د. ماذا لو حصل لطفلك عارض سلبي؟

إن المركــز الطبــي فــي الجامعــة الأمريكيــة فــي بيــروت ســوف يغطّــي تكــاليف العــلاج فــي المركــز للعــوارض الطبيّــة الســلبية الناجمــة مباشــرة عــن الأدويــة و/أو الإجــراءات الطبيّــة الخاصّــة بهــذه الدّراســة البحثيّــة. فيمــا عــدا ذلــك، لــن يقــوم المركــز الطبّــي بتغطيــة تكــاليف العنايــة الطبّيــة لأيّــة حالــة أو مشكلة مرضيّة. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جدًّا .

ذ. هل هناك تكاليف من خلال المشاركة في هذه الدّراسة؟

لــيس ثمّــة أي تكــاليف مرتبطــة بالمشــاركة. ســتغطّي الدّراســة تكــاليف الاجــراءات المطلوبــة لتحقيــق أهــدافها: ســحب الــدمّ، التحليــل الجينــي والأشــعّة الســينيّة التــي ســتؤخذ لطفلكــم و لأيّ فــرد قــد ينضــم للدّراســة بالإضــافة إلــى كشــف ســريري مــن أجــل التحقّــق إن كــان عــلاج تقــويم الأســنان ضــروريًا. لن تتمّ تغطية أي تكاليف أخرى.

ر. هل ستتقاضون أي مردود مالي مقابل مشاركة طفلكم في الدّراسة؟

لن تحصلوا على أي مردود مالي مقابل مشاركة طفلكم في الدّراسة.

ز. الإتفاق على التواصل من أجل أبحاث أخرى مستقبليّة:

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أحرف القاصر:

- ☐ أوافق على أن يتم"الاتصال بي من أجل أن يشارك طفلي في دراسات بحثية مستقبليّة.
- ☐ أنا لا أوافق على أن يتم الاتصال بي من أجل أن يشارك طفلي في دراسات بحثيّة مستقبليّة.

س. موافقة الباحث:

لقد استعرضت، بالتفصيل، وثيقة الموافقة المسبقة لهذه الدّراسة البحثيّة مع

____

(اسم: _المريض، _الممثل القانوني، _أو والد، والدة / وصي)، الغرض من الدّراسة، مخاطرها وفوائدها.
لقد أجبت على كل أسئلة المريض بوضوح. سأبلغ المشارك في حال طرأت أي تغييرات على البحث.

______________________   ______________________

اسم المحقق أو من ينوب عنها   التوقيع

____

التاريخ و السّاعة

ش. موافقة المشترك:

لقد قرأنا وفهمنا كل جوانب الدّراسة البحثية وقد تمّت الإجابة على جميع أسئلتنا .ونحن نوافق طوعا على السّماح لطفلنا بالمشاركة في هذه الدراسة البحثية ونعلم أنه يمكننا الاتصال بالدكتور جوزيف غفري على الرقم 01350000 مقسم 5707 أو بمن ينوب عنه في الدّراسة في حال لدينا أي سؤال.
إذا شعرنا أنهّ لم يتم الاجابة على أسئلتنا، يمكننا الاتصال بلجنة الأخلاقيات على الرقم 01350000 مقسم5445 ونحن نفهم أننا أحرار في سحب هذه الموافقة والتوقف عن المشاركة في هذا المشروع في أي وقت، حتى بعد التوقيع على هذا النموذج، وأن ذلك لن يؤثّر على رعاية طفلنا .كما وأنه يحق للباحث الرئيسي إنهاء مشاركة طفلنا فالدّراسة في أي وقت .نعلم أننا سنحصل على نسخة من هذه الموافقة الموقعة.

______________________   ______________________

اسم الوالد   توقيع الوالد

____

التاريخ و السّاعة

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اسم الوالدة   توقيع الوالدة

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التاريخ و السّاعة

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14 JUL 2020
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أحرف القاصر:

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اسم الشاهد

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التوقيع التاريخ و الساعة

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10 JUL 2020
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