AMERICAN UNIVERSITY OF BEIRUT

ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR MICROGNATHISM IN AN EASTERN MEDITERRANEAN POPULATION

by
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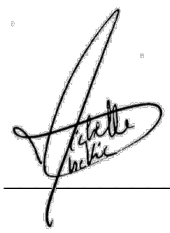

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"I can no other answer make but thanks, and thanks, and ever thanks"
William Shakespeare

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AN ABSTRACT OF THE THESIS OF

Michelle Rabih El-Chekie for Master of Science
Major: Orthodontics

Title: Association between genes and familial mandibular micrognathism in an Eastern Mediterranean population.

Background:

Mandibular micrognathism (MM) is characterized by an underdeveloped mandible leading to a chinless-type face and a convex profile which is characteristic of the Class II/division1 malocclusion. The etiology of MM is multifactorial, where environmental and genetic/familial factors interact over time, thus favoring the polygenic model of inheritance. Only one genome-wide family-based linkage has been carried out in the Hispanic population, exposing that data on the genetic determinants behind MM is nevertheless fragmentary.

Aims:

1. Explore the inheritance pattern and identify the candidate genes and loci involved in the development and familial transmission of MM in an Eastern Mediterranean population; 2. Evaluate the skeletal and dento-alveolar cephalometric characteristics of affected individuals compared to a sample of non-affected with normoclusion.

Methods:

Out of the 11 pedigrees of Eastern Mediterranean families which included probands affected with MM, 5 families (4 Lebanese and 1 Syrian/Jordanian) accepted to undergo a detailed data and biospecimen collection procedure. The diagnosis of the probands was based on a clinical and radiographic (lateral cephalogram) examination. 5cc of blood was collected from both affected and non-affected individuals (as control), and genomic DNA was isolated from blood cells to investigate protein-coding regions via whole exome sequencing (WES) performed on a NovaSeq6000 Illumina platform in Korea.

Results:

Most of the pedigrees suggest a Mendelian inheritance pattern and segregate in an autosomal-dominant manner, while one family (which underwent biospecimen) displayed an X-linked inheritance pattern of the trait. Pedigree analysis indicated an equal number of reported generations per family (n=3), an equal average number of reported affected males and females (n=2.36) per family and an equal number of families with males and females predominance (n=4). Averaged cephalometric

measurements on affected individuals from the 5 families confirmed a reduced mandibular length (mandibular micrognathism), normal maxillary length, a tendency to a hyperdivergent facial pattern, a skeletal Class II malocclusion underlined by an orthognathic maxilla and retrognathic mandible. This was accompanied by dento-alveolar compensations and an increased overjet. Genetic screening didn't show any aberration in the previously reported genes linked to ClassII/ division1, mandibular retrognathism or MM, but did point out to 8 potentially novel genes (GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7 and WNT6) that could be implicated in mandibular development and lead mainly to MM.

Conclusion:

This study reaped its novelty from its design as it is the first genetic study on large families with MM worldwide and more specifically in the Eastern Mediterranean population using NGS to better understand the variations and risks for MM. GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7 and WNT6 are novel genes discovered to be associated with familial MM, which emphasizes on the complexity of the trait . This exploration will help understand the variation in mandibular growth, potential prediction of the final manifestation of the growth pattern which would help in treatment planning by incorporating such information into clinical-decision making process.

CONTENTS

ILLUSTRATIONS

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ABBREVIATIONS

MM	Mandibular Micrognathism
NGS	Next Generation Sequencing
NHANES	National Health and Nutrition Examination Survey
US	United States
DNA	Deoxyribonucleic Acid
A	Adenine
T	Thymine
C	Cytosine
G	Guanine
mRNA	Messenger RNA
SNP	Single Nucleotide Polymorphism
INDEL	Insertion or Deletion
dNTPs	Deoxynucleotide Triphosphates
ddNTPs	Dideoxynucleotide Triphosphates
WGS	Whole Genome Sequencing
WES	Whole Exome Sequencing
Q	Quality score
MAF	Minor Allele Frequency
GC	Guanine-Cytosine
HGP	Human Genome Project
GWAS	Genome Wide Association Study
PCR	Polymerase Chain Reaction
OFD1	Orofacialgigital 1
GHR	Growth Hormone Receptor
NOG	Noggin
GHR	Growth Hormone Receptor
ACTN3	α-actinin-3
FGFR2	Fibroblast Growth Factor 2
SNAI2	Snail Family Transcriptional Repressor 2
MATN1	Matrilin-1
MYO1H	Myosin 1H
CBC	Capillary Blood Collection
AW	Wash Buffer
AUBMC	American University of Beirut Medical Center
OJ	Overjet
H	True horizontal
S	Sella turcica
N	Nasion
Ba	Basion
Ar	Articulare
Po	Porion
Co	Condylion
Go	Gonion
Or	Orbitale

Gn	Gnathion
Pog	Pogonion
Me	Menton
ANS	Anterior nasal spine
PNS	Posterior nasal spine
Ptm	Pterygomaxillary point
D	Center of the symphysis
LFH	Lower face height
TFH	Total face height
MP	Mandibular pane
PP	Palatal plane
OB	Overbite
U1	Most proclined maxillary incisor
U6	Maxillary first molar
L1	Most proclined mandibular incisor
L6	Mandibular first molar
VCF	Variant Call Format
Bp	Base pairs
Mbp	Mega base pair

CHAPTER I
INTRODUCTION

A. Background

Mandibular micrognathism (MM) is characterized by an underdeveloped mandible leading to a chinless-type face and a convex profile. This reduction in mandibular length results in the development of a positive overjet. MM is one of the characteristic features of the Cl II, division 1 malocclusion.

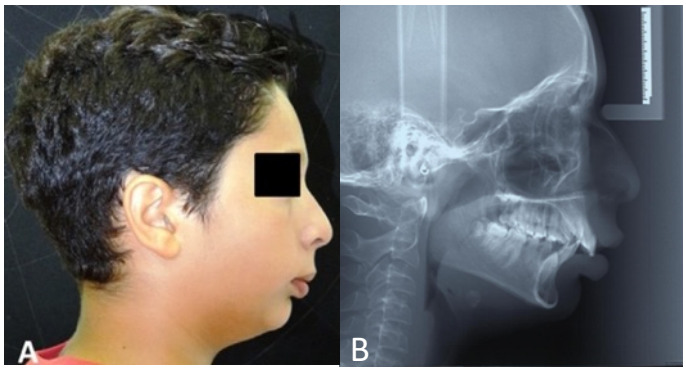


Figure I-1: A) Mandibular micrognathism on profile view and B) on lateral cephalogram

Among the various reports on Cl II, division 1 malocclusion, mandibular retrognathism is thought to be the most common phenotypic feature (Anderson & Popovich, 1983; Kerr, 1987; McNamara, 1981; Varrela, 1998). Further skeletal findings revealed the same mandibular skeletal pattern accompanied by short mandibular length(Baccetti, 1997; Drelich, 1948; Harris, Kowalski, & Walker, 1975). Noteworthy is the imperative differentiation between mandibular micrognathism and mandibular retrognathism, where the mandible is well-developed but in a posterior position relative

to the cranial base(Henry, 1957). During the diagnosis, it is crucial to evaluate the components of this specific phenotype to end up with a targeted treatment plan that achieves the best functional, esthetic, and stable results (Ghafari & Macari, 2014).

MM may cause psychosocial problems because of facial appearance, alter oral function (mastication, speech, etc.), and is found to be linked with dental trauma to the protruding maxillary incisors (W. R. Proffit, Henry W. Fields, and David M. Sarver. , 2014). The receding mandible with a short mandibular body length has been furthermore associated with obstructive sleep apnea and other respiratory problems due to the impingement on the airway space by the posterior displacement of the soft tissue attached to the mandible (Temani, Jain, Rathee, & Temani, 2016).

Behind the soft tissue drape of the patient's face is a dynamic process that can be influenced by our heritage and altered by our environment. According to the study by Lundstrom (1984), investigations published prior to that article have suggested that about 40% of common anomalies in tooth position and the relationship between maxillary and mandibular dental arches are due to genetic differences between individuals. Looking at the importance of environmental vs. inherited factors in the etiology of malocclusions, it was suggested that urbanization (and evolution) influence malocclusions, making them more severe. The evolutionary factors involved are a decrease in the size of the jaws, size, and number of the teeth. We have no control over these evolutionary factors (as well as the hereditary factors), whereas the environmental factors can often be eliminated through preventive or interceptive treatment at the appropriate time (Lundström, 1984). The etiology of MM is thought to be multifactorial, whereby environmental and genetic/familial factors interact over time,

thus supporting the polygenic model of inheritance. Data on the genetic determinants behind MM is still fragmentary.

B. Significance

Recent progress in molecular genetics, like the genome-wide linkage scan technology, has allowed the investigation of susceptibility genes that underlie the development of the maxilla and mandible, resulting in the different types of malocclusion.

Studies have largely focused on Cl III malocclusion, featured with either maxillary retrognathism, mandibular prognathism, or both, and fewer are the data on the Cl II, division 1 malocclusion specifically when starred with MM. Only one family-based linkage has been investigated in the Hispanic population, and no previous study was undergone in the eastern Mediterranean population, highlighting the fact that the genetic determinants of MM remain ambiguous.

The exploration of specific genes contributing to MM will help us understand the variation in mandibular growth along with potential prediction of the final manifestation of the growth pattern or the severity of the malocclusion conferred by a particular genotype, and particularly in the eastern Mediterranean population.

Precision Medicine is a new buzz phrase as patients are separated into different groups based on their predicted response or risk of disease in order to select the appropriate and optimal therapies based on the patient's genetic content (Gaw, 2016). Thus, the identification of the genetic implication and the mechanisms governing the genic transmission behind this malocclusion would help in the treatment. Variants predicting the risk for this condition have the potential to help the orthodontist to reduce

uncertainties in clinical decision-making, personalize health care delivery, and improve outcomes. A skeletal Cl II malocclusion is usually maintained if left untreated, and sometimes the mandibular deficiency can be worsened (Stahl, Baccetti, Franchi, & McNamara Jr, 2008). This was consistent with the findings of Bishara, who found that untreated Cl II patients presented greater profile convexity, and even if the growth trends are similar to those of normal Cl I patients, there is always a likelihood toward a retrusive mandible (Bishara, 1998). Thus, by early forecast of the condition, earlier treatment may be instituted if needed. More importantly, present intervention may be foregone in favor of a later orthognathic surgery when severe mandibular Micrognathism/retrognathism is genetically determined as a "certainty."

C. Research objectives

The aims of this study are to:

1. Explore the inheritance pattern of MM in mostly Lebanese families and identify candidate loci and gene(s) responsible for the development and familial transmission of MM in the Eastern Mediterranean population.
2. Evaluate the skeletal and dentoalveolar cephalometric characteristics of affected individuals.

D. Hypothesis

Our main hypothesis is that specific candidate loci and genes have an etiological role in the susceptibility to mandibular micrognathism in the Eastern Mediterranean population.

CHAPTER II
LITERATURE REVIEW

A. Definitions and concepts

1. Malocclusion

Malocclusion is an appreciable deviation from the ideal occlusion (Hassan & Rahimah, 2007). It is the outcome of complex interactions between genetics and environmental factors, resulting in disturbance of the developmental pathway(s) involved in the formation of the orofacial region (Graber, Vanarsdall, Vig, & Huang, 2016). The concept of occlusion emerged in the late 1800s due to the necessity of making suitable prosthetic replacement of teeth (W Proffit, 2013).

Such imbalance may entail:

1. The position of teeth (misalignment) where the teeth in the arch occupy a deviated position,
2. Facial bones with a skeletal discrepancy affecting the maxilla and/or the mandible,
3. Soft tissues (lips, cheek, and tongue) ((Sampson & Sims, 1992);(W. R. Proffit, 1986)).

The World Health Organization(WHO, 1987) had included malocclusion under the heading of Handicapping Dento-facial Anomaly, which was defined as an anomaly causing mutilation or which impedes function, thus requiring treatment "if the disfigurement or functional defect was likely to be an obstacle to the patient's physical or emotional well-being."

Edward Angle published the first classification of malocclusion in the early 1900s, based on the relationship of the mesiobuccal cusp of the maxillary permanent first

molar and the buccal groove of the mandibular permanent first molar. He described three basic types of malocclusion (Figure II.1):

1. Cl I malocclusion: the mesiobuccal cup of the maxillary first molar occludes in the central groove of the mandibular first molar (molar neutroclusion).
2. Cl III malocclusion: the maxillary first molar is positioned posteriorly to the buccal groove of the mandibular first molar with an incisal edge-to-edge relationship or anterior crossbite (molar mesioclusion)
3. Cl II malocclusion: the maxillary first molar is anterior to the buccal groove of the mandibular first molar (molar distoclusion). He further specified two divisions of class II depending on the position and inclination of the maxillary incisors:
    - Cl II, division 1 characterized by the proclination of the maxillary incisors
    - Cl II, division 2, where the maxillary incisors, where retroclined (Angle, 1899).

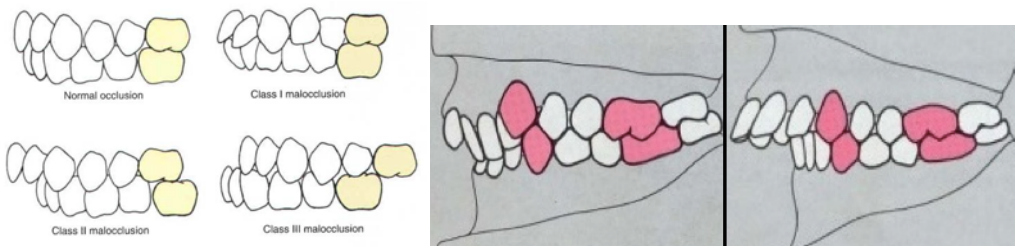


Figure II-1: a) schematic presentation of the types of malocclusion. b) Cl II, division 2 versus Cl II, division 1 malocclusion

Malocclusion can be complicated by the presence of an underlying skeletal discrepancy necessitating dentoalveolar compensation (Figure II.2).

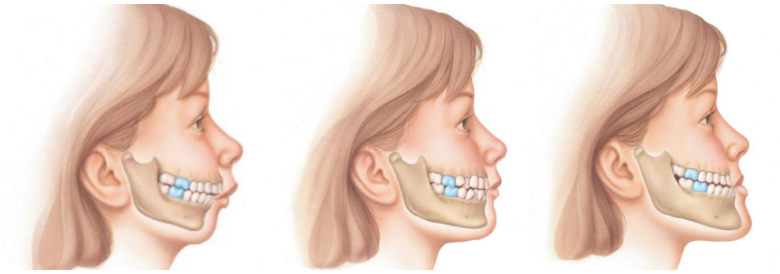


Figure II-2: Associated faces with the three types of malocclusion.

Malocclusion arises from the combined interactions of genetic and environmental factors overtime on the developmental pathways involved in the formation of the orofacial region. However, even if a patient's craniofacial growth is influenced heavily by one gene as opposed to multiple genetic factors, there is no guarantee that future growth will necessarily or absolutely be predetermined. Nor does it mean that growth will proceed on a particular immutable track, although traits with a monogenic influence may be less amenable to environmental (treatment) intervention than traits influenced by multiple genes (J. K. Hartsfield Jr, 2011).

2. Genome, genotype, and genes

a. Human genome

The human genome is the complete set of nucleic acid for humans, encoded as DNA. The genetic material is stored in two organelles: nucleus and mitochondria. The molecules that make up the DNA are called nucleotides. The base structure of the nucleotide includes a phosphate group, a sugar group, and a nitrogen base. There are four types of nitrogen bases: adenine (A), thymine (T), guanine (G), and cytosine (C). The genetic code is governed by the order of these bases. Around 3.2 billion base pairs are packed in the nuclear genome which consists of 22 pairs of autosomes and two sex chromosomes (X and Y) (Makałowski, 2001)

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 essential role in regulating when and where the cells will use portions of the genetic information contained in the genome (Golbabapour, Abdulla, & Hajrezaei, 2011)

The first human genome sequences were published in nearly complete draft form in February 2001 by the Human Genome Project and Celera Corporation. Completion of the Human Genome Project's sequencing effort was announced in April 2003.

From a functional point of view, genomic sequences can be divided into coding and non-coding DNA sequences. Coding DNA is defined as the sequences that can be transcribed into mRNA and translated into proteins. Coding DNA is also known as Exons. Non-coding DNA is made up of all those sequences that are not used to encode proteins which accounts for 98% of the genome (Figure II.3) (Wang, 2016).

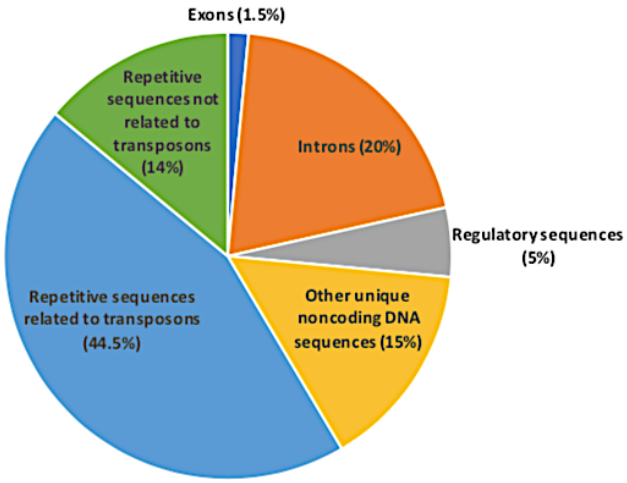


Figure II-3: Composition of the Human genome (X. Wang: Next-Generation Sequencing Data analysis 2016)

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 (J. K. Hartsfield Jr, 2011).

b.Genes:

Looking closer at chromosomes, they are further organized into smaller units called genes, which represent the most minor physical and functional unit of inheritance. A gene can be defined as “the complete DNA sequence that codes 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)." Genes are inherited as units, where each offspring receives the divided copies of his/her parents' genes. Each individual has two copies of each of their genes, but each egg or sperm cell only gets one of those copies for each gene. An egg and sperm join to form a complete set of genes. The resulting offspring has the same number of genes as their parents, but for any gene, one of their two copies comes from their father and one from their mother.

Out of our whole genome, genes only make up for 2% and estimated to be at the number of 25000. Each gene is 3000 nucleotide base pairs in length.

Within the human genome, each gene occupies a specific location on a chromosome called locus. The alleles are referred to as the genes at the same locus on a pair of homologous chromosomes, where one allele would be a copy of the maternal allele and the other a copy of the paternal allele. Homozygotic locus is when both

alleles are identical. The individual is said to be heterozygous for a locus when the two alleles are distinctive in the DNA sequence (J. K. Hartsfield Jr, 2011).

c. Epigenome and epigenetics

The epigenome is the complete description of all the chemical modifications to DNA and histone proteins that control the expression of genes within the genome. These modifications occur without fundamental changes in the primary DNA sequence and are necessary for key biologic processes, thus, a change in the phenotype but not the genotype. The most common mechanisms of epigenetic modification include DNA methylation, histone modifications, and transcription of small non-coding RNA (Morgensztern, Devarakonda, Mitsudomi, Maher, & Govindan, 2018). These modifications of the DNA double-helix back-bone act to open or close regional chromosome structure to enhance or shut down gene expression (J. K. Hartsfield Jr, 2011).

Epigenetics is a mechanism for regulating gene activity independent of DNA sequence that determines which genes are turned on or off in a particular cell type, different disease states, and in response to a physiological stimulus. These regulatory activities are heritable (Holliday, 1987).

d. Genetic variation

Genetic variation refers to the genomic differences seen in a population or species, and as previously mentioned, between any two humans, the amount of genetic variation is about 0.1 %. DNA variants leading to monogenic diseases are usually rare

in a population due to the process of natural selection. Neutral DNA variants occur at variable frequencies within populations as a result of genetic drift.

Sequence variants which are present at a frequency of less than 1% in a population are arbitrarily designated as mutations, and those at a higher incidence are referred to as polymorphisms (Schafer & Hawkins, 1998)

Genetic variation in the human genome is present in many forms and occurs at different frequencies throughout the genome. The various forms of genetic variation include tandemly repeated DNA, single nucleotide polymorphisms (SNPs), small insertions/deletions, large-scale mutations, transposable elements, fragile sites and null alleles (Talseth-Palmer & Scott, 2011)

The most abundant type of genetic variations in the human genome is the SNP. It occurs approximately every 100 to 300 bases and accounts for roughly 90% of all human genetic variation. Most SNPs are located in non-coding regions, thus not altering the phenotype (Shastry, 2002). Contrariwise, SNPs can be causative of altering protein expression when occurring in a gene. Consequently, the phenotype is modified and may manifest as a disease (Sunyaev et al., 2001). Moreover, a point mutation kind of SNP in a DNA sequence that results in a premature stop codon within the mRNA is called nonsense mutation. This results in signaling a termination of translation into proteins (Program., 2003)

Small insertions and deletions (INDELS) account for the vast majority of genetic variation observed in DNA, together with SNPs. Small INDELS (1-30 bp) in the coding regions of genes can, but not always, lead to frame-shift mutations causing a severely altered and potentially non-functional protein (Talseth-Palmer & Scott, 2011)

e. Genotype:

The genotype is the individual's unique set of all the genes. It is the complete heritable genetic identity represented by the chemical composition of the organism's DNA (referring to to the alleles, or variant forms of a gene). Two organisms whose genes differ at even one locus are said to have different genotypes (J. K. Hartsfield Jr, 2011).

3. Phenotype

The phenotype is the ensemble of all traits of the organism's observable physical properties, including appearance, development, and behavior. In a sophisticated manner, the genotype regulates the phenotype, meaning that the phenotype can be thought of as a clinical expression of an individual's specific genotype, superimposed with environmental influences upon these genes. Organisms with identical genotypes, such as identical twins, ultimately express non-identical phenotypes due to the power of environmental factors, because each organism encounters unique environmental influences as it develops.

4. Inheritance and penetrance

a. Monogenic traits

Traits that develop primarily due to the influence of a single gene locus are termed monogenic or mendelian traits. In order to apprehend patterns of disease transmission of monogenic traits, it is crucial to learn about the basic laws of inheritance.

i. Mendel's laws of inheritance

It's the set of three laws, proposed by Gregor J. Mendel in the mid-1860s, to explain the biological inheritance/ heredity. These laws comprise the law of segregation, the law of independent assortment, and the law of dominance, and they shape the core of classical genetics to date.

The first law is the "Law of Dominance," which states that, in a heterozygous condition, the allele whose characters are expressed over the other allele is called the dominant allele and the traits of this dominant allele are called dominant characters.

The "Law of Segregation" comes second and asserts that when two traits come together in one hybrid pair, the two characters do not mix with each other and are independent of each other. Each gamete receives one of the two alleles during meiosis of the chromosome.

Last, the "Law of Independent Assortment" describes how different genes independently segregate from one another, as well as of other traits, when reproductive cells develop. Also, at the time of gamete and zygote formation, the genes are independently passed on from the parents to the offspring (Gautam, 2018).

ii. Modes of inheritance

Single-gene diseases are as a rule inherited in a particular pattern, depending on the location of the gene (e.g., chromosomes 1-22 or X and Y) and whether one or two normal copies of the gene are needed for proper protein activity.

Five basic modes of inheritance for single-gene diseases exist: autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive, and mitochondria (Genetic & Services, 2009).

- Autosomal dominant: individuals that are carrying one mutated copy of the gene in each cell will be affected by the disease. Having one affected parent is enough, and the trait tends not to skip a generation in an affected family (Figure II.4) (Genetic & Services, 2009)

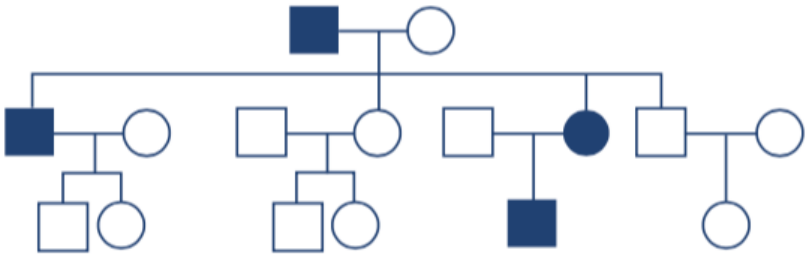


Figure II-4: Three-generation pedigree of a family with an autosomal dominant trait (J. K. Hartsfield Jr, 2011)

- Autosomal recessive: the expression of the trait necessitates two mutated copies of the gene. Parents are usually referred to as "carriers,"; being unaffected and carrying each one a single copy of the mutated gene. It is not stereotypically seen in every generation (Figure II.6) (Genetic & Services, 2009)

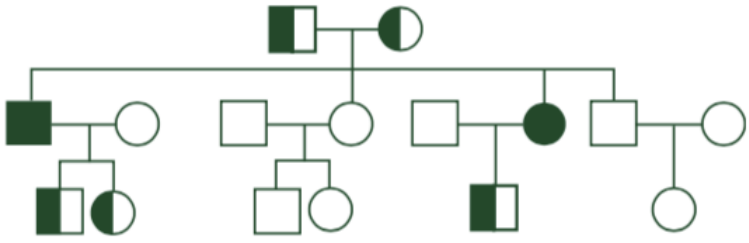


Figure II-5: Three-generation pedigree of a family with an autosomal recessive trait. The symbols for presumed carriers (heterozygotes) of the autosomal recessive gene are filled in halfway (J. K. Hartsfield Jr, 2011)

- X-linked dominant: the gene accountable for a genetic disorder is positioned on chromosome X, and only one copy of the allele is sufficient to cause the disease. Affected fathers by an X-linked dominant disorder will necessarily have affected daughters but not affected sons. However, if the mother is affected then sons and daughters will have a chance of being affected, depending on which chromosome is passed on (Figure II.6) (Genetic & Services, 2009)

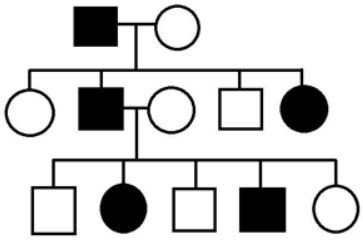


Figure II-6: Three-generation pedigree of a family affected with an X-linked dominant trait (J. K. Hartsfield Jr, 2011)

- X-linked recessive: in a family with an X-linked recessive disorder, males are necessarily affected in each generation since they are unavoidably hemizygous for the gene mutation because they have one X and one Y chromosome. Whereas, only females who are homozygous for the variation in concern are affected. An affected daughter has to have both parents carriers (the father being positively affected), while only the mother is a carrier when the son is affected since the father cannot pass X-linked trait to his son (Figure II.7) (Genetic & Services, 2009).

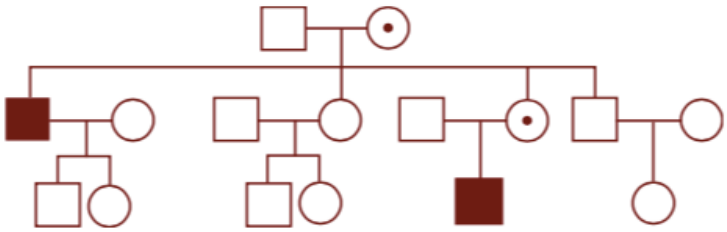


Figure II-7: Three-generation pedigree of a family with an X-linked recessive trait. The symbols for presumed female carriers of the X-linked recessive gene have a dot in the middle of the circle (J. K. Hartsfield Jr, 2011).

iii. Penetrance and modes of penetrance

The penetrance of a genotype is described as the probability that a person carrying it will present clinical manifestations. Penetrance has been until recently a term used almost exclusively for dominant disorders because analysis of the pedigrees alone allowed one to diagnose healthy obligatory carriers (Zlotogora, 2003).

Typically, it refers to the degree to which some individuals of a mutant genotype display the associated phenotype. Penetrance may vary from 0 to 1 (Brenner, Miller, & Broughton, 2002).

- Complete penetrance is when clinical symptoms of the trait are present in an individual carrying the trait-causing mutation.
- Incomplete or reduced penetrance is when the individual fails to express the trait though he/she carries the allele. Therefore, the individual (considered as a carrier) does not outwardly show the trait but is able to have offspring with the trait (Figure II.8).

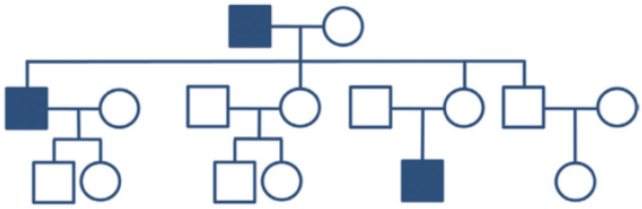


Figure II-8: Three-generation pedigree of a family with an autosomal dominant trait showing incomplete penetrance (J. K. Hartsfield Jr, 2011)

- High penetrance: the trait that a highly penetrant allele produces will roughly always be apparent in an individual carrying the allele.
- Low penetrance: An allele with low penetrance, it is challenging to distinguish environmental from genetic factors as it only sporadically produces the trait with which it is associated (Miko, 2008).

b. Complex traits

Complex traits, also known as polygenic traits, obtained their terminology to reflect their complex etiologic interaction among genes from more than one locus and environmental factors. Each gene involved in creating the trait is thought to have a minimal effect by itself but that the effect of all genes involved is additive. Thus, these traits do not adhere to the Mendelian (monogenic) inheritance pattern.

A change in the phenotype depends on the result of the genetic and environmental factors present over time (Figure II.9). Consequently, environmental factors can play a variable and generally greater role in complex traits than in monogenic traits. Complex traits will be more amenable to change (or a greater change) following environmental/treatment modification when contrasted with monogenic traits (J. K. Hartsfield Jr, 2011).

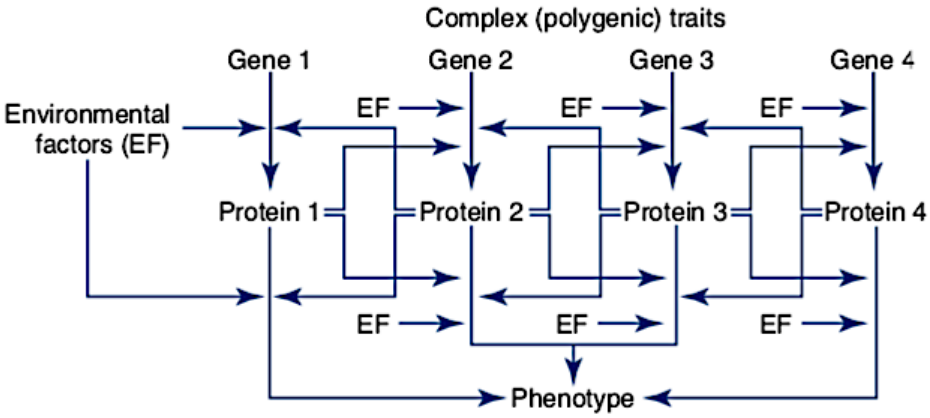


Figure II-9: Interactions of genetic and environmental factors on a complex trait (J. K. Hartsfield Jr, 2011)

5. Sanger and Next Generation Sequencing techniques

DNA sequencing is the process of defining the sequence of nucleotide bases (As, Ts, Cs, and Gs) in a piece of DNA.

In order to sequence an entire genome, shearing the DNA into many smaller pieces is required. The pieces are subsequently amplified and then sequenced. The whole thing is then assembled into a single long "consensus." However, with the developing technologies over the past two decades, genome sequencing is now much faster and less expensive (Palladino, 2002).

a. Sanger sequencing

Sanger sequencing, also known as the chain termination method or dideoxy method, was first introduced by Frederick Sanger in 1977.

It encompasses the use of a purified DNA polymerase enzyme to synthesize DNA chains of varying lengths in a mixture of chain-terminating dideoxynucleotide triphosphates (ddNTPs). ddNTPs are referred to as chain-terminating because they

inhibit further strand extension since they lack the 3' hydroxyl (OH) group needed to form the phosphodiester bond between one nucleotide and the next to come.

To sequence a single sample, four parallel sequencing reactions are conducted, where each reaction involves a single-strand template, a specific primer (whichis a short piece of single-stranded DNA that binds to the template DNA, acting as a starter for the polymerase), DNA polymerase, and normal deoxynucleotides triphosphate (dNTPs). Complementary bases are added to the single-stranded template by the polymerase, synthesizing a double-stranded DNA molecule.

One of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP) is then added to each reaction at a lower concentration than the dNTPs. The ddNTPs that terminate the strands have specific fluorescent labels covalently attached to them. The growing DNA terminates, producing an assortment of strand lengths for analysis.

The resulting fragments are then subjected to gel or capillary tube electrophoresis through four columns; fragments line up according to size (differentiating the strands by even one nucleotide). 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 ddNTP-specific emitted fluorescence, a computer keeps track of the order and identity of the terminating nucleotide. By knowing which base is at the end of each fragment, researchers are able to read the DNA sequence (Sanger, Nicklen, & Coulson, 1977).

Sanger sequencing allows the study of DNA stretches up to about 900 bp. Thus, making this technique inefficient for large-scale projects as well as expensive when it comes to sequencing large genomes. Additionally, it has a low throughput as it

permits the sequencing of a limited number of samples per run. This is why mass sequencing techniques that are faster and less expensive have been introduced.

b. Next Generation sequencing

Next-generation sequencing (NGS), also known as massively parallel or high-throughput sequencing, is the technology that allows the sequencing of millions of small fragments of DNA in parallel.

NGS technologies have been introduced in 2009 and have been continually improving to become faster, more efficient, and cheaper. Each of the three billion bases in the human genome is sequenced multiple times, providing high depth to deliver accurate data and insight into unexpected DNA variation.

Two major platforms have been introduced: Illumina (reversible dye-terminator sequencing technology) and Ion-torrent (semiconductor sequencing technology). Illumina's platforms have primarily dominated. They differ in their details but typically follow a similar general paradigm: library preparation, clonal amplification, followed by cyclical rounds of massively parallel sequencing. Library preparation with Illumina relies on the bridge amplification method, whereas an emulsion polymerase chain reaction (PCR) is employed with Ion-torrent (Reuter, Spacek, & Snyder, 2015).

The technique of NGS will be described in detail in the material and methods section.

Table II-1: Comparison between Sanger sequencing and NGS

	Sanger Sequencing	Targeted NGS
Benefits	• Fast, cost-effective sequencing for low numbers of targets (1–20 targets) • Familiar workflow	• Higher sequencing depth enables higher sensitivity (down to 1%) • Higher discovery power • Higher mutation resolution • More data produced with the same amount of input DNA • Higher sample throughput
Challenges	• Low sensitivity (limit of detection ~15–20%) • Low discovery power • Not as cost-effective for high numbers of targets (> 20 targets) • Low scalability due to increasing sample input requirements	• Less cost-effective for sequencing low numbers of targets (1–20 targets) • Time-consuming for sequencing low numbers of targets (1–20 targets)

6. Terms related to the genetic analysis

a. Quality score

Quality score (Q) is the most common metric used to evaluate the base calling accuracy, meaning that it defines how likely it is that a base call is erroneous.

Q is defined by a logarithmic equation. Higher scores designate a smaller probability of error, whereas lower scores may yield in a significant portion of the reads being unusable.

Q30 is considered a benchmark for quality in NGS, since, with a Q30, there is a 1 in 1000 times probability of incorrect base call, and the base call accuracy is at 99.9%. Virtually, all of the reads are perfect, having zero errors and ambiguities. (Illumina, 2011).

b. Filter status

Filtering is one step that is executed prior to cluster analysis or genetic network analysis, where clusters are required to pass filters on a flow cell to eliminate genes that are judged irrelevant from the set of genes for the analysis.

When a position passes all filters, the filter status is marked as PASS, while a specific code for filters that fail is written (HumanGenomeProgram, 2008).

c. Putative impact

Accurate prediction of the impact of genetic variants improves our understanding of how genetic information is conveyed to molecular and cellular functions.

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

d. Allele count and Minor Allele Frequency (MAF)

Allele count is the counts of each alternate allele for each site across all samples. In most cases, there is only a single alternate and so the count is the number of observations of this allele across all chromosomes of the samples.

The MAF Minor allele frequency (MAF) is the frequency at which the second most common allele occurs in a given population., obtained by dividing the number of appearances of the minor variant by the total number of alleles and expressed in %. A low MAF designates that the variant is less likely to be present in the normal population, while the variant is present in the normal population if the MAF is high.

Thus, it provides information to differentiate between common and rare variants in the population (Hernandez et al., 2019).

e. Read depth

Depth of coverage (N) refers to the number of unique reads that align to or cover a known reference base. With higher levels of coverage, base calls can be done with a higher degree of confidence as each base is covered by a larger number of aligned sequence reads. Sequence coverage requirements depend on the sequencing method to be employed (whole genome sequencing, whole-exomes sequencing, RNA sequencing, ChIP-Seq). (Sims, Sudbery, Ilott, Heger, & Ponting, 2014)

h. GC-content

G-C (guanine-cytosine) bps have stronger interactions than A-T (adenine-thymine) bps arising from their ability to form three hydrogen bonds in water. A-T base-pairing yields only two hydrogen bonds. That is why the melting point of double stranded DNA is higher for high G-C content DNA as well as for longer pieces of DNA. High GC content makes the DNA molecule more stable (HumanGenomeProgram, 2008).

B. Incidence and prevalence

Class II malocclusion was shown to be the most prevalent between all the malocclusions with a frequency of 5% to 29% (Massler & Frankel, 1951; Woodside, 1968). Differences are attributed to race and ethnicity, where the highest incidence has been observed in Caucasians of European descent (Brunelle, Bhat, & Lipton, 1996).

In a sample of Lebanese orthodontic population, Kassis et al. (Kassis, 2010) found that Class II (division 1 and 2) malocclusion was the most frequently seen malocclusion with a prevalence of 49%. Class I was 20.98%, Class III 7.32%, Class II subdivision 20.49%, and Class III subdivision 2.2% the least common (Figure II.10).

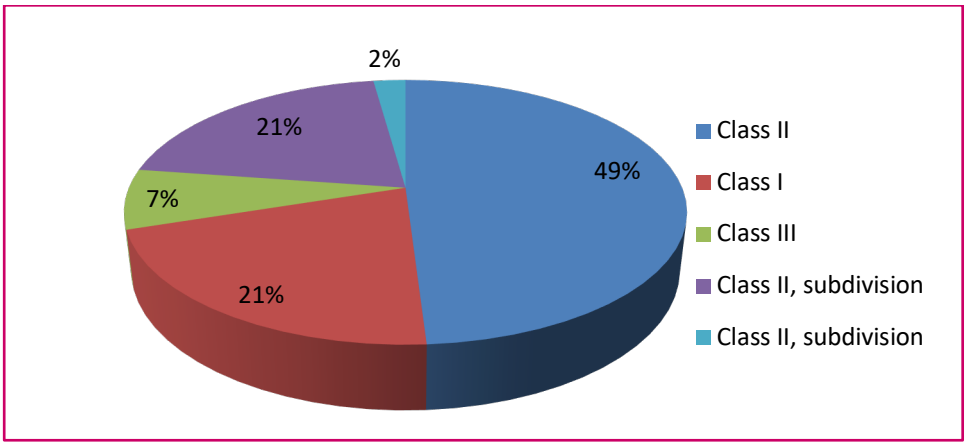


Figure II-10: Prevalence of malocclusion in Lebanese orthodontic population

According to NHANES III, the prevalence of malocclusion of the U.S. population is the following: 50% to 55% fall into the class I malocclusion which constitute the largest group, followed by the class II malocclusion encompassing around 15% of the population and finally the class III malocclusion which represents less than 1% of the total population (WR Proffit, Fields, & Moray, 1998)

C. Craniofacial and dental morphological features

Cl II, division 1 is clinically heterogeneous and can be associated with many combinations of skeletal and dental components that lead to the recessive positioning of the mandible relative to the maxilla.

1. Craniofacial features

Fisk et al. (1953) affirmed that multiple morphological variations are responsible for the development of Cl II. Division 1:

a. Prognathic maxilla along with anteriorly positioned maxillary dentition relative to the cranium,
b. Orthognathic maxilla along with anteriorly positioned maxillary dentition,
c. Small orthognathic mandible (micrognathia)
d. Retrognathic mandible but of normal size,
e. Orthognathic mandible, along with posteriorly positioned mandibular dentition.

Skeletal discrepancies as such are typically associated with dentoalveolar compensations, which can be therapeutically challenging insofar as their severity (Fisk, 1953).

After comparing several components from various studies, Ghafari et al. (2014) observed that the anatomical structures that contribute to a Cl II, division 1 malocclusion encompass both jaws and the cranial base. Based on the literature and with the advent of cephalometrics, selected structures consist of:

a. Flexure of the cranial base (Saddle angle): Increased flexure and shorter lower cranial heights were observed in Class II subjects compared with subjects with class I,
b. Sagittal jaw relations (ANB, WITS appraisal): Most studies reported no statistically significant differences in the skeletal and dentoalveolar positions of the maxilla in the Cl II compared with Cl I samples.
c. Vertical jaw relations (angle of divergence PP/MP),

d. Total mandibular length and position relative to the cranial base: Shorter and retrusive mandibles have been reported in patients with Cl II malocclusion,
e. Mandibular dentoalveolar/skeletal relation,
f. The shape of the chin and its soft tissue thickness: The shape of the chin makes a significant difference in the perception of convexity. Similar malocclusions treated alike may disclose more or less favorable esthetics depending on the form of the chin "button" (Ghafari & Macari, 2014).

2. Dental features

The dental characteristics include an increased overjet and a Cl II relationship between molars, as previously stated. Proclination of the mandibular incisors reflects dentoalveolar compensation within the intimate association between dentoalveolar and skeletal parts. Often retroclination of maxillary incisors is also observed (Ghafari & Macari, 2014).

D. Etiology of Cl II, division 1 malocclusion and mandibular micrognathism

There is clearly a genetic influence in the dentofacial morphology and the etiology of malocclusion (P. A. Mossey, 1999a). Craniofacial development is a highly regulated process under tight genetic control, involving coordinated events of patterning, proliferation, and differentiation of embryonic tissues from multiple origin. Genetic mechanisms are presumably predominant during embryonic craniofacial

morphogenesis, but the environment is also thought to have an influence postnatally, particularly during facial growth (P. A. Mossey, 1999a).

Environmental modifications may alter the developing phenotype at a particular moment; however, gross structural morphology, already present, may not change readily unless the environmental factor is sufficient to amend preexisting structure(Buschang & Hinton, 2005). Thus, it is difficult to prove a single major cause of malocclusion as it evolves throughout the growth of the child, and the development of occlusion is very vulnerable to many stimuli.

In spite of the fact that it was ascribed to Cl II, division 1 malocclusion being heritable, the etiology is considered to be multifactorial where environmental and genetic/familial factors interact over time, resulting in the characteristic facial phenotype (Howe, 2012). At least two environmental factors have been recognized in the development and worsening of Class II, division 1 malocclusion. Sustained digit sucking would lead to protrusion of maxillary incisors and retrusion of mandibular incisors, producing an increased overjet, which is the most essential expression of the malocclusion (Ghafari & Macari, 2014). On the other hand, sustained mouth breathing is known to cause a cascade of events that alters the myofunctional equilibrium and leads to changes in some skeletal and soft tissue components of the face (head, mandible and tongue posture), causing lip incompetency, a steep mandibular plane, large gonial angles and increased anterior lower facial height (Harvold, Tomer, Vargervik, & Chierici, 1981). In extreme situations, these manifestations will lead to the development of the long face syndrome (also known as adenoid facies) (Figure II.11), which will aggravate the phenotypic expression of a present Cl II, division 1 malocclusion (Ghafari & Macari, 2014).

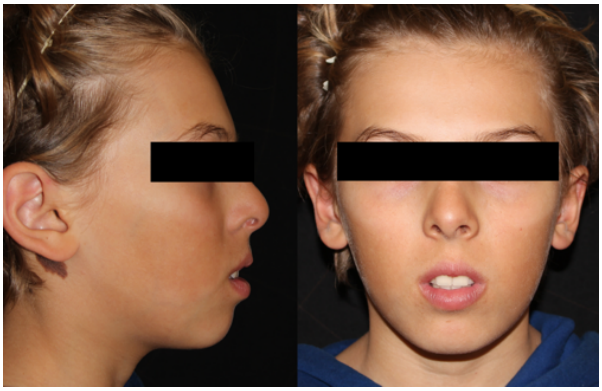


Figure II-11: Extra-oral photographs of a 12-year-old boy with chronic mouth breathing resulting in the typical adenoid facies

Sometimes, past events or a systemic condition can lead to the development of this malocclusion. It has been known that mandibular growth is substantially impaired by ankylosis of the temporomandibular joint (TMJ), defined as a fusion across the joint so that motion is prevented (growth totally stops) or limited (growth is impeded). One possible cause of TMJ ankylosis is a severe infection in the area of the joint, leading to the destruction of tissues and ultimate scarring (Figure II.12). (W Proffit, 2013).

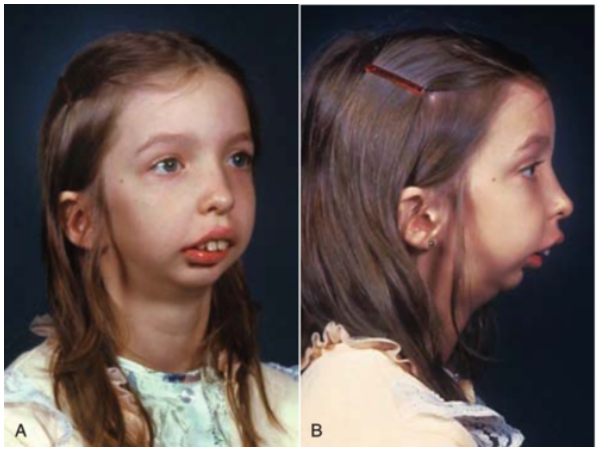


Figure II-12: Oblique (A) and profile (B) views of a girl with a severe infection of the mastoid air cells involving the TMJ of the mandible with an evident restriction of mandibular growth (W Proffit, 2013).

Another cause is trauma, which can result in a growth deficiency with the stipulation that enough soft tissue injury is present, leading to scaring that consequently impedes motion as the injury heals. Therefore, the mechanical restriction hinders translation of the mandible as adjacent soft tissues grow, leading to decreased growth of the mandible (W Proffit, 2013).

Mandibular underdevelopment may be related to some genetic syndromes. Pierre Robin sequence (PRS) refers to the association of micrognathia, glossoptosis, and airway obstruction (Figure II.13A). Typically, a broad U-shaped cleft palate is also associated with PRS; in large series, a cleft palate is reported in up to 73–90% of cases (Costa et al., 2014). Recent work has shown that multiple non-coding elements contribute to the craniofacial regulation of SOX9 expression; in PRS, these craniofacial regulatory elements are the site of deletions, contributing to the typical phenotype (Gordon et al., 2014). Treacher-Collins syndrome affects the orofacial complex and the signs/ symptoms of this disorder range from nearly imperceptible to severe. Most affected individuals have underdeveloped cheekbones and micrognathia (Figure II.13B). Some people with this condition also have a cleft palate. Mutations in the TCOF1, POLR1C, or POLR1D gene can cause Treacher Collins syndrome (Katsanis & Jabs, 1993).

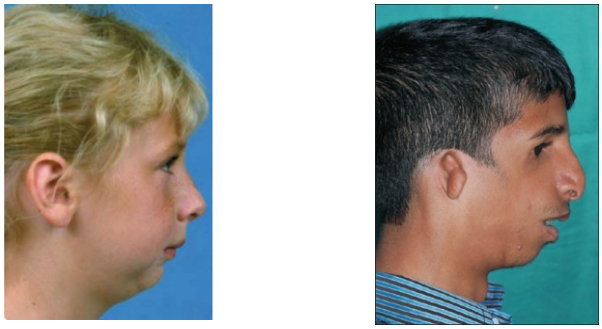


Figure II-13: Female diagnosed as having the Pierre Robin Sequence (A) Male exhibiting the Treacher-Collins Syndrome (B) Note the underdeveloped mandible in both (W Proffit, 2013).

E. Management of Cl II, division 1 and MM

Longitudinal studies reported that Cl II malocclusion does not tend to self-resolve solely with growth, which explains the rationale behind an orthopedic treatment to normalize the underlying skeletal dysplasia (Stahl et al., 2008). Thus, such an approach should idyllically be implemented during the active period of growth, aiming to enhance differential growth between both jaws with a forward mandibular growth in favor (W Proffit, 2013). The success of the treatment is highly reliant on the growth potential of the patient, as individual differences exist in term of the amount of skeletal and dentoalveolar response to treatment inherent to each jaw, whereby the mandibular growth is exceptionally unpredictable, and most of the correction is due to maxillary changes (West, 1957). The compliance of the patient throughout the treatment is similarly a key factor to success.

Treatment modalities in growing patients include a combination of extra-oral forces and a variety of removable or fixed functional appliances designed to modify the mandibular position resulting in a maximum advantage of growth (W Proffit, 2013). Whenever the malocclusion is mostly due to mandibular retrognathism/ micrognathism, functional appliances are favored under the assumption of stimulating mandibular growth, whereas if the diagnosis reveals maxillary prognathism, targeting the maxilla requires the use of direct distal extra-oral force provided by the headgear (Ghafari & Macari, 2014)

In non-growing individuals, a dentoalveolar compensation is implemented which comprises: extraction of maxillary premolars (mandibular premolars as well in case of crowding) or distalization of the maxillary arch (by means of mini-screws or conventional extra-oral traction) and depending on the severity of the discrepancy,

mandibular incisor proclination is considered (Ghafari & Macari, 2014). However, when the amount of skeletal dysplasia is judged to be severe enough that any dentoalveolar compensation is deemed to be detrimental on the face and profile (Figure II.14), an adjunctive orthognathic surgery is advised to achieve a straight profile with proper facial esthetics (W Proffit, 2013).

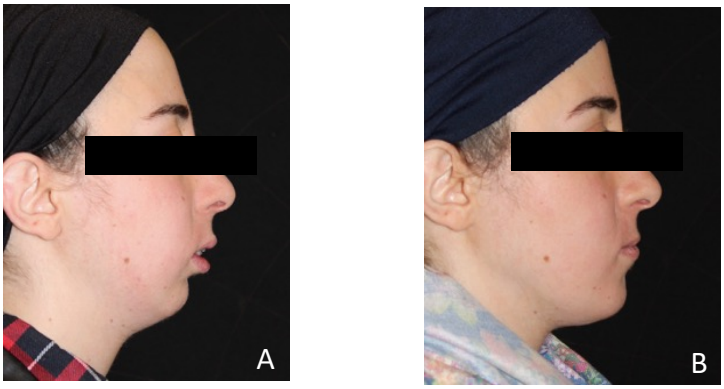


Figure II-14: Female with severe mandibular micrognathism (A) pre-surgical, (B) post-surgical

In syndromic MM, where the receding and small mandible may be altering oral functions and respiration, lengthening of the mandible via distraction osteogenesis has been adopted by many at an early stage (where orthognathic surgery is not indicated) (Figure II.15). This approach allows major changes in mandibular length (a centimeter or more). Nevertheless, the precise positioning of the jaw is not possible.

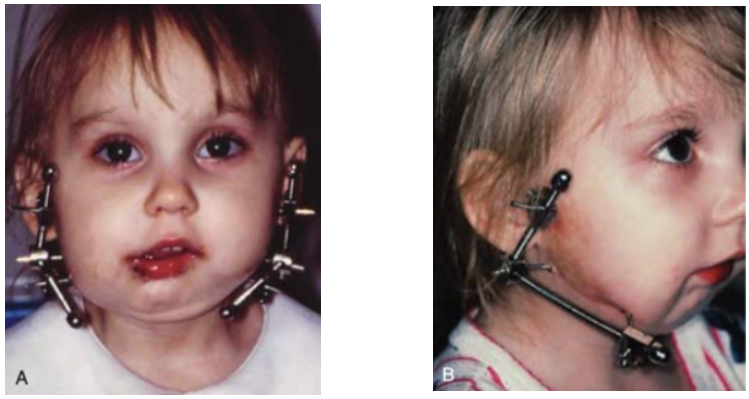


Figure II-15: external fixation for lengthening of the mandible by distraction osteogenesis (W Proffit, 2013).

F. Inheritance pattern

Findings on the inheritance pattern of the Cl II, division 1 strongly support the polygenic model (Fig.6A) meaning that the phenotypic expression, which can be also affected by environmental factors, is determined by the summated effect of a large number of genes, thus, as previously mentioned, not adhering to patterns of Mendelian inheritance which refers to the expression of inheritance of monogenic trait (J. K. Hartsfield Jr, Morford, L. A., & Otero, L. M., 2012) (Figure II.16). However, variable data supporting this model is still fragmentary.

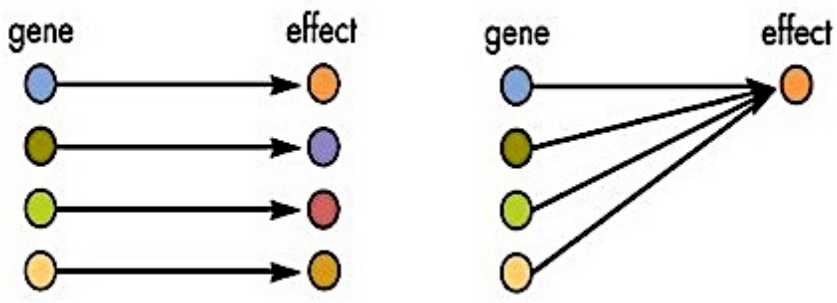


Figure II-16: Monogenic traits, where each gene has a distinct biological effect versus polygenic trait, where many genes contribute to a single outcome.

An autosomal dominance model with incomplete penetrance and variable expressivity (Harris et al., 1975) and no evidence of sex-linked or sex-influenced inheritance (Hughes, 1942) have been suggested as well for the Cl II, division 1.

F. Nature versus nurture

If genetic factors refer to the actual DNA code that is inherited (nature) and environmental factors, in contrast, include such things as diet, living conditions, stress, and learned behaviors that may influence a person's mindset, perception, and/or

epigenetic landscape (nurture), one may reckon that growth and development are not merely the results of nature or nurture working in complete absence or independence of other, it is instead an individual response to environmental changes while influenced by genetic factors.

In a revisitation of the functional matrix theory, Moss concluded that neither genetic nor epigenetic factors alone are sufficient, and only their interactive activities provide the necessary and sufficient causes of growth and development (while genetic factors being intrinsic and environmental/ epigenetic causes being extrinsic factors) (J. K. Hartsfield Jr, 2011).

G. Prediction and importance of familial data

Genes and environment, as stated earlier, are the contributing factors to the malocclusion. The finest evidence in ascertaining the relative impact of these factors is from familial and twin studies.

The influence of genetics varies according to the trait under consideration (P. A. Mossey, 1999b) and it's evident to say that the skeletal pattern is more directly allied to genetic factors as the correlation for skeletal measurements between parents and offspring were strong (Nakasima, Ichinose, Nakata, & Takahama, 1982).

Siblings commonly display similar types of malocclusion. Thus, screening and inspecting family members may postulate illuminating data. One way to obtain information concerning the treatment need for a child, including early treatment of malocclusion, is the examination of parents and older siblings (Litton, Ackermann, Isaacson, & Shapiro, 1970). Extensive cephalometric studies have shown a higher

correlation between an individual and his/her direct family than data from random pairings of unrelated siblings (P. A. Mossey, 1999b).

Harris and co-workers disclosed that the craniofacial skeletal patterns of children with Cl II malocclusions are heritable while a high resemblance to the skeletal patterns occurring in their siblings with normal occlusion. From this, it was concluded that multiple genetic factors possibly contribute to this resemblance, and family skeletal patterns were used as predictors for the treatment prognosis of the child with a Cl II malocclusion (J. K. Hartsfield Jr, 2011).

Data on the Cl II, division 1 revealed that the craniofacial skeletal patterns in children are familial, meaning that associated traits occur in multiple members with a higher resemblance to the skeletal models (J. K. Hartsfield Jr, Morford, & Otero, 2012). Hence, exploring family patterns of similarity, while taking a family history, especially for traits that have or can have monogenic inheritance, may alert the practitioner to the increased likelihood of the same trait developing in their patient (J. K. Hartsfield Jr, 2011).

Genetic epidemiology has traditionally concentrated on the study of patterns of familial resemblance and identifying the factors that explain the observed patterns (Morris, Elston, Barnholtz-Sloan, & Sun, 2015). Albeit that each child inherits half of his/her genes from each parent, the usual correlation for facial proportions between parents and their children is only 30% due to interactions between multiple genetic factors with the varying effects of the environment. Thus, yielding even less predictive power (J. K. Hartsfield Jr et al., 2012).

With the discovery and advances of SNP arrays and next-generation sequencing, genome-wide association studies (GWAS) allowed the exploration of the

genetic determinants of diseases. GWAS have primarily used samples of unrelated individuals and let the reveal of many variants, nevertheless, not out coming the importance of family-based study designs in untangling the complex web of environmental and genetic factors leading to the disease. Table 1 summarizes the advantages and disadvantages of using family-based approaches (Morris et al., 2015).

Table II-2: Summary of advantages and disadvantages of using family-based approaches in genetic epidemiology

Advantages	Disadvantages
Discovery of highly penetrant rare segregating alleles	An added layer of complexity at all stages from design to analysis
The ability to overcome confounding factors (i.e. population stratification)	Specialized statistical methods and software are often needed
Discover important biology informing us about disease	
The evidence for a variant co-segregating with a phenotype to be associated with the disease is strengthened.	

H. Genetic studies

Recent progress in molecular genetics, like the genome-wide linkage scan technology, has allowed the investigation of susceptibility genes that underlie the development of maxilla and mandible.

Genetic mapping studies performed in different ethnic populations have identified several chromosomal regions or loci that might harbor susceptibility genes for various maxillary and mandibular dysmorphogenesis. Studies have primarily focused on Class III malocclusion, featured with either maxillary retrognathism, mandibular prognathism, or both, and fewer are the data on the Class II/ division1 malocclusion.

1. Cl II and mandibular micrognathism

a. Animal model

Animals provide beneficial models for the study of diseases and conditions afflicting humans, hence providing novel insight. This significantly improved our understanding of the initiation and perpetuation of human diseases. One example of that is a study conducted by Abdel Al-Lami et al. (2016), where they focused on the development of micrognathia in the ciliopathy orofaciodigital syndrome caused by the mutation of the OFD1 gene in mice knowing that the mouse model simulates the human phenotype. It was suggested that cilia are required for neural crest migration during jaw outgrowth.

b. Human studies

One genome-wide family-based linkage has been done (Gutierrez et al., 2010), where an association was found between SNP rs 1348322 within the human Noggin gene (NOG) and mandibular Micrognathism, however, the exact effect of the polymorphism was uncertain. The NOG gene is a protein-coding gene in the region 17q22. The secreted polypeptide binds and inactivates members of the transforming growth factor-beta (TGF-beta) superfamily signaling proteins, such as bone morphogenetic protein-4 (BMP4) in osteoblast, which can limit their effects on skeletal cells. The mouse knockout of the ortholog results insinuate that it is implicated in a range of developmental processes, such as neural tube fusion and joint formation.

Yamaguchi et al. in 2001 investigated the role of the d3/fl-GHR SNP of GHR and five SNPs in exon 10 of GHR with regard to craniofacial morphology. Japanese individuals with P561T variants at the GHR gene locus (which affected the cytoplasmic

domain of GHR) had a significantly smaller mandibular ramus length (condylion-gonion) than those who were wild type at this locus. Thus, this variant has no momentous role in regulating the final body height (Yamaguchi, Maki, & Shibasaki, 2001). Likewise, Sasaki et al. in 2009 explored whether mandibular growth is affected by the P561T heterozygous missense mutation during early childhood in mandibular protrusion and normal occlusion. It was reported that this missense mutation might function as an inhibitory factor in the process of mandibular growth (mandibular linear parameters tended to be smaller in subjects carrying the heterozygous mutation than in normal and wild types). Yet, it did not account for the difference between mandibular protrusion and normal occlusion (Sasaki et al., 2009). GHR gene, located at 5p13.1-p12, encodes a member of the type I cytokine receptor family, a transmembrane receptor for growth hormone. An inter and intracellular signal transduction pathway is activated once the growth hormone is bound to the receptor, leading to growth. Thus, GHR gene may be a candidate for mandibular morphogenesis.

Moreover, Zebrick et al. explored the SNP rs1815739 of α-actinin-3 gene (ACTN3), where a common non-sense mutation at residue 577 results in a stop codon (R577X). It was found that ACTN3 R577X is associated with Class II and deep-bite skeletal malocclusions (Zebrick et al., 2014). ACTN3 gene at 11q13,2 encodes a protein that is primarily expressed in skeletal muscle and is involved in crosslinking actin containing thin filaments. Deficiency in α-actinin-3 has been proven to influence bone remodeling markers and reduction in bone mass or bone mineral density. Arun et al. explored the link between 3 polymorphisms of the MYO1H gene at 12q24.11 and mandibular retrognathism. rs3825393 polymorphism was found to be associated with an increased risk for mandibular retrognathism. It's interesting to mention that rs10850110

was associated with mandibular prognathism, which provides further support for the potential role of MYOH1 in regulating maxillomandibular growth(Arun, Lakkakula, & Chitharanjan, 2016). The effect of these muscle-related genes on bone and maxillomandibular growth may be justified by the fact that malocclusion is influenced by combinations of transcription and growth factors acting on bone, teeth, and skeletal muscles.

Da Fontura et al. evaluated the associations of types of skeletal malocclusion with craniofacial candidate genes/loci. The SNP rs4287555 within SNAI3 gene was linked with a severe class II phenotype and accentuated convex profile. SNAI3 is a member of the SNAIL family of zinc-finger transcription factors, which are essential in epithelial to mesenchymal transitions that contribute to the formation of the mesoderm and the neural crest. Neural crest-specific deletion in mice leads to multiple craniofacial defects, including mandibular deficiency similar to Pierre Robin sequence, indicating that SNAIL genes may modulate jaw growth. Additionally, an increased risk of class II over class I was allied with rs11200014 in FGFR2. FGFR2 mutations are found in patients with Apert (OMIM 101200) and Crouzon syndrome (OMIM 123500)(da Fontoura et al., 2015). These associations that were observed could indicate that SNPs within FGFR2 modulate risk for abnormal maxillomandibular discrepancies in general.

More recently, Balkhande et al. were interested in Matrilin-1, a cartilage extra-cellular matrix protein encoded by MATN1 gene at 1p35.2, since an endochondral ossification determines the condylar growth of the mandible. The SNPs rs1149048, rs1149042, and rs1065755 of MATN1 were genotyped by PCR (polymerase chain reaction)-restriction fragment length polymorphism. They came to the result that rs1149042 polymorphism was associated with the mandibular measurement SNB and

exhibited a significant association with mandibular retrognathism in dominant and allelic models. Thus, the results suggest an association between MATN1 gene polymorphisms and mandibular retrognathism in a South Indian population (Balkhande, Lakkakula, & Chitharanjan, 2018).

Candidate genes associated with mandibular micrognathism, mandibular retrognathism, and Class II phenotype found in different ethnic populations are summarized in Table II-3. This review shows that the genetic determinants of Cl II, division 1, more specifically with mandibular micrognathism, are still poorly explored.

Table II-3: Summary of the susceptibility genes in different ethnic populations

Gene Name	Effect	Population	Reference
GHR P561T	Shorter mandibular ramus height (Co-Go)	Japanese	Yamaguchi et al., 2001
GHR P561T	Negative impact on mandibular growth	Japanese	Sasaki et al., 2009
NOG rs1348322	Mandibular micrognathia	Hispanic	Gutierrez et al., 2009
ACTN3 rs1815739	Class II	French	Zebrick et al. 2014
SNAI3 rs28755	Class II, accentuated convex profile	Caucasian	Da fontoura et al., 2015
FGFR2 rs11200014	Increased risk of Cl II	Caucasian	Da fontoura et al., 2015
MYO1H rs3825393	Mandibular retrognathism	Indian	Arun et al., 2016
MATN1 rs1149042	Mandibular retrognathism	South Indian	Balkhande et al., 2018

2. Other malocclusions

Genetic studies have primarily focused on Class III malocclusion, featured with either maxillary retrognathism, mandibular prognathism (MP), or both, whilst highlighting the genetic heterogeneity in different ethnic populations.

MP has been said to be polygenic. However, in the majority of cases, this trait appeared to have an autosomal dominant inheritance, such as the European noble

families (e.g., Hapsburg family). Considerable variations exist in the clinical expression of the trait, despite the high penetrance (Wolff, Wienker, & Sander, 1993).

Table II-4: Summary of mapping studies for Class III malocclusion

Gene name	Phenotype	Population	Reference
HSPG2, MATN1and ALPL	MP	Korean and Japanese	Yamaguchi et al., 2005
MATN1	MP	Korean	Jang et al., 2010
EPB41	MP	Chinese	Xue et al., 2010a
EVC and EVC2	MP	Chinese Han	Li et al., 2010
TGFβ3 and LTBP2	MP	Chinese Han	Li et al., 2011
MYO1H (rs10850110)	MP	White, African American, Hispanic, and Asian	Tassopoulou-Fishell et al., 2012
DUSP6	MP, MD	Estonian	Nikopensius et al., 2013
PLXNA2 and SSX2IP	MP	Japanese	Ikuno et al., 2014
COL2A1	MP, MD	Chinese	Xue et al., 2014
ADAMTS1	MP	Chinese	Guan et al., 2015
Gly1121Ser variant in ARHGAP21	MP	Italian	Perillo et al., 2015
IGF1	MD	Hispanic	Frazier-Bowers et al., 2009
C1orf167, NBPF8, and NBPF9	MP	Eastern Mediterranean	Genno et al., 2019

MP: mandibular prognathism; MD: maxillary deficiency

Yet, rare are the studies on Cl II and Cl I malocclusions. For Cl, I malocclusion, delayed tooth eruption, and irregularities were linked to HOXB rs 6504340. Moreover, SNPs within EDA, XEDAR rs 372024, and BMP2 exhibited significant associations with crowding of more than 5mm.

As for Cl II, division 2 malocclusion, evidence of a genetic component with a polygenic model was based on a twin study in which all 20 monozygotic pairs were concordant for Class II/2, while almost 90% of the dizygotic twins were discordant (Markovic, 1992). Accordingly, more than one genetic factor contributes to this malocclusion with a relative risk of first-degree relatives to have a Cl II, division 2 ranging from 3.3 to 7.3 (J. K. Hartsfield Jr et al., 2012).

3. Tooth size and agenesis

Phenotypic variation of overall crown size was demonstrated to be associated more with genetic variation than was the morphology of the occlusal surface. Based on studies of epithelial-mesenchymal interactions during tooth generation, sonic hedgehog (SHH) gene expression appears to have a significant influence on crown width and cusp number.

Dental agenesis has been seen to segregate in families over many generations and usually observed as an isolated trait (non-syndromic). It may also be sporadic, meaning that due to a newly introduced mutation. Dental agenesis may involve one tooth or can extend to oligodontia with multiple teeth being missing. Modes of inheritance have ranged from being autosomal dominant or autosomal recessive or X-linked to having a multifactorial inheritance (Mostowska, Kobielak, & Trzeciak, 2003). A polygenic influence on the size and patterning of the dentition has been suggested, where a general trend of having the mesio-distal size crowns of the teeth present to be relatively small (notably if more teeth are missing) was observed. Despite the fact that genetic factors are implicated, epigenetics, and environment can also be involved in the etiology (Brook et al., 2009). Mutations in paired box 9 (*PAX9*), muscle segment homeobox 1 (*MSX1*), and Axis inhibitor 2 gene (*AXIN2*) have been shown to be involved in human dental agenesis. The characteristic pattern of dental agenesis caused by *PAX9* mutations widely affects molars in both dental arches and second premolars most often in the maxilla than the mandible, occasionally presenting with missing or peg-shaped mandibular central incisors and/or maxillary lateral incisors. *MSX1* and *AXIN2* gene mutations can lead to hypodontia or oligodontia (J. K. Hartsfield Jr, 2011).

J. Orthodontic studies on heritability of malocclusion

Heritability is a descriptive of variances within a sample at a given time, 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, thus, it is not predictive (J. K. Hartsfield Jr et al., 2012). It relies principally on correlations between relatives and on the statistical procedure of analysis of variance and it is expressed as a percentage (Peter A Mossey, 1999).

Heritability estimates only include additive genetic influences and do not take into account genetic and environmental interactions. In addition, heritability estimates refer to a specific sample and do not necessarily pertain to the situation of a given individual, even from within the sample. Thus, they do not allow one to tell to what degree a particular trait was determined by genetic or environmental factors in a single individual. (J. K. Hartsfield Jr et al., 2012).

Heritability in craniofacial and dental morphology can be determined by the twin method or by familial studies (Peter A Mossey, 1999).

Twin studies have provided much useful information on complex genetic traits by evaluating the observed differences in monozygotic (identical) twins (MZ) and attributing the differences to environmental factors. With dizygotic (fraternal) twins (DZ), who share 50% of their total gene complement, differences are concluded to be due to genetic and environmental factors. This provides an insight into the role of genetic effects and the effects of shared and unique environmental effects. When comparing monozygotic to dizygotic twins (noting that the twin pairs were raised in relatively identical environments), traits that show greater similarity in MZ than in DZ are undoubtedly allocated to a shared genetic basis for the trait in concern. Francis

Galton in 1889 was the pioneer in discussing the assets of the twin investigations (Patel, Gupta, & Sharma, 2012).

1. Growth and development

The co-twin method was employed by Picacintini on six sets of same-gender triplets in whom the zygosity had previously been determined to a high degree of probability. It disclosed significantly lesser intra-pair differences in MZ compared to DZ for combined increment growth of maxilla and mandible, ascertaining the role of heredity in reigning the growth of both jaws (Piacentini, 1962).

Dental development was also proven to be genetically determined for its most part, when studying the dental developmental stages for mandibular 1st and 2nd molars in two sets of triplets (Garn, Lewis, & Polacheck, 1960).

2. Craniofacial complex

The morphology of all the bones of the craniofacial complex was found to be under strict control of heredity in a twin study where roughly complete concordance was found in the craniofacial complex in MZ triples, however, at a lower degree in DZ triplets (Kraus, Wise, & Frei, 1959).

Lower facial height is also highly determined by heredity as it displayed great variability, while the upper facial height is the more stable element, not contributing to the genetic variability of the face as a whole. This was confirmed in a cephalometric study in 56 twin pairs. Moreover, the anterior cranial base, mandibular body length disclosed significant genetic variations (Horowitz, Osborne, & DeGeorge, 1960).

3. Tooth size and occlusal variations

Heredity is a vital factor in malocclusion. Lundstrom uncovered that in Cl I cases, 87.3% of MZ twins and 84.6% of DZ twins had concordance, versus 67.7% and 10% respectively in Cl II (Lundström, 1948).

Another study carried out on 54 pairs like sexed adult twins came to conclude a strong genetic component of variability of the four maxillary and mandibular incisors, with a low hereditary component of variability for canines (Horowitz, Osborne, & DeGeorge, 1958).

As for occlusal variations, teeth displacement and crossbite were observed to be the most significant heritable criteria in a sample of 32 MZ and 28 DZ twin-pairs. No correlations were found for overjet, buccal segment relation, rotations/displacements and overbite, signifying a substantial increased environmental component of variance in occlusion (Corruccini & Potter, 1980).

CHAPTER III
MATERIAL AND METHODS

A. Target population

1. General characteristics

This is a prospective case-control study where 11 Mediterranean families were approached given that features of MM have been seen to segregate over at least 2 generations, since they were or still ongoing treatment at the Division of Orthodontics and Dentofacial Orthopedics of the American University of Beirut Medical Center, AUBMC. Pedigrees of the 11 families were drawn, displaying affected and non-affected individuals. 5 families including 29 subjects agreed to undergo the data and biospecimen collection procedure. The Institution Review Board (IRB) at the American University of Beirut approved the protocol of the study (Protocol Number: BIO.2018.0289) prior to initiation of the study. No potential risk of breach of confidentiality was present in view of the fact that the privacy of the subjects was only evaluated by the research group members. Medical Practice Plan (MPP) and the University Research Board (URB) funded the research.

2. Inclusion criteria

Families that were included comprised affected probands over at least 2 generations and with an average of 5 individuals per family to start our genetic screening (in small families, all the members were included). Family members were blood relatives (no stepchildren) and part of the eastern Mediterranean population. Affected probands were identified through the routine radiographic measurements of all

orthodontic patients exhibiting Cl II, division 1 malocclusion within the Division of Orthodontic and Dentofacial Orthopedics. Subjects considered as affected had an ANB angle $\geq$ 4.5 degrees and/or a positive wits appraisal +2.0 mm accompanied with a reduced mandibular length (Co-Gn and Co-Go) below at least one standard deviation of the norm for their age and a dental Cl II malocclusion at least on one side (with an increased OJ). Non-affected family members were also included as controls, where mandibular linear measurements were within one standard deviation and the inter-jaw relationship indicates a Cl I malocclusion and a straight profile.

3. Exclusion criteria

Subjects having a Cl II, division 1 malocclusion yet, with the mandible being retrognathic (of normal mandibular length) were excluded. Pregnant women and individuals with a general physical disease or congenital disorders (such as cleft lip and palate and syndromic conditions) were disqualified.

Table III-1: Selection criteria for affected individuals

Inclusion Criteria	Exclusion Criteria
- ANB angle of equal or greater than 4.5 degrees, - Positive Wits appraisal greater than + 2.0 mm, - Mandibular linear measurements less than 1 SD, - Bilateral full Class II molar/canine relationship.	- Subjects having Class II with mandibular retrognathia, but of normal mandibular length, - General physical disease or congenital disorders such as cleft lip/palate and syndromic conditions - Pregnant women

Table III-2: Selection criteria for non-affected individuals

Inclusion Criteria	Exclusion Criteria
- Inter-jaw relationship indicating a Class I malocclusion (Normal overjet and straight lower face profile). - ANB angle of 2 ± 2 degrees, - Wits appraisal of 0 ± 1 mm, - Mandibular linear measurements within 1 SD.	- General physical disease or congenital disorders such as cleft lip/palate and syndromic conditions - Pregnant women

B. Families selection and recruitment process

11 individuals from the eastern Mediterranean population diagnosed with MM (because of previous or ongoing treatment at the Division of Orthodontics and Dentofacial Orthopedics, AUBMC) were approached by their treating orthodontist and then the study coordinator structured the pedigrees of their corresponding families in order to clarify the families' structure in term of number of affected and non-affected males and females, mode of inheritance and any consanguinity.

The recruitment process was as follows:

a- After being first approached by the treating orthodontist, patients were then addressed by the study coordinator and asked if they agree to participate in the research project.

b- Written informed consent was signed in a private environment by the selected subjects, upon their agreement to take part in the research project. The study protocol was explained to them in details. Consent forms were constructed to each age category (child between 7-12 years, adolescent between 13-17 years, adult and parental consents) and signed suitably. Explicit information about the aims of the study, the procedure, the risks and benefits and a confidentiality section were detailed in the consent forms.

c- Study coordinator asked about the medical history of each individual and it included demographics: gender, date of birth, age, family origin or racial/ethnic background, health status: congenital disorders, genetics: consanguinity, previous genetic test, number of affected subjects over at least 2 generations**.**

d- Flyers (invitation to participate in a study) were given to subjects in concern to distribute them to their affected and non-affected relatives. The flyers include general information about the study, its purpose, benefits, location…

e- Interested relatives in the study contacted the research team (at 01- 350000 ext. 5702) for further information and/or participation process. They were then requested to come to the Division of Orthodontics and Dentofacial Orthopedics, AUBMC to sign the consent form. In the case of minors, the consent form was signed by a parental guardian.

f- The subjects were then diagnosed by means of a clinical examination and a lateral cephalometric radiograph (done only on subjects on whom features of a MM are noted clinically). The lateral cephalometric radiograph was taken in our division in natural head position and with the patients' jaws in centric occlusion (posterior teeth in maximum intercuspation). Patients and/or guardians benefited from a free diagnosis and treatment was suggested. Subjects already under treatment would have had the series of records taken before initiation of therapy. No radiographs were obtained from patients who have had one within the past year.

Biospecimen collection consisted of 5cc of blood withdrawal from both affected and non-affected individuals. 5 eastern Mediterranean families (4 Lebanese, 1 Syrian/Jordanian) including a total of 29 individuals (16 affected, 13 non-affected) agreed to enroll. Biospecimen collection entailed all 29 individuals and, as previously stated, the lateral cephalogram was taken on the 14 affected individuals. Each one of the selected 29 individuals was assigned a specific code that includes the family code (A-E) followed by an Arabic numeral (1-12) (Tables III.3-III.4)

Table III-3: Distribution of the ethnicities included in this study.

Families who were first approached and their pedigree drawn	**Families who had data and biospecimens collected**
11 (10 Lebanese and 1 Syrian/ Jordanian)	5 (4 Lebanese and 1 Syrian/ Jordanian)

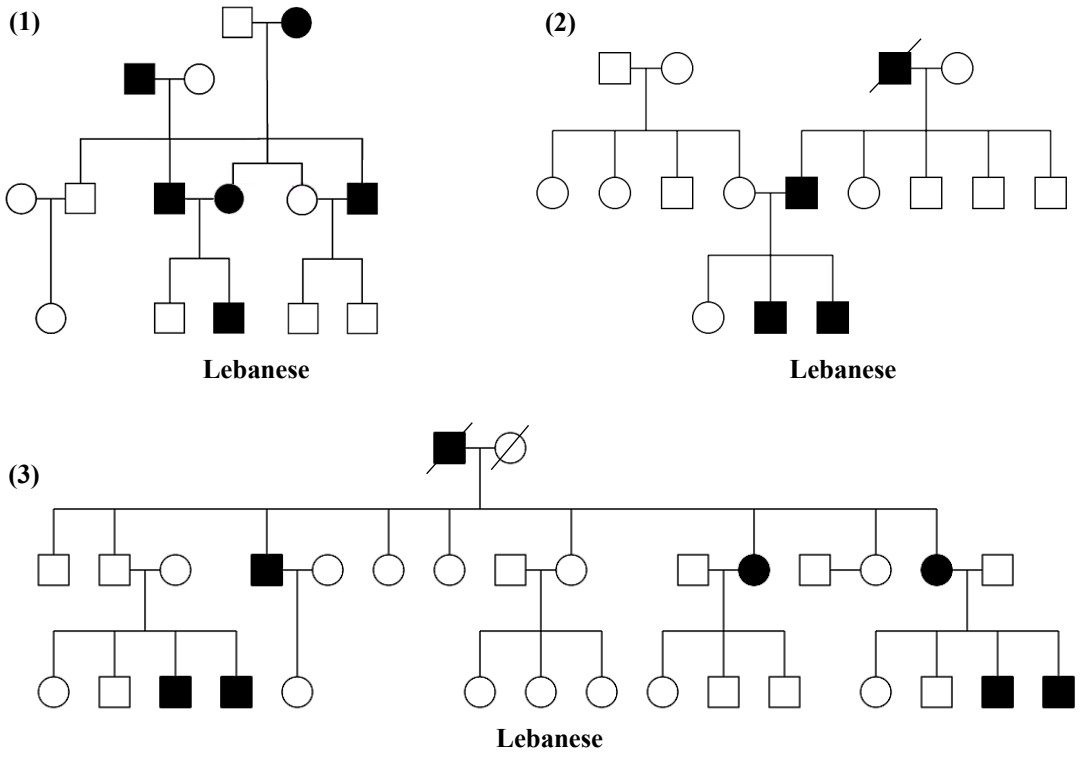
Table III-4: Summary of the demographic characteristics of the 5 families.

	Ethnicity	Blood collection				Lateral cephalometric 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	8	4	6	6	4	1
Family (B)	Lebanese	2	2	2	2	2	0
Family (C)	Lebanese	1	4	2	2	1	1
Family (D)	Syrian/ Jordanian	2	2	3	1	1	1
Family (E)	Lebanese	2	2	2	2	1	1
Total		**15**	**13**	**15**	**13**	**9**	**4**
TOTAL		**28**		**28**		**13**	

C. Families' structure (pedigrees)

1. Pedigrees of the 11 approached families

Pedigrees of the 11 eastern Mediterranean families that were approached are illustrated below (Figure III-1):



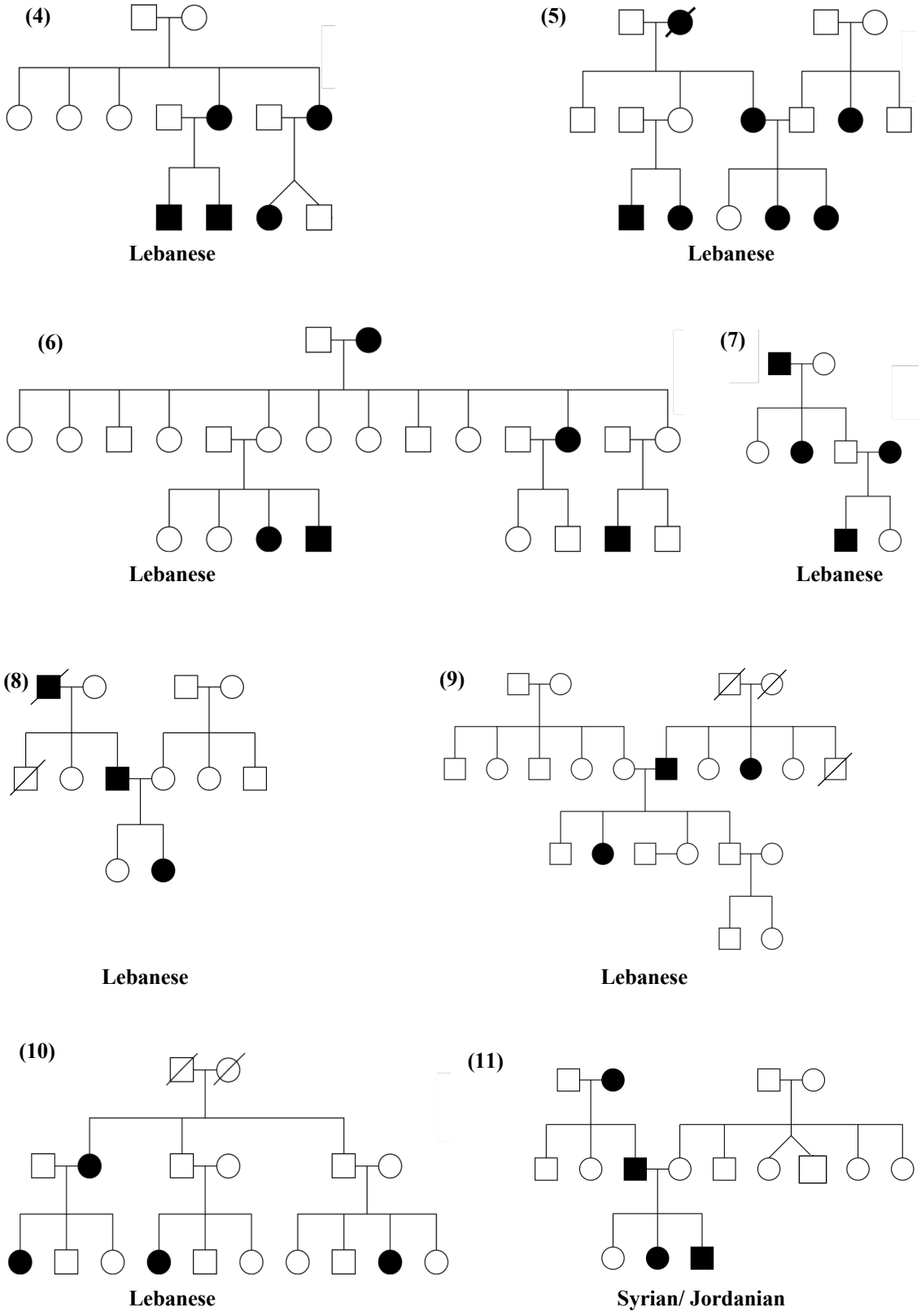


Figure III-1: Pedigrees of the 11 Eastern Mediterranean families

The 5 families that underwent data and biospecimen collection procedure correspond to the following pedigrees: 1, 2, 7, 8, 10 (4 Lebanese, 1 Syrian/ Jordanian)

2. Pedigrees of the 5 selected families

The 5 families that accepted to enroll in the study are illustrated below (Figures III.2-III.7), with the subjects from whom blood was collected underlined in red and on whom a lateral cephalogram was taken underlined in green. The selected subjects are numbered using Arabic numerals. 4 families are Lebanese and 1 family is Syrian/ Jordanian.

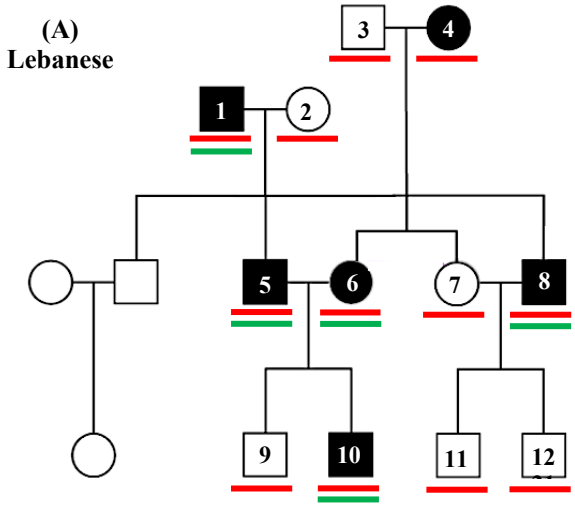


Figure III-2: Pedigree of the selected family (A)

Family's structure pedigree comprised of 3 generations with a total of 15 individuals including 6 affected individuals (4 males versus 2 females) and 9 non-affected. Consanguinity is not noted in the family. The mode of transmission is autosomal dominant. Blood was collected from 12 subjects (6 affected and 6 non-affected) and a lateral cephalogram was taken on 5 of the affected individuals.

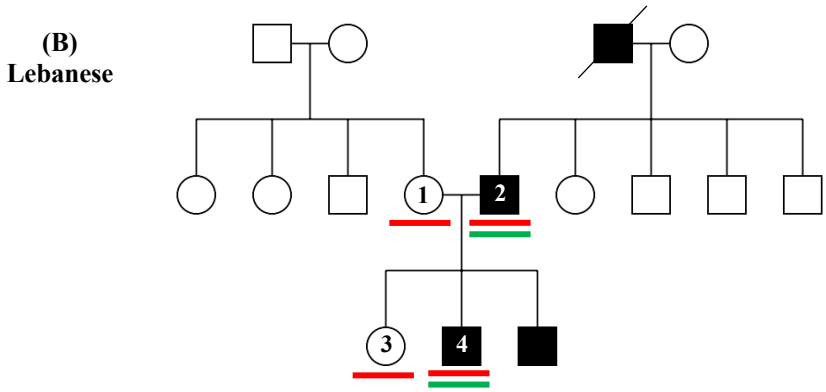


Figure III-3: Pedigree of the selected family (B)

Family's structure: pedigree comprised of 3 generations with a total of 17 individuals including 4 affected (all males) and 13 non-affected. Consanguinity is not noted in the family. The mode of inheritance is autosomal dominant. Blood was collected from 4 individuals (2 affected and 2 non-affected) and a lateral cephalogram was taken on 2 affected subjects.

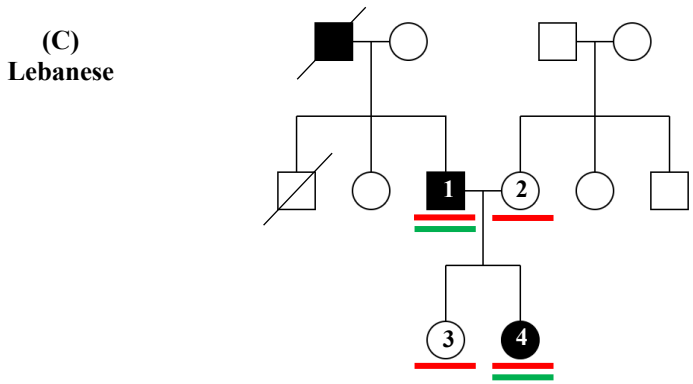


Figure III-4: Pedigree of the selected family (C)

Family's structure: pedigree comprised of 3 generations with a total of 12 individuals including 3 affected (2 males and 1 female) and 9 non-affected. Consanguinity is not noted in the family. The mode of inheritance is autosomal dominant. Blood was collected on 4 individuals (2 affected and 2 non-affected) and a lateral cephalogram was taken on 2 affected subjects.

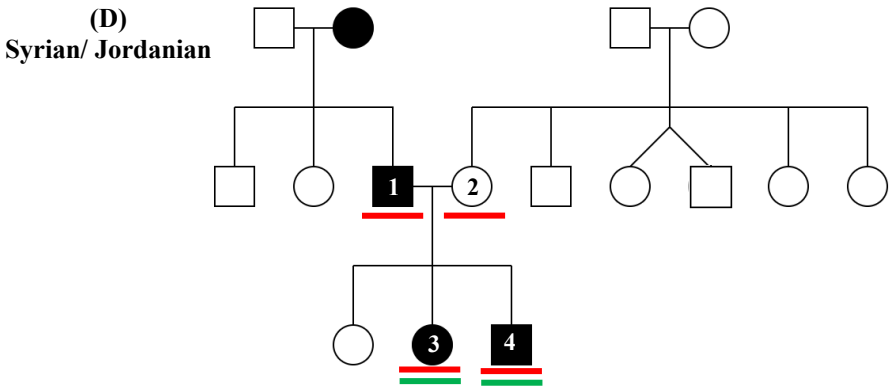


Figure III-5: Pedigree of the selected family (D)

Family's structure: pedigree comprised of 3 generations with a total of 16 subjects including 4 affected (2 males and 2 females) and 12 non-affected. Consanguinity is not present in the family. The mode of inheritance is autosomal dominant. Blood was collected on 4 subjects (2 affected, 2 non-affected) and a lateral cephalogram was taken on 2 affected individuals.

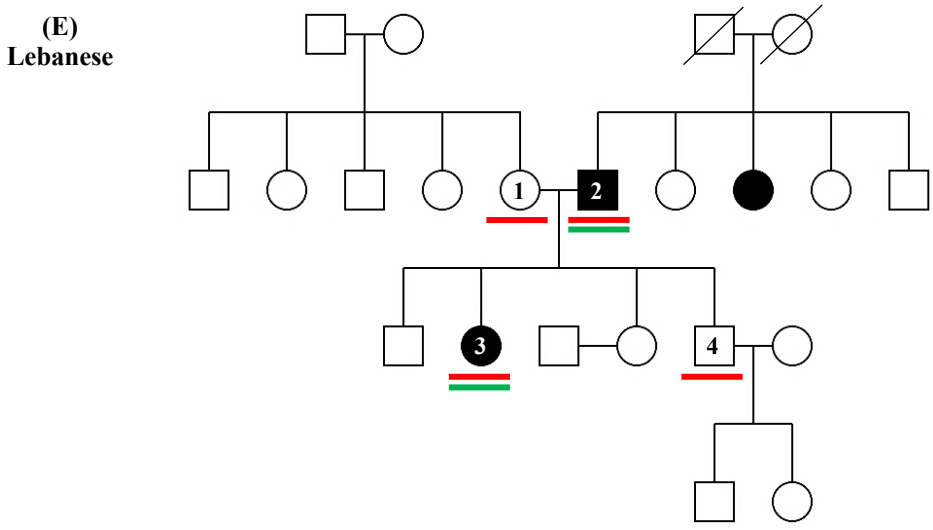


Figure III-6: Pedigree of the selected family (E)

Family's structure: pedigree comprised of 4 generations with a total of 22 subjects including 3 affected (2 females and 1 male) and 19 non-affected. Consanguinity is not present in the family. The mode of inheritance is autosomal dominant. Blood was

collected on 4 subjects (2 affected, 2 non-affected) and a lateral cephalogram was taken on 2 affected individuals.

In summary, the genetic analysis was performed on 28 subjects (15 affected and 13 non-affected) after withdrawal of 5cc of blood. A lateral cephalogram was taken on 13 affected individuals. Consanguinity was not present in any family and the mode of inheritance was autosomal dominant.

D. Clinical examination and cephalometric analysis

1. Description of the means of diagnosis

The probands who are part of the 11 families were diagnosed following clinical examination where features of MM were noted clinically. A lateral cephalogram was taken only on subjects exhibiting characteristics of the phenotype. Subjects already under treatment have had the series of records taken before initiation of therapy. No radiographs were obtained from patients who have had one within the past year. The lateral cephalogram was taken in our division in natural head position with the patients' jaws in centric occlusion (posterior teeth in maximum intercuspation) and their lips in a gentle touch. The radiographs were taken in the division of Orthodontics and Dentofacial Orthopedics, AUBMC and stored in the corresponding bank of radiographs generated and housed in the corresponding radiologic software (CLINIVIEW). Subsequently, they were copied by the study coordinator (MC) and placed in a separate digital folder named ''lateral cephalogram'' on a computer in our division and codes were applied so that the folder can only be accessed by the study coordinator.

The lateral cephalograms were digitized and analyzed by the study coordinator using the Dolphin Imaging program (version 11.5, La Jolla, California) and Cliniview 9.3. The advantages of using the imaging program include:

1. The computer software is consistent and decreases the operator time and efforts. It is easily manipulated and provides accurate measurements and instant reading of linear and angular measurements of corresponding landmarks.

2. The use of mathematical algorithms to generate digitized cephalometric tracings.

3. The available options of enhancing the tracings for adequate assessment of the bony and soft tissue structures.

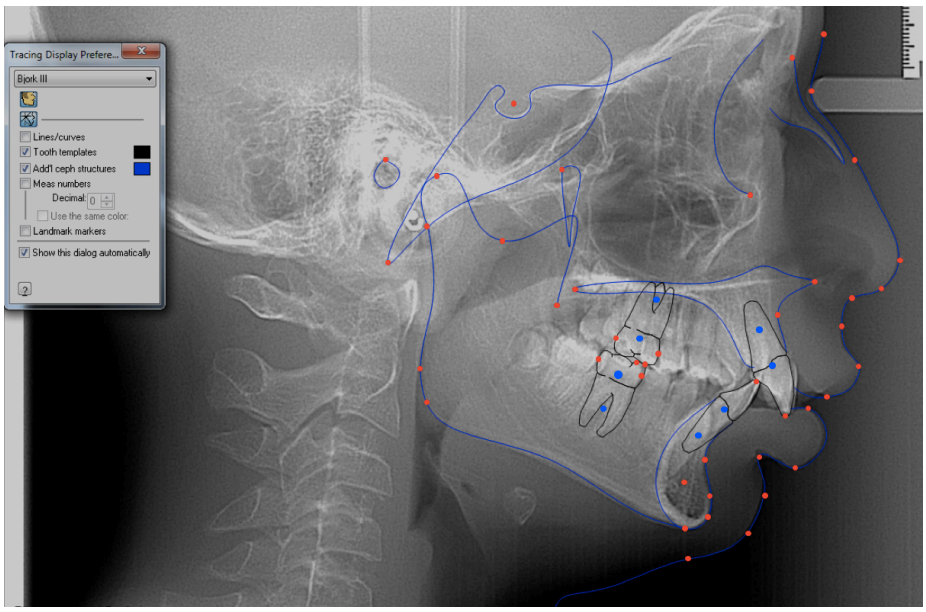


Figure III-7: Digitized lateral cephalogram with soft and hard tissue landmarks

2. Cephalometric landmarks

All landmarks used for the digitization are shown in Figure.III.7, and the corresponding definitions are displayed in Tables III.5, 6 and 7.

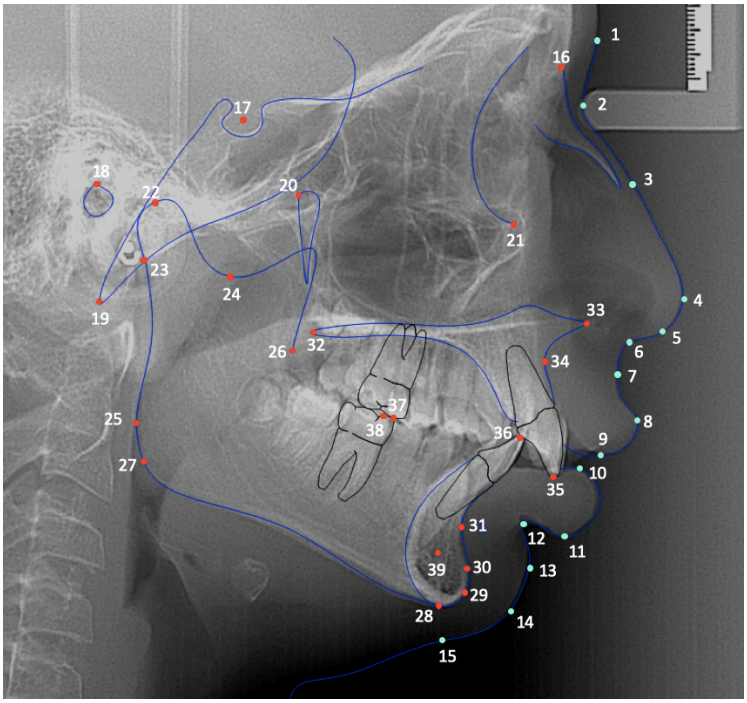


Figure III-8: Lateral cephalogram tracing displaying the used anatomic landmarks

Table III-5: Soft tissue landmarks

Landmark	Number	Definition
Glabella	1	Most anterior point in the mid-sagittal plane of the forehead at the level of the superior orbital ridges
Soft tissue nasion	2	Point of intersection of the soft-tissue profile with a line drawn from the center of sella turcica through nasion
Bridge of nose	3	Mid-way between the soft tissue N and tip of nose
Tip of nose	4	Most prominent or anterior point of the nose tip
Columella	5	Most anterior soft tissue point on the columella
Subnasale	6	Midpoint of the columella base at the apex of the angle where the lower border of the nasal septum and the upper lip surface meet
Soft tissue A point	7	Deepest point on the upper lip determined by an imaginary line joining subnasale with the laberale superius
Superior lip	8	Midpoint of the upper vermilion line
Stomion superior	9	Most inferior point located on the upper lip
Stomion inferior	10	Most inferior point located on the lower lip
Lower lip	11	Midpoint of the lower vermilion line
Soft tissue B point	12	Point at the deepest concavity between laberale inferius and soft-tissue pogonion
Soft tissue pogonion	13	Most anterior point on the soft tissue chin in the mid-sagittal plane
Soft tissue gnathion	14	Midpoint between soft tissue pogonion and soft tissue menton
Soft tissue menton	15	Most inferior point on the soft tissue chin

Table III-6: Hard tissue landmarks

Landmark	Number	Definition
Nasion (N)	16	Middle point of the junction between the frontal and the two nasal bones (frontonasal suture)
Sella (S)	17	Center of sella turcica, located by inspection
Porion (Po)	18	Highest point on the roof of the external auditory meatus

Basion (Ba)	19	Most inferior point on the anterior margin of the foramen magnum in the midsagittal plane
Pterygoid point (Ptm)	20	Most posterior point on the outline of the pterygopalatine fossa
Orbitale (Or)	21	Lowest point on the lower margin of the orbit.
Condylion (Co)	22	Most posterior and superior point on the mandibular condyle
Articulare (Ar)	23	Intersection of the radiographic image of basi-occipital (middle structure) and the radiographic image of the posterior border of the condylar process
Sigmoid notch	24	Deepest point on the most inferior border along the top of the ramus
Ramus point	25	Most posterior point up the border of the ramus
Mid ramus	26	Most concave point of the inferior of the ramus
Gonion (Go)	27	External angle of the mandible, located by bisecting the angle formed by tangents to the posterior border of the ramus and the inferior border of the mandible
Menton (Me)	28	Most inferior point on the mandibular symphysis of the mandible, in the median plane
Gnathion (Gn)	29	Midpoint between Me and Pog on the contour of the chin on the mid-sagittal plane
Pogonion (Pog)	30	Most anterior point on the mid-sagittal symphysis
B point	31	Deepest (most posterior) midline point on the bony curvature of the anterior mandible, between infradentale and pogonion. Also called supramentale (Downs)
Posterior nasal spine (PNS)	32	Most posterior point on the contour of the bony palate
Anterior nasal spine (ANS)	33	Most anterior point of the nasal floor; tip of the premaxilla on the midsagittal plane
A point	34	Deepest (most posterior) midline point on the anterior contour of the maxilla [curvature between ANS and prosthion (dental alveolus)]. Also called subspinale (Downs)
D point	39	Center of the symphysis

Table III-7: Dental landmarks

Landmark	**Number**	**Definition**
U1	35	Most proclined maxillary incisor (at the incisal edge)
L1	36	Most proclined mandibular incisor (at the incisal edge)
U6	37	Maxillary first molar
L6	38	Mandibular first molar

3. Cephalometric measurements

In order to select and confirm subjects affected with MM, angular and linear measurements were constructed on the lateral cephalogram. Characteristics of the cranial base and each jaw, as well as the relationships of the jaws to the cranial base and to each other was also gauged.

a) Measurements of the: cranial base, maxilla, facial heights, vertical and sagittal relationships between the jaws and dento-alveolar relationships (Table III.8; Fig. III.8)

Table III-8: Definitions of cephalometric measurements related to the cranial base, maxilla, jaws and teeth.

	Measurement	Definition	Category
1	SN	Length of the anterior cranial base	Cranial base
2	S-Ar	Length of the posterior cranial base	
3	SN/H	Angle between anterior cranial base and the true horizontal	
4	N-S-Ar	Saddle angle	
5	ANS-PNS	Maxillary length	Maxilla
6	N-ANS	Linear measurement between nasion and anterior nasal spine	Facial heights
7	ANS-Me	Anterior facial height	
8	PFH	Posterior facial height	
9	LFH/TFH	Ratio between lower facial height and total facial height	
10	AFH/PFH	Facial height index: ratio between anterior and posterior facial heights	
11	MP/SN	Angle between cranial base cant and mandibular plane (MP)	Vertical relationship between the jaws (facial divergence)
12	MP/H	Angle between mandibular plane (MP) and the true horizontal	
13	PP/MP	Angle between palatal plane (PP) and mandibular plane (MP)	
14	PP/H	Angle between palatal plane (PP) and the true horizontal	
15	SNA	Angle between anterior cranial base cant and point A	Sagittal relationship between the jaws
16	SNB	Angle between anterior cranial base cant and point B	
17	ANB	Angle between points A and B	
18	AOBO	Distance between the perpendiculars drawn from A and B points to the occlusal plane	
19	I/NA	Angle between maxillary incisor long axis and a line joining nasion and A point	Dento-alveolar relationships
20	I-NA	Distance between maxillary incisor long axis and a line joining nasion and A point	
21	I/SN	Angle between maxillary incisor long axis and anterior cranial base	
22	I/PP	Angle between maxillary incisor long axis and palatal plane	
23	i/NB	Angle between mandibular incisor long axis and a line joining nasion and B point	
24	i-NB	Distance between mandibular incisor long axis and a line joining nasion and B point	
25	i/MP	Angle between mandibular incisor long axis and mandibular plane (MP)	
26	I/i	Angle between maxillary and mandibular incisors long axes	
27	OJ	Horizontal distance between the incisal edges of maxillary and mandibular incisors	
28	OB	Vertical distance between the incisal edges of maxillary and mandibular incisors	

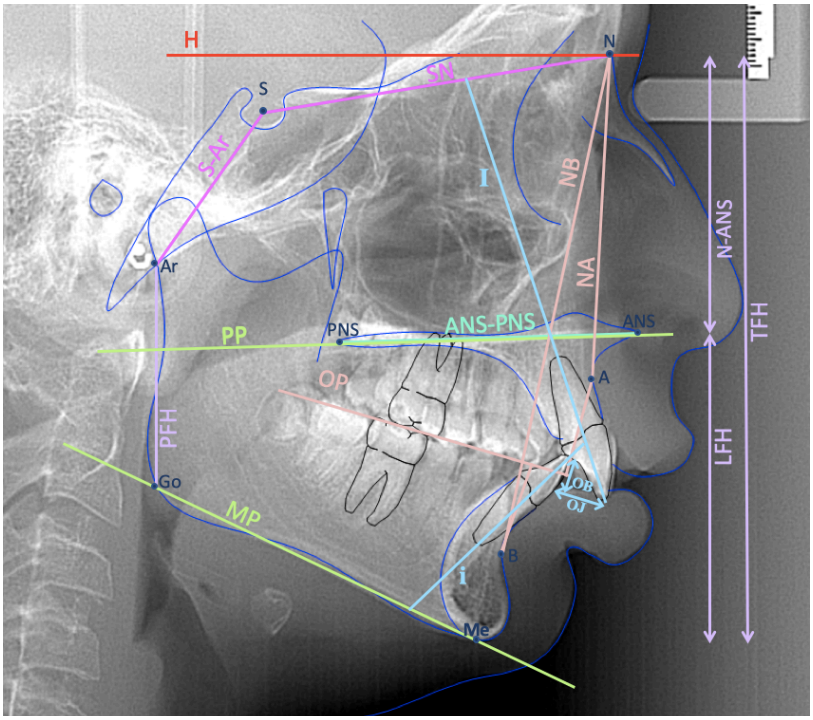


Figure III-9: Lateral cephalometric tracing with landmarks and angles describing the cranial base, maxilla, facial heights, vertical and sagittal relationships between the jaws and dento-alveolar relationships.

b) Measurements at the level of the mandible (Table III.9, Fig. III.9)

Table III-9: Definitions of cephalometric measurements related to the mandible.

	Measurement	Definition
1	Ar-Go-Gn	Angle of the mandible between the ramus and the mandibular plane
2	Ar-Go-Me	Angle of the mandible between the ramus and the mandibular plane
3	Co-Go-Me	Angle of the mandible between the ramus and the mandibular plane
4	Ar-Gn	Length of the mandible
5	Co-Gn	Length of the mandible
6	Co-Pog	Length of the mandible
7	Co-Go	Length of the ramus of the mandible
8	Ar-Go	Length of the ramus of the mandible
9	Go-Me	Length of the body of the mandible
10	Go-Gn	Length of the body of the mandible
11	Go-Pog	Length of the body of the mandible

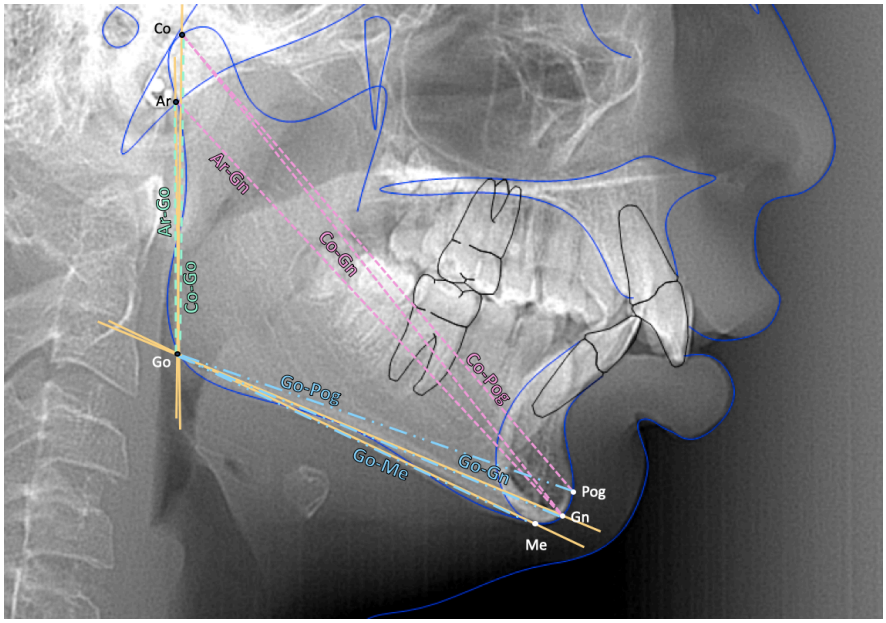


Figure III-10: Lateral cephalometric tracing with mandibular landmarks and angles.

c) Measurements at the level of the symphysis (Table III.10,11; Fig. III.10A-10B)

Table III-10: Definitions of cephalometric measurements related to the symphysis.

	Measurement	Definition
1	Chin width at the level of the mandibular incisor apex	Line through the apex, parallel to the horizontal, intersecting anterior and posterior contours of symphysis
2	Chin width at the level of point D	Line through D parallel to the horizontal, intersecting anterior and posterior contours of symphysis
3	Distance between point D and mandibular incisor apex	D to apex
4	Distance between point D and menton	D to Me

Table III-11: Definitions of cephalometric measurements constituting the components of the chin.

	Measurement	Definition
1	Anterior slope plane	Through Pogonion and B points
2	Posterior slope plane	Through Pogonion 1 (Po1: most convex point on the posterior symphyseal cortical) and point B1 (intersection of the parallel to Po–Po1 through B and the posterior cortical of the symphysis)
3	Ant slope of the chin/ V	Angle between the anterior slope of the chin and the vertical
4	Post slope of the chin/ V	Angle between the posterior slope of the chin and the vertical
5	Angle anterior/posterior slopes	Angle between the anterior slope plane and the posterior slope plane

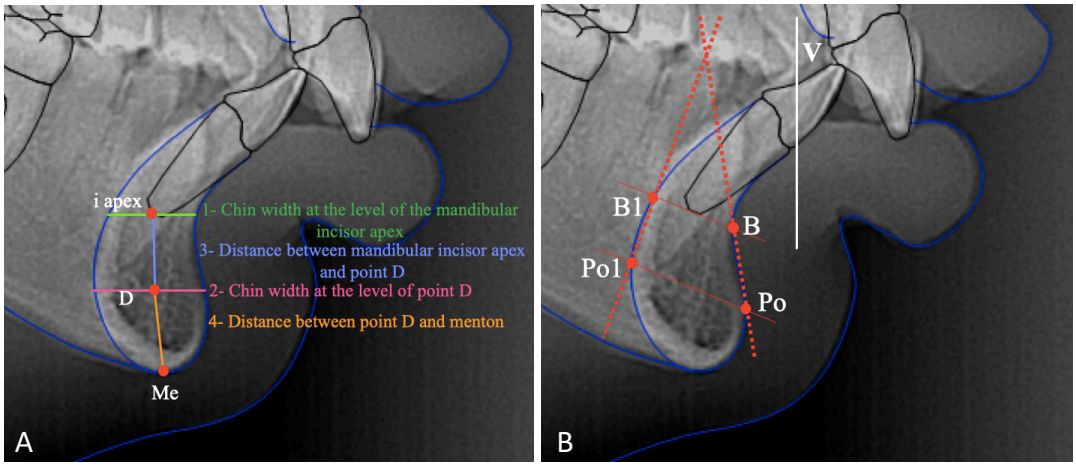


Figure III-11: Cephalometric tracing describing (A) the relationship between point D, mandibular incisor and menton (B) component analysis of the symphysis.

The potential risk associated with the lateral cephalogram is the radiation dose. However, the effective dose of a single cephalogram is 1.7 mrem, which is considered minor. As previously mentioned, the lateral cephalogram was only taken for subjects who exhibited the phenotype in question. This radiograph is part of the pre-treatment records normally taken and justified for proper diagnosis for patients. For other subjects, the radiograph may represent an opportunity for evaluation of present or past problems in the relations between the jaws. If they had had the same radiograph in the last year with a good quality, no additional exposure would be needed.

Subjects were classified as affected if, as mentioned in the inclusion criteria, the cephalometric analysis revealed a positive ANB of $\geq$ 4.5 degrees with a positive Wits appraisal $\geq$ +2mm and associated with a reduced mandibular length (Co-Gn) below one standard deviation of the corresponding norm to their age. An increased overjet was also noted. Subsequently, an accurate pedigree was established for every family that exhibited multiple affected individuals to help establish the mode of inheritance. The Z score was calculated for each individual, depending on the value of

his/her mandibular length (Co-Gn). The Z score is the difference between the value and the mean for the specific age, divided by the standard deviation.

4. Repeated measurements

Intra-examiner reliability of the measurements was assessed by choosing randomly, re-digitizing and analyzing 10 lateral cephalograms (43% 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 0.956.

5. Statistical analysis

The cephalometric analysis aimed at finding the differences at the level of cephalometric measurements between subjects affected with MM and a control group (that consisted of unaffected individuals with a straight profile and a Cl I malocclusion, retrieved from the pool of patients at the Division of Orthodontics and Dento-facial orthopedics). The differences were gauged using a two-sample independent *t-test.* An ANOVA test was used to determine the p value; the results were considered statistically different if the p value was ≤ 0.05. Then, a descriptive analysis and an ANOVA test were performed on the measurements of the 5 selected families to evaluate differences between them.

E. Genetic procedure

In order to explore the genetic implication behind MM, isolation of genomic DNA from whole blood cells was required from both affected and non-affected subjects in order to facilitate the analysis by associating the genotype to the underlying phenotype. The extracted DNA was evaluated for quantity and quality using the

Nanodrop at the American University of Beirut Molecular Core Facility. The experimental genetic procedure was then carried out by Macrogen in Korea (dna.macrogen.com) using the NovaSeq 6000 platform. The NovaSeq 6000 System leverages proven Illumina sequencing by synthesis (SBS) technology to deliver accurate data and robust performance. This proprietary reversible terminator–based method enables the massively parallel sequencing of billions of DNA fragments, detecting single bases as they are incorporated into growing DNA strands. The method significantly reduces errors and missed calls associated with strings of repeated nucleotides (homopolymers). Whole exome sequencing (WES) was employed to efficiently identify coding variants. Data generated from WES was analyzed by Dr. Georges Nemer (Professor and head of Basic Science Affairs and genetic unit at FM), who has ample expertise in this field, by means of special softwares.

1. Blood collection

This procedure entailed withdrawal of 5cc of blood from the identified affected individuals along with non-affected individuals from the same family. Non-affected subjects served as controls.

The following protocol was adopted:

1. Patient was positioned in the chair.
2. A tourniquet was placed 3 to 4 inches above the puncture site on the patient and a suitable site for venipuncture was selected.
3. A vein is selected after palpation (Figure III.11A) and then the area was cleaned using an alcohol pad in a circular motion beginning at the site and working outward.
4. While the area was allowed to dry, the patient was requested to make a fist and his arm was held using the thumb.

5. The needle with the vacutainer was inserted through the skin into the lumen of the vein at a 15-30 degrees angle with the surface. For young subjects, the butterfly set, or winged infusion set, was used as it tolerates more movements (Figure III.11B- III.11C)

6. The tourniquet was removed as the Capillary Blood Collection (CBC) tube was filling. CBC tubes that were used were with a purple/lavender top color (i.e. the interior of the tube wall is coated with EDTA K2 or K3).

7. The needle was removed promptly, and a gauze was placed immediately on the puncture site along with some pressure to avoid formation of hematoma.

8. A fresh piece of gauze with a tape or a Band-Aid was placed over the puncture site.

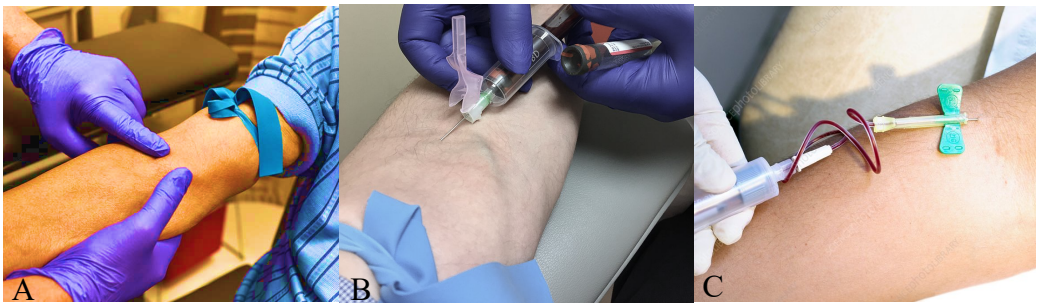


Figure III-12: Various steps of blood withdrawal (A) vein palpation (B) venipuncture with a needle and vacutainer (C) Winged infusion set

Blood samples were stored at 4°C temperature in a refrigerator, pending DNA extraction which was completed within 0 to 10 days after blood withdrawal.

Any potential risk associated with blood withdrawal (bruising, pain, hematoma, and slight possibility of infection or fainting) was considered minimal since the procedure was done at the hospital (AUBMC), by a specialized nurse or physician, using a clean needle and the AUBMC Laboratory Medicine rules and regulations were followed, including any information provided by this department on a routine basis.

AUBMC was to cover the cost of treating, on its premises, medical adverse events resulting directly from the medical procedures of this research study. However, no incidents were encountered with any participant.

2. DNA extraction

Genomic DNA was isolated using the Qiagen Blood-Midi kit (Qiagen Science Inc., Germantown, MD), as per the manufacturer recommendations. The workflow was as follow:

a. Cell lysis

1. Pipet 200 µl QIAGEN Protease (5.5ml H_2O to the powder) (to denature the proteins and keep the DNA intact) into the bottom of a 15 ml centrifuge tube (conical centrifugation tube).

2. Add 2 ml blood and mix briefly.

3. Add 2.4 ml of lysis buffer, and mix thoroughly by inverting the tube 15 times, followed by additional vigorous shaking for at least 1 min. To ensure adequate lysis, the sample must be mixed thoroughly with the buffer to yield a homogenous solution.

4. Incubate at 70°C for 10 min. DNA yield reaches a maximum after lysis for 10 min at 70°C, but longer incubation times will not adversely affect yield.

5. Add 2 ml of ethanol (96–100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking for 30 seconds. This allows the DNA to precipitate from the lysed cells.

To ensure efficient binding, it is essential that the sample is mixed thoroughly after addition of ethanol to yield a homogeneous solution.

b. DNA purification: removal of the cellular debris

1. Carefully transfer one half of the solution (≈ 3ml) from step 5 onto the QIAamp Midi column placed in a 15 ml centrifuge tube. Close the cap and centrifuge at 3600 rpm for 10 minutes at 15°C to separate the DNA from the reagents and proteins during the cell lysis step.

If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed.

2. Remove the QIAamp Midi column, discard the filtrate, and place the QIAamp Midi column back into the 15 ml centrifuge tube. Load the remainder of the solution from step 5 onto the QIAamp Midi column. Close the cap and centrifuge again at 3600 rpm for 10 minutes at 15°C.

3. Carefully, without moistening the rim, add 2 ml washing buffer 1 (buffer AW1) to the QIAamp Midi column to clean the reagent. Close the cap and centrifuge at 3600 rpm for 10 minutes.

4. Carefully, without moistening the rim, add 2 ml washing buffer 2 (buffer AW2) to the QIAamp Midi column, also to clean the reagent. Close the cap and centrifuge at 3600 rpm for 10 minutes. At this stage, the DNA moved to the bottom of the tube.

5. Air-dry for 7 minutes to evaporate the ethanol as residual ethanol in the eluate may cause inhibition of PCR leading to false-negative results.

c. DNA elution: removing the DNA from the filter

1. Place the QIAamp Midi column in a clean 15 ml centrifuge tube and discard the collection tube containing the filtrate.

2. Pipet 150 µl od dilution buffer or distilled water, equilibrated to room temperature (15–25°C), directly onto the membrane of the QIAamp Midi column and close the cap. Incubate at room temperature for 5 min, and centrifuge at 3600 rpm for 10 minutes. Repeat the elution step if the quantity is small.

3. Quantify with a nanodrop to know the concentration of DNA in the blood.

4. Store at -20°C with an elution buffer to stabilize the DNA while protecting it from degradation.

3. Genetic analysis

The human exome represents less than 2% of the genome but contains ~85% of known disease-related variants, making the WES a cost-effective method. Exome sequencing has gained recognition in the scientific community as a powerful method for discovering potential causative variants for genetically driven conditions. The NovaSeq 6000 platform was employed for this purpose (Figure III-12). The Illumina sequencing workflow is composed of four basic steps: library preparation, target enrichment, sequencing and data analysis. The first three steps were executed at Macrogen (dna.macrogen.com), while data analysis was carried out the department of biochemistry and molecular genetics at AUB.

Figure III-13: NovaSeq 6000 Illumina sequencer (© 2017 Illumina, Inc.)

a. Library preparation:

The samples were prepared per "Twist Human Core Exome Kit" (provided by Twist Bioscience) preparation guide. Library preparation usually begins with fragmentation, which is basically cutting up the large genomic DNA (gDNA) into smaller-sized fragments. This is important since NGS Platforms like Illumina can only sequence small fragments, averaging 300 bp in length. So instead of sequencing the entire length of the genome one base at a time, Illumina reads millions of smaller fragments in parallel, then assembles them back later.

Next, the fragmented DNA was subjected to a 3-part process: Blunting, A-tailing and adapter ligation. The process of blunting consists of producing "blunt" ends of equal length (which are often of uneven length on the double strand of the fragmented DNA). This is achieved by either removing or filling in base pairs, depending on which strand the overhang is on (Figure III-13).

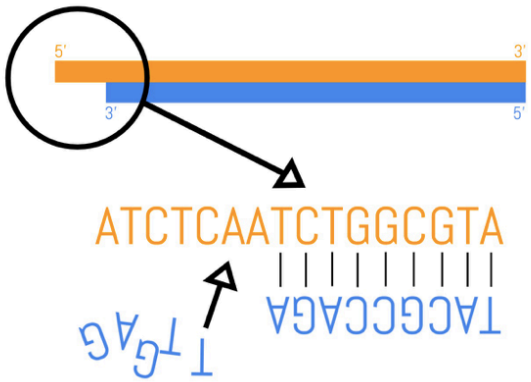


Figure III-14: Blunting of the fragmented DNA during library preparation *(Adapted from source: https://www.twistbioscience.com. Accessed: May 29. 2020).*

The blunted ends were then modified by adding a single adenine (A) nucleotide that forms an overhanging "A-tail". Next, where there was an overhanging A nucleotide, adapters with an overhanging thymine (T) nucleotide formed base-pair

interactions. A covalent bond was established between these two interacting fragments. Each genome fragment now possessed the same sequencing adapters which will incite the DNA reading reaction (Figure III-14). Once adapters were ligated onto each genomic fragment, the genomic library was prepared for the next stage of the exome sequencing protocol: Target Enrichment.

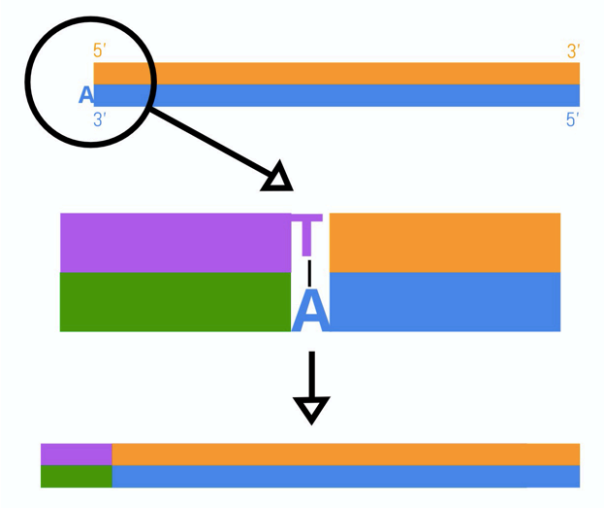


Figure III-15: Sequencing adapters with an overhanging T-tail are ligated to each fragment with an A-tail *(Adapted from source: https://www.twistbioscience.com. Accessed: May 29. 2020).*

b. Target enrichment

Target enrichment is the process of isolating and separating relevant regions of the genome for focused analysis by NGS. Our genomic region of interest is the protein-coding exome.

A hybridization-based target enrichment was carried. The "Twist Human Core Exome Kit" contains double-stranded DNA probes (varying in length between 50 to 120 bp) designed to bond complementary to the exome DNA sequences. The probes were mixed with the genomic sample and then heated to above 95°C to melt the base pair interactions in the double-stranded genomic DNA, forming a pool of single

stranded DNA. Bringing the temperature down allowed the genomic DNA to start to form back into complementary double stranded molecules. As the probes are designed to be complementary with the exome, they also formed base pair interactions with the genomic DNA.

The probes are manufactured to include a biotin molecule on one end. Biotin is a molecule that binds to a protein called streptavidin with one of the strongest interactions known to biology. Magnetic beads coated in streptavidin were then added to the mixture. Once the coated beads were firmly bound to the biotinylated probes, a magnet was used to pull the bound exome DNA out of solution (Figure III-15).

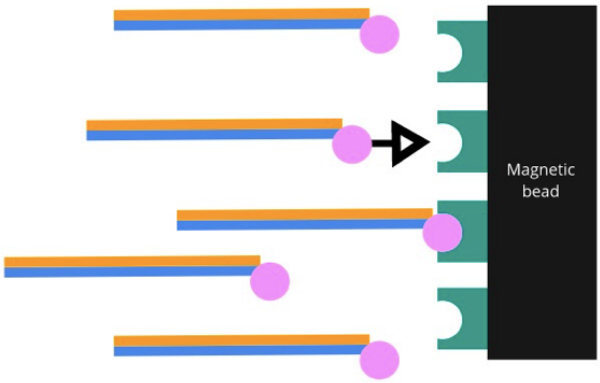


Figure III-16: Biotin (pink) and Streptavidin (green) form one of the strongest molecular interactions in nature. this interaction is used to affix the target capture probes to the magnetic beads. *(Adapted from source: https://www.twistbioscience.com. Accessed: May 29. 2020).*

c. Next Generation Sequencing (NGS)

The enriched target DNA was loaded onto a flow cell and the adapters hybridize to DNA strands affixed into each well on the Illumina flow cell. One single genome fragment is captured per well.

Once the exonic fragments have been captured in the wells by their adapters, the Illumina sequencer begins a process called clustering. Clustering is the process where fragments from the DNA libraries are isothermally amplified. The NovaSeq 6000 system offers the standard workflow featuring fully automated onboard cluster generation for ease of use and reduced hands-on time. Prepared libraries are loaded directly into a sample tube that sits in a preconfigured reagent cartridge, which is loaded directly onto the system for fully automated cluster generation. Priming occurred as the opposite end of a ligated fragment bent over and "bridged" to another complementary oligo on the surface. Repeated denaturation and extension cycles (similar to PCR) resulted in localized amplification of single molecules into millions of unique, clonal clusters across the flow cell, which is sufficient for reporting incorporated bases at the required signal intensity for detection during sequencing. Each fragment was thus amplified into distinct, clonal clusters through bridge amplification (Figure III-16). When cluster generation was completed, the templates were ready for sequencing.

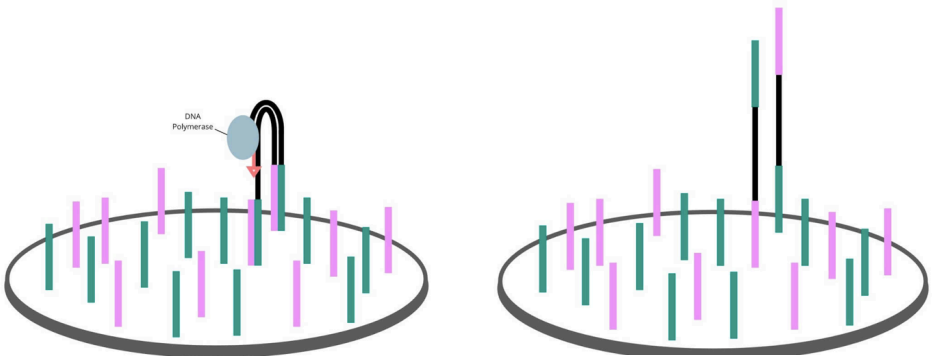


Figure III-17: Cluster generation through bridge amplification *(Adapted from source: https://www.twistbioscience.com. Accessed: May 29. 2020).*

The amplified libraries underwent then WES to determine the exact sequence of nucleotides (adenine, guanine, cytosine and thymine). As previously mentioned, NovaSeq 6000 leverages proven Illumina sequencing by synthesis (SBS) chemistry—

the most widely adopted NGS technology worldwide. SBS technology uses four fluorescently labeled nucleotides to sequence the tens of millions of clusters on the flow cell surface in parallel. During each sequencing cycle, a single labeled dNTP is added to the nucleic acid chain. The nucleotide label serves as a "reversible terminator" for polymerization: after dNTP incorporation, the fluorescent dye is identified through laser excitation and imaging, then enzymatically cleaved to allow the next round of incorporation. Base calls are made directly from signal intensity measurements during each cycle. This proprietary, reversible, terminator–based method enables the parallel sequencing of millions of DNA fragments, detecting single bases as they are incorporated into growing DNA strands. Once the sequencing run was finished, the sequencer compiled all of the spots from all of the images it had taken and converted it into millions of short sequences of DNA letters. These short reads were exported as a FASTQ file along with other details about the read quality, ready to be taken forward for data analysis.

d. Data analysis:

Several softwares 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. Those softwares annotate 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. Illumina variant studio software

The reads generated from the FastQ files were aligned to the hg19 human genome using Novoalign and variants were called by the VariantStudio software by Illumina. Single nucleotides and deletion/insertion variants were generated using this software. The average total number of single nucleotide variants (SNV) and Indels in all samples was around 100 000 and these were not found in the normal population according to the gnomAD database.

The first filtering covered a comparison between the results of this study and those of previous studies present in the literature (Tables II-3 and 4) to assess if common genes are present.

During the first round of stringent filtering, only the passed variants having a high putative impact (either missense mutations or disruptive mutations: frameshift/ stop codon inside the coding region) and a coverage read >20 were analyzed. We also included all the synonymous variants, while excluding all the intronic regions and variants in the non-coding regions. All variants with a minor allele frequency (MAF) in the gnomAD database of less than 5% are included in this first round of filtering against the normal population. The variant call software (VariantStudio software by Illumina) was used for this purpose.

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.

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 MM. In other words, the

genes that do not have a role in the formation of the jaws were filtered out, and those that are related to the formation of the jaws, were considered as candidate gene(s) for MM.

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 inbredness) 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 11 eastern Mediterranean families with affected individuals suggest a Mendelian inheritance pattern and segregate in an autosomal dominant manner. Analysis of those pedigrees show (Tables IV.1,2):

- Equal number of reported generations per family (n=3).
- Equal number of families with males and females predominance (n=4)
- Families with equal number of reported affected males and females (n=2)
- More families with affected males in the 1st generation (5 males versus 4 females.
- 6 families having affected siblings in the youngest generation; 2 of them accepted to enroll in the genetic analysis.

Table IV-1: Number and average of generations, affected males and females in the 11 Eastern Mediterranean families.

	Generations	Affected females	Affected males
Total number in 11 families	33	26	26
Average	3	2.36	2.36

Table IV-2: Pedigree analysis of the 11 Eastern Mediterranean families.

	N	%		N	%
Families with more affected females	4	36.3	**Families with more affected males**	4	36.3
Families with at least 3 affected females	3	27.3	**Families with at least 3 affected males**	3	27.3
Families with no affected females	1	0.9	**Families with no affected males**	1	0.9
Families with affected females in the 1st generation	4	36.3	**Families with affected males in the 1st generation**	5	45.5
Families with affected siblings in the youngest generation	6	54.5	**Selected families with affected siblings in the youngest generation**	2	40

B. Genetic analysis

Whole Exome Sequencing (WES) identified the genetic variants in exonic regions of the 28 subjects (15 affected and 13 non-affected) that are part of the 5 enrolled families. 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….

1. Families characteristics

Below are displayed tables that summarize in detail the average characteristics of each selected family in terms of number of reads and quality scores (Tables IV.15→18).

Family B

Family B has an average total read bases of 7,226,151,055, 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 (Table IV.15).

Table IV-3: Summary of the average characteristics of family B

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	7,226,151,055	737984762.3	6,777,628,960	8,329,596,088
Total number of reads	47,855,305	4887316.307	44,884,960	55,162,888
GC-content (%)	52	0.13301	51.44	51.74
Q20 (%)	98	0.0613052	97.74	97.87
Q30 (%)	94	0.1114675	93.96	94.2

Family C

Family C has an average total read bases of 7,223,106,820, 52% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 97% and 94% respectively, indicating that 97% of the fragments have 20 copies read and more and 94% of the fragments have 30 copies read and more (Table IV.16).

Table IV-4: Summary of the average characteristics of family C

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	7,223,106,820	437460435.4	6,642,412,990	7,573,915,078
Total number of reads	47,835,145	2897088.976	43,989,490	50,158,378
GC-content (%)	52	0.100457288	52.09	51.85
Q20 (%)	97	0.07410578	97.38	97.45
Q30 (%)	94	0.176894507	93.52	93.9

Family D

Family D has an average total read bases of 7,211,528,819, 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 (Table IV.17).

Table IV-5: Summary of the average characteristics of family D

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	7,211,528,819	873723858.8	6,192,170,552	8,310,933,696
Total number of reads	47,758,469	5786250.72	43,989,490	50,158,378
GC-content (%)	52	0.130256158	51.77	52.06
Q20 (%)	98	0.071414284	97.52	97.67
Q30 (%)	94	0.146144905	93.65	93.96

Family E

Family C has an average total read bases of 6,691,180,630, 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 (Table IV.18).

Table IV-6: Summary of the average characteristics of family E

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	6,691,180,630	255092308.8	6,387,680,822	6,917,842,728
Total number of reads	44,312,455	1689353.038	42,302,522	45,813,528
GC-content (%)	51	0.179698822	51.21	51.63
Q20 (%)	98	0.096609178	97.68	97.9
Q30 (%)	94	0.200229036	93.87	94.31

2. Comparison with previous studies

Before the filtering steps, a comparison was done between the results of the present study and those of previous studies that discovered the following candidate genes implicated in craniofacial development: *GHR, NOG, ACTN3, SNAI3, FGFR2, MYO1H, MATN1, HSPG2, MATN1, ALPL, EPB41, EVC2, TGFβ3, LTBP2, MYO1H, DUSP6, PLXNA2, SSX2IP, COL2A1, ADAMTS1, ARHGAP21 and IGF1*. The present variations in these candidate genes, loci or variants were either intronic or synonymous and were found in both affected and non-affected individuals, meaning that none of

these variations can be disease causing and cannot be associated with MM in our present study.

3. Filtering results

As previously mentioned, we started our stringent filtering analysis by keeping only the variants that have a PASS filter status, a coverage read >20, a Minor Allele Frequency (MAF) ≤ 0.005 and a high putative impact on protein structure and function. Therefore, the number of the remaining variants was reduced from 100000 to 2000-3000 on average in all families.

Following the 2nd filtering, during which a comparison was done between individuals of the same family, many genes were found to be common between the affected individuals. The shared variants in specific genes between the affected individuals that may segregate with the phenotype were highlighted. The numbers came as follow:

- 3 in family A: GLUD2, ADGRG4 and ARSH
- 1 in family B: TGIF1
- 1 in family C: FGFR3
- 1 in family D: ZNF181
- 2 in family E: INTS7 and WNT6 (Tables IV-19 and 20)

Table IV-7: Summary of the shared variants in specific genes between affected individuals of each family (that segregate with the phenotype) following the 2nd filtering (high putative impact) and function check.

	Family A	**Family B**	**Family C**	**Family D**	**Family E**
Gene 1	*GLUD2*	*TGIF1*	*FGFR3*	*ZNF181*	*INTS7*
Gene 2	*ADGRG4*	-	-	-	*WNT6*
Gene 3	*ARSH*	-	-	-	-

Table IV-8: Summary of the characteristics of the shared variant in a specific gene (that segregate with the phenotype) following the 2nd filtering (high putative impact) and function check in all families.

Family	Gene Name	Chromosome	Positions	HGVSp	dbSNP	Zygosity	Effect
A	***GLUD2***	X	120183030	p.Ser498Ala	rs9697983	HET	missense_ variant
	ADGRG4	X	135428968	p.Glu1035Lys	rs149243520	HET	missense_ variant
	ARSH	X	2928174	p.Arg66Trp	rs148749736	HET	missense_ variant
B	**TGIF1**	18	3452167	p.Pro64Ser	-	HET	missense_ variant
C	**FGFR3**	4	1808029	p.Arg671Gly	-	HET	missense_ variant
D	**ZNF181**	19	35232206	p.Asn310Ilefs Ter14	-	HET	frameshift _variant
E	**INTS7**	1	212154515	p.Gln384Ter	-	HET	Stop_ gained
	WNT6	2	219724783	p.Arg8Pro	-	HET	missense_ variant

Worth dissecting is family A as there were two subfamilies (Figure IV-3). The analysis was carried out first assuming one gene mutation and assuming an autosomal dominant genotype-phenotype inheritance. The results came out negative. Thereafter, an X-linked potential inheritance was suggested, and again there was no common variant between all affected patients. Nevertheless, when the families were dissected into two subfamilies, we indeed found two missense mutations that are X-linked and could potentially cause the phenotype:

- The GLUD2 mutation is found on X-chromosome of the male affected offsprings from subfamily 1 and is heterozygous recessive in the mother.
- The ARSH mutation is also found on X-chromosome but is inherited as heterozygous dominant in subfamily 2.

- The ADGRG4 mutation was only found in one individual in subfamily 1, subsequent to an additional sorting of the variants. There are no worldwide reports on this mutation.

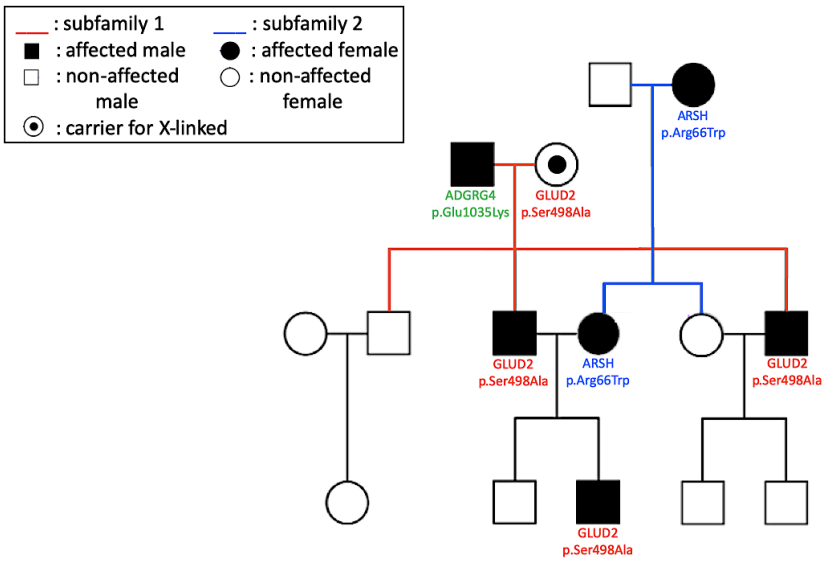


Figure IV-1: Pedigree of family **A** exhibiting the mode of transmission of the candidate gene. Subfamily 1 displaying an X-linked recessive pattern of inheritance, while subfamily 2 revealing an X-linked dominant inheritance.

All the remaining families had an autosomal dominant mode of inheritance. The corresponding pedigrees are displayed below (Figures IV-4 to 7).

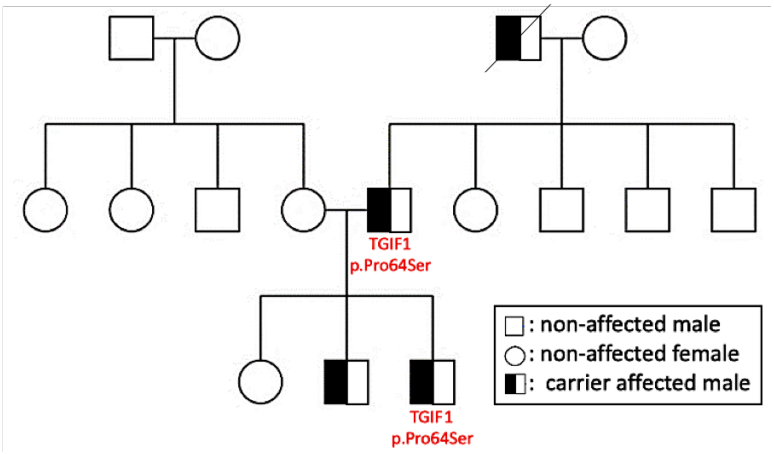


Figure IV-2: Pedigree of family **B** displaying an autosomal dominant pattern of inheritance.

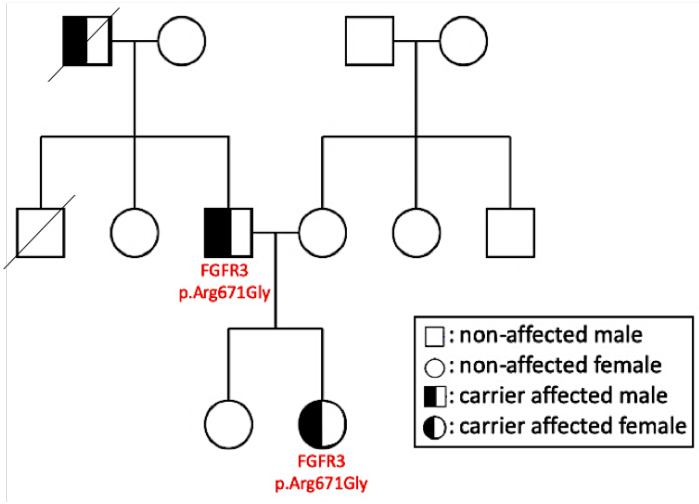


Figure IV-3: Pedigree of family **C** displaying an autosomal dominant pattern of inheritance. The interaction of both potential candidate genes results in the phenotype.

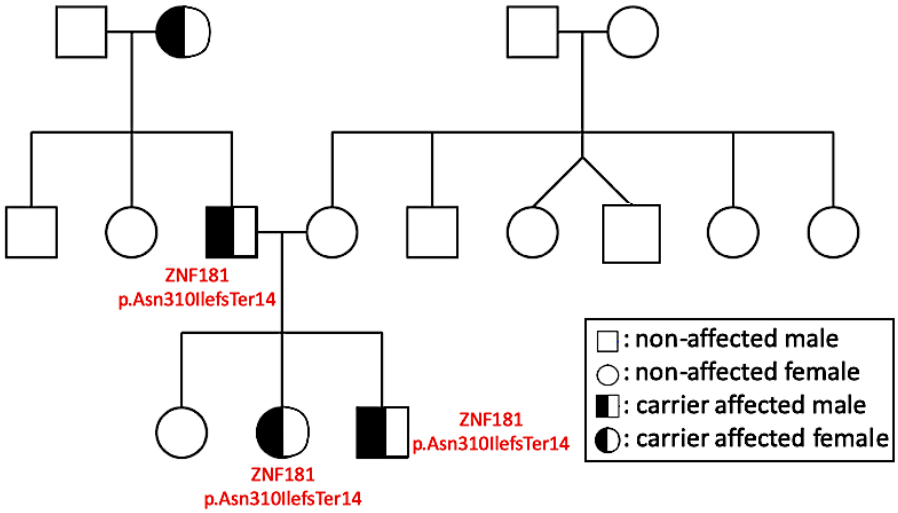


Figure IV-4: Pedigree of family **D** displaying an autosomal dominant pattern of inheritance.

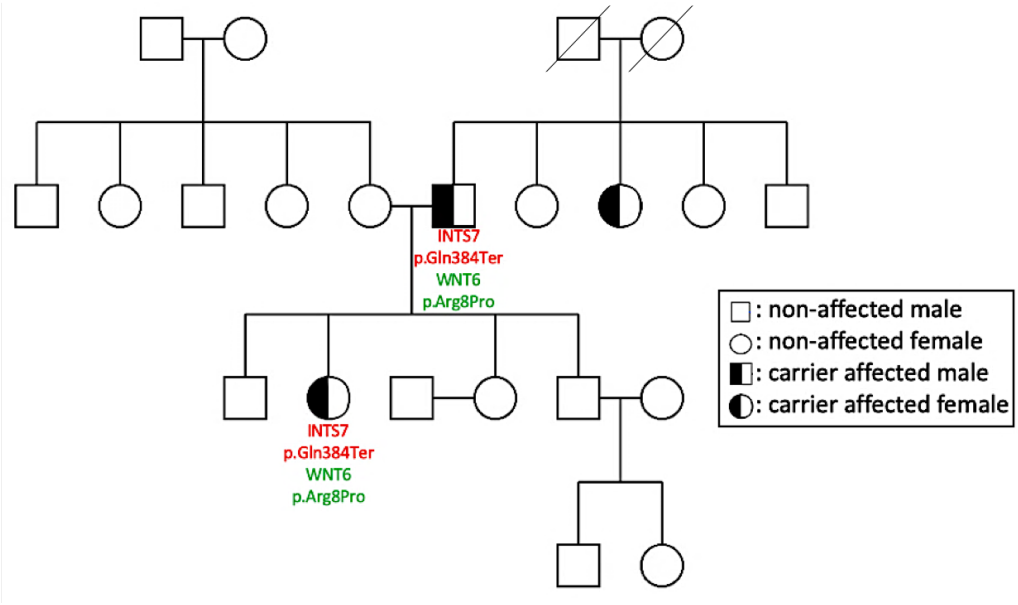


Figure IV-5: Pedigree of family **E** displaying an autosomal dominant pattern of inheritance. The interaction of the potential candidate genes results in the phenotype.

Subsequent to the several steps of filtering in families A-E, while accounting for the fact that we filtered for frameshift and stop gained (non-sense) mutations primarily (that are not present in the normal population according to the gnomAD database) and missense mutations secondarily, 8 potential candidate genes (GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7 and WNT6) that may segregate with the phenotype were highlighted and their functions checked. Worth mentioning is that frameshift and non-sense mutations impact protein function and are likely to be deleterious by significantly altering protein function or expression levels. The missense mutations may have no effect, or it may render the protein non-functional. Nevertheless, based on SIFT and Polyphen loss-of-function and gain-of-function predictive software, they were considered of high importance because of their correlation with the phenotype inside the families while being absent in other members of the family and the normal population.

C. Cephalometric analysis

1. Measurements related to the 11 approached families

When statistical analysis was performed on measurements related to the 23 individuals that are part of the 11 approached families, the following results were noted. Most importantly, mandibular length is represented by the average Co-Gn and Co-Pog, which is 100.64 mm and 97.97 mm respectively. Maxillary length is denoted by the average ANS-PNS that is equal to 52.65 mm. Facial divergence is represented by the average MP/SN, which is equal to 36.7°, showing, on average, a tendency toward a hyperdivergent facial pattern with an upward inclination of SN relative to the horizontal (N is higher than S), where SN/H was equal to 13.2°. Measurements related to the sagittal relationship of the jaws show an average SNA angle of 81.57°, SNB angle of 73.86°, ANB angle of 7.7° and AO-BO of 6.22 mm denoting, on average, an orthognathic maxilla, a retrognathic mandible, denoting a skeletal Cl II. Dento-alveolar measurements show retroclined and retruded maxillary incisors versus proclined and protruded mandibular incisors as a dento-alveolar compensation of the skeletal Cl II malocclusion. Finally, the average OJ is equal to 6.94 mm (Table IV.3).

Table IV-9: Measurements related to individuals that are part of the 11 approached families (n=23).

Measurement	Average	Standard deviation	Maximum	Minimum
SN	66.49	4.49	72.9	57.5
S-Ar	34.05	3.17	40.6	27.2
SN/H	13.2	4.06	22.3	3.9
N-S-Ar	127.5	7.3	140.5	115.7
Ar-Go-Gn	124.07	6.94	140.4	113.6
Ar-Go-Me	126.98	6.84	144.4	116.6
Co-Go-Me	121.33	7.09	139.7	109.7
Ar-Gn	100.92	7.54	111.7	85.7
Co-Gn	100.64	6.55	112.3	87.3
Co-Pog	97.97	6.30	109.9	84.4

Co-Go	50.8	6.54	61.9	42
Ar-Go	42.96	6.69	54.8	32.3
Go-Me	70.72	5.36	79	61.8
Go-Gn	69.33	4.12	77	61.2
Go-Pog	64.25	9.52	75.7	27.2
ANS-PNS	52.65	3.88	58.5	45.2
N-ANS	50.4	3.55	59	44.4
ANS-Me (AFH)	63.36	6.71	81.4	52.4
PFH	42,2	6.07	52.5	31.2
LFH/TFH	55.43	2.31	61.6	49.9
AFH/PFH	140.14	17.13	164.2	112.3
MP/SN	36.7	7.16	53.5	24.2
MP/H	29.7	7.16	46.5	17.2
PP/MP	27.43	6.82	40.4	16.3
PP/H	-3.93	3.11	1.5	-15.1
SNA	81.57	4.97	91.4	74
SNB	73.86	4.71	84.8	64.6
ANB	7.7	2.28	13.3	4.7
AOBO	6.22	4.55	16	0.2
I/NA	19.84	8.11	37.4	6
I-NA	2.98	2.49	8.2	-0.8
I/SN	101.4	8.27	116.1	88.7
I/PP	110.66	8.24	126.5	94
i/NB	31.91	5.66	40.7	18
i-NB	6.8	2.33	12.7	5.3
i/MP	101.36	7.11	112.1	98.2
I/i	120.54	9.66	137.9	114.4
OJ	6.94	2.34	12.8	5.6
OB	3.96	2.12	8.2	2.5
Chin width at apex of i	8.92	1.7	12	5.9
Chin width at level of D	12.07	2.12	14.7	5.8
D- i apex	9.36	1.96	13	5.7
D-Me	9.5	2.16	13.3	3.9
Ant slope of the chin/ V	7.77	6.36	24.4	0.8
Post slope of the chin/ V	20.8	6.09	36.2	9.4
Angle Ant/ Post slopes	28.57	6.19	60.6	12.8

2. Measurements related to the 5 enrolled families

Mandibular length (Co-Pog) value of the initial affected patient of each selected family confirms the presence of a mandibular micrognathism. The difference between the patients' values and the norm ranges between -1.5 and -16.7, indicating a decreased mandibular length that is more than 1SD below the norm. The average Co-Pog value of the 5 initial affected patients is 98.28 mm, which is lesser than the norms by at least 1SD (Table IV.4).

Table IV-10: Mandibular length analysis of the initial affected patient of each selected family.

Initial affected patient	Age	Co-Pog value	Average of 5 initial affected patients	Difference: Co-Pog value - average affected	Co-Pog Norm (per age)	Difference: Co-Pog value-norm
Family A	10.5	95	98.28	-3.28	100.5	-5.5
Family B	12.8	97		-1.28	106.3	-9.3
Family C	48.6	109.9		11.62	116.7	-6.8
Family D	12.4	94.7		-3.58	98.5	-3.8
Family E	23	94.8		-3.48	106	-11.2

Statistical analysis performed on measurements related to the 13 individuals that are part of the 5 selected families confirms the affection status and presence of characteristics of MM in those subjects. Specifically, an average Co-Pog of 99.67 mm and Co-Gn of 102.29 indicate a short mandibular length (mandibular micrognathism). An average ANS-PNS of 53.23 designates a normal maxillary length and an average MP/SN of 35.56 denotes a tendency to a hyperdivergent facial pattern with upward inclination of SN relative to the horizontal (N is higher than S), where SN/H was equal to 13.78°. An average SNA angle of 81.43°, SNB angle of 74.08°, ANB angle of 7.36° and AoBo of 6.16 mm refer to a skeletal Class II malocclusion underlined by an orthognathic maxilla and retrognathic mandible. Dento-alveolar measurements indicate also retroclined maxillary incisors and proclined mandibular incisors as a dento-alveolar compensation of the Class II malocclusion. Finally, an average OJ of 5.81mm is accompanied by a deep overbite with an average of 3.78 mm.

Table IV-11: Measurements of individuals that are part of the 5 enrolled families (n=13).

Measurement	Average	Standard deviation	Maximum	Minimum
SN	68.09	2.94	72.9	62.9
S-Ar	33.42	3.39	37.7	27.2
SN/H	15.82	4.85	22.3	3.9
N-S-Ar	132.08	7.14	140.5	117.9
Ar-Go-Gn	126.04	6.26	134.7	113.6
Ar-Go-Me	128.7	6.00	137.1	116.8
Co-Go-Me	123.26	6.44	130.2	109.9
Ar-Gn	101.77	6.36	111.7	94.4
Co-Gn	101.69	6.06	112.3	94.1
Co-Pog	99.24	5.74	109.9	92.5
Co-Go	51.52	6.65	61.9	43.6
Ar-Go	44.16	6.46	54.8	34.4
Go-Me	70.50	5.04	79	64.1
Go-Gn	68.39	3.84	77	62.9
Go-Pog	59.62	12.23	75.7	27.2
ANS-PNS	53.28	3.27	58.5	47.5
N-ANS	51.2	3.64	59	44.9
ANS-Me (AFH)	64.27	6.89	81.4	54.4
PFH	43.24	5.69	52.5	36.5
LFH/TFH	55.44	2.39	61.6	51.7
AFH/PFH	138.77	16.37	159.3	112.3
MP/SN	38.28	7.92	53.5	24.2
MP/H	31.28	7.92	46.5	17.2
PP/MP	26.91	7.04	40.4	16.3
PP/H	-4.45	3.92	1.5	-15.1
SNA	78.40	5.48	91.4	74
SNB	71.26	5.52	84.8	64.6
ANB	7.17	2.19	11.1	4.7
AOBO	6.51	4.92	16	0.5
I/NA	19.30	8.75	37.4	6
I-NA	2.63	2.47	7.2	-0.8
I/SN	97.72	8.83	113.7	88.7
I/PP	109.07	8.79	124.8	94
i/NB	31.82	6.87	40.7	18
i-NB	6.88	2.91	12.7	2.3
i/MP	102.31	5.94	111.6	88.5
I/i	121.70	10.76	137.9	100.7
OJ	5.93	1.72	9.8	3.7
OB	3.61	2.17	8.2	-0.8
Chin width at apex of i	8.29	1.77	10.6	5.9
Chin width at level of D	11.26	2.47	14.2	5.8
D- i apex	9.92	1.99	13	6.4
D-Me	9.22	2.23	13.3	5
Ant slope of the chin/ V	9.24	7.41	24.2	-4.3
Post slope of the chin/ V	21.36	6.65	36.2	9.4
Angle Ant/ Post slopes	30.60	7.33	48.7	20.5

3. Comparison between individuals and families

A two-sample independent T test compared the cephalometric measurements of group1, comprising the 23 affected subjects from the 11 recruited families, and group 2, involving 30 non-affected subjects (skeletal Class I) that were included as controls. These controls were collected from the data base of the department of orthodontics and dentofacial orthopedics-AUBMC. The objective was to assess the differences between the 2 groups. The results of the statistical test are displayed in Table IV.6. Statistically significant difference ($p \leq 0.05$) is noted for the following measurements:

SN/H	I/NA
N-S-Ar	I-NA
Ar-Gn	I-SN
Go-Gn	I-PP
Co-Pog	i-NB
Go-Gn	i-MP
Go-Pog	OJ
ANS-PNS	OB
PP/H	chin width at point D
SNB	distance D-i apex
ANB	Post slope of the chin/V
AoBo	Angle Ant/Post slopes

Table IV-12: Comparison between measurements of the affected subjects and a group of controls

Measurement	Mean (group 1)	STD	Mean (group 2)	STD	p-value
SN	66.49	4.49	66.0	4.14	0.689
S-Ar	34.05	3.17	34.01	2.94	0.969
SN/H	13.2	4.06	10.79	2.70	0.015
N-S-Ar	127.5	7.3	123.83	5.55	0.049
Ar-Go-Gn	124.07	6.94	127.15	4.08	0.054
Ar-Go-Me	126.98	6.84	129.83	3.77	0.066
Co-Go-Me	121.33	7.09	123.61	3.32	0.136
Ar-Gn	100.92	7.54	106.45	7.82	0.014
Co-Gn	100.64	6.55	107.63	7.67	0.001
Co-Pog	97.97	6.30	104.49	7.55	0.002
Co-Go	50.8	6.54	52.83	5.35	0.229
Ar-Go	42.96	6.69	42.36	4.94	0.713
Go-Me	70.72	5.36	71.38	9.24	0.743
Go-Gn	69.33	4.12	74.27	4.82	0.000
Go-Pog	64.25	9.52	70.21	4.56	0.005
ANS-PNS	52.65	3.88	48.89	4.49	0.003
N-ANS	50.4	3.55	50.33	4.56	0.941
ANS-Me (AFH)	63.36	6.71	61.05	4.49	0.194
PFH	42,2	6.07	42.40	3.96	0.895
LFH/TFH	55.43	2.31	54.67	5.87	0.209
AFH/PFH	140.14	17.13	140.42	5.35	0.950
MP/SN	36.7	7.16	36.19	1.94	0.758
MP/H	29.7	7.16	29.19	14.97	0.758
PP/MP	27.43	6.82	26.44	4.32	0.527
PP/H	-3.93	3.11	-1.04	4.18	0.002
SNA	81.57	4.97	81.25	3.00	0.778
SNB	73.86	4.71	79.05	3.00	0.000
ANB	7.7	2.28	2.21	2.96	0.000
AOBO	6.22	4.55	-2.45	1.02	0.000
I/NA	19.84	8.11	27.05	1.75	0.000
I-NA	2.98	2.49	5.41	4.62	0.000
I/SN	101.4	8.27	108.31	1.50	0.001
I/PP	110.66	8.24	118.06	4.92	0.000
i/NB	31.91	5.66	29	4.51	0.081
i-NB	6.8	2.33	5.59	5.91	0.046
i/MP	101.36	7.11	93.77	1.87	0.000
I/i	120.54	9.66	121.73	6.11	0.651
OJ	6.94	2.34	3.00	8.92	0.000
OB	3.96	2.12	1.71	1.16	0.000
Chin width at apex of i	8.92	1.7	9.70	1.30	0.119
Chin width at level of D	12.07	2.12	13.17	1.80	0.025
D- i apex	9.36	1.96	7.52	1.24	0.001
D-Me	9.5	2.16	10.46	1.41	0.055
Ant slope of the chin/ V	7.77	6.36	7.08	1.64	0.678
Post slope of the chin/ V	20.8	6.09	25.33	6.13	0.011
Angle Ant/ Post slopes	28.57	6.19	32.41	5.41	0.030

Group 1: 23 affected subjects from the 11 families.
Group 2: 30 non-affected subjects (controls)
p-value ≤ 0.05: statistically significant.

This denotes the presence of more severe skeletal features accompanied with dento-alveolar compensations in group 1when compared to group 2.

Descriptive statistics, including means, standard deviations and p-values, was performed on cephalometric measurements related to the 5 families in order to evaluate differences between them. The results are displayed in Tables IV.7.A and IV.7.B.

Table IV-13A: Descriptive statistics of cephalometric measurements of families A-C

Measurement	Family A (n= 5)		Family B (n= 2)		Family C (n= 2)	
	μ	σ	μ	σ	μ	σ
SN	68.74	3.03	69.95	1.20	70.60	3.25
S-Ar	33.48	2.57	31.75	3.89	35.25	2.90
SN/H	9.68	4.07	13.00	0.99	16.15	1.34
N-S-Ar	123.06	3.22	136.30	1.41	137.80	3.82
Ar-Go-Gn	120.58	3.99	116.60	4.24	130.70	5.66
Ar-Go-Me	123.86	3.78	119.35	3.61	131.00	2.83
Co-Go-Me	116.96	4.26	113.95	5.73	127.55	3.75
Ar-Gn	106.52	2.59	103.45	6.58	103.75	10.54
Co-Gn	103.56	3.81	101.50	5.23	103.20	12.87
Co-Pog	100.50	3.35	98.95	4.60	101.20	12.30
Co-Go	53.70	4.79	54.30	9.90	53.05	12.52
Ar-Go	47.58	3.19	48.30	9.19	45.60	10.04
Go-Me	73.58	3.48	74.00	7.07	68.10	5.66
Go-Gn	72.40	3.59	70.90	1.13	67.35	4.17
Go-Pog	69.94	3.90	68.50	2.69	40.00	18.10
ANS-PNS	53.74	3.01	55.25	1.34	55.70	5.96
N-ANS	50.06	3.84	49.30	2.55	54.10	6.93
ANS-Me (AFH)	61.90	3.48	60.50	2.83	62.05	4.45
PFH	46.62	3.31	47.35	7.28	44.45	9.97
LFH/TFH	54.94	0.83	55.05	0.007	53.50	1.41
AFH/PFH	124.68	10.56	120.10	9.19	131.70	14.28
MP/SN	30.26 (28.58)	4.10	30.05 (25.05)	5.02	38.20 (30.05)	2.40
MP/H	23.26	4.10	23.05	5.02	31.20	2.40
PP/MP	23.42	2.42	18.80	3.54	23.35	7.71
PP/H	-2.88	1.54	-1.75	0.49	-1.30	3.96
SNA	86.80 (88.48)	4.06	77.90 (82.90)	0.57	75.15 (83.30)	1.63
SNB	78.98 (80.66)	4.88	71.55 (76.55)	1.77	70.00 (78.15)	0.85
ANB	7.76	1.76	6.45	1.20	5.20	0.71
AOBO	7.50	7.72	7.80	3.11	4.95	3.04
I/NA	16.90	5.49	20.80	13.58	30.10	10.32
I-NA	2.18	1.95	3.20	4.24	5.25	2.76
I/SN	103.66 (105.34)	7.15	98.75 (103.75)	12.94	105.25 (113.40)	11.95
I/PP	110.50	5.64	109.95	14.50	120.10	6.65
i/NB	34.04	5.92	29.70	6.22	32.10	5.80
i-NB	7.24	2.09	4.80	0.99	5.95	0.92
i/MP	104.78	5.39	108.15	3.04	103.90	2.55
I/i	121.26	6.47	123.05	21.00	112.65	16.90
OJ	5.76	1.23	6.75	4.31	6.40	1.84
OB	4.26	2.64	4.00	0.99	1.85	3.75

Chin width at apex of i	9.54	0.73	9.35	0.07	10.50	2.12
Chin width at level of D	12.38	1.56	13.50	0.14	13.65	0.49
D- i apex	7.72	1.09	8.05	0.92	9.90	1.27
D-Me	10.08	1.13	8.80	0.71	7.20	3.11
Ant slope of the chin/ V	7.43	4.73	18.30	8.63	4.70	2.26
Post slope of the chin/ V	20.34	4.79	21.05	4.60	27.60	12.16
Angle Ant/ Post slopes	27.68	3.28	39.35	13.22	32.30	9.90

( ) Corrected values relative to SN/H

Highest value **Lowest value** **Statistically significant: p-value < 0.05**

Table IV-7B: Descriptive statistics of cephalometric measurements of the families D-E.

Measurement	**Family D (n= 2)**		**Family E (n=2)**		**P value**
	μ	**σ**	**μ**	**σ**	
SN	64.90	2.83	67.00	1.98	0.307
S-Ar	37.45	0.35	29.25	2.00	0.107
SN/H	13.80	0.57	21.35	1.34	0.016
N-S-Ar	125.00	8.63	130.65	2.05	0.010
Ar-Go-Gn	128.30	2.83	129.15	1.20	0.018
Ar-Go-Me	132.95	5.87	132.00	0.14	0.018
Co-Go-Me	125.60	2.12	126.65	0.35	0.016
Ar-Gn	95.15	1.06	104.05	10.82	0.367
Co-Gn	96.95	0.34	104.80	10.47	0.773
Co-Pog	94.55	0.21	102.05	10.25	0.769
Co-Go	44.10	0.71	54.30	4.81	0.521
Ar-Go	35.60	1.70	46.60	6.08	0.227
Go-Me	67.25	2.05	72.35	8.70	0.507
Go-Gn	67.90	1.41	66.95	5.73	0.327
Go-Pog	65.20	1.56	63.40	5.52	0.013
ANS-PNS	50.70	4.53	51.50	3.82	0.509
N-ANS	49.50	1.70	52.20	2.55	0.679
ANS-Me (AFH)	58.95	6.43	76.15	7.42	0.032
PFH	36.90	0.57	43.75	6.01	0.336
LFH/TFH	54.15	3.46	59.15	3.46	0.104
AFH/PFH	149.50	12.87	156.20	4.38	0.026
MP/SN	36.65 (30.85)	3.61	49.60 (36.25)	5.52	0.005
MP/H	29.65	3.61	42.60	5.52	0.006
PP/MP	27.30	3.82	38.80	2.26	0.060
PP/H	-4.45	0.78	-10.60	6.36	0.021
SNA	80.10 (85.90)	0.00	78.95 (92.30)	5.30	0.054
SNB	74.00 (79.80)	1.98	68.10 (81.45)	4.95	0.038
ANB	6.10	1.98	10.85	0.35	0.977
AOBO	5.80	4.24	7.65	0.07	0.216
I/NA	16.60	5.80	10.15	5.87	0.293
I-NA	2.25	0.78	-0.20	0.85	0.318
I/SN	96.70 (102.50)	5.94	89.10 (102.45)	0.57	0.222
I/PP	106.00	6.08	99.90	8.34	0.235
i/NB	24.70	9.48	40.05	0.92	0.044
i-NB	4.80	3.54	11.80	1.27	0.141
i/MP	94.10	7.92	102.40	0.28	0.521
I/i	132.60	5.66	118.90	10.76	0.921
OJ	5.50	1.70	5.15	1.06	0.731

OB	4.95	0.07	3.55	1.48	0.005
Chin width at apex of i	7.15	0.49	5.95	0.07	0.005
Chin width at level of D	10.30	0.14	7.35	2.19	0.007
D- i apex	9.45	1.20	12.65	0.49	0.005
D-Me	8.30	3.11	12.45	1.20	0.122
Ant slope of the chin/ V	11.55	8.84	2.60	9.76	0.214
Post slope of the chin/ V	16.50	10.04	20.40	6.22	0.641
Angle Ant/ Post slopes	28.05	1.20	23.00	3.54	0.202

( ) Corrected values relative to SN/H

Highest value **Lowest value** **Statistically significant: p-value < 0.05**

A summary of the cephalometric measurements of the families having the most severe skeletal and dento-alveolar features of MM is presented in Table IV.8. The most severe skeletal values are noted in families D and E. Family C presented the most proclined maxillary incisors (one of the characteristic variations of a Cl II, div1).

Table IV-14: Families with the most severe cephalometric measurements related to MM

	Family with most severe values	**Average value in family D**	**Average value in family E**
SN	D	64.90	67
Co-Gn	D	96.95	104.80
Co-Pog	D	94.55	102.05
Go-Gn	E	67.90	66.95
Go-Pog	C	65.20	63.40
ANS-PNS	D	50.70	51.50
MP/SN	E	30.85	36.25
SNA	E	85.90	92.30
SNB	B	79.80	81.45
ANB	E	6.10	10.85
AOBO	E	5.80	7.65
I/NA	C	16.60	10.15
I-NA	C	2.25	-0.20
I/SN	C	102.50	102.45
I/PP	C	106.00	99.90
i/NB	E	24.70	40.05
i-NB	E	24.70	40.05
i/MP	B	94.10	102.40
OJ	B	5.50	5.15

——— Separation between skeletal and dento-alveolar measurements.

The average main features of each selected family are presented in Tables IV.9.A-B. Each family expresses MM features with mainly an orthognathic/ prognathic maxilla, an orthognathic/ retrognathic mandible and a dento-alveolar compensation. However, the starting point is the initial affected patient who shares mostly the same characteristics.

Table IV-15: Cephalometric characteristics of the 5 families A-E.

		Family A (n=5)	Family B (n=2)	Family C (n=2)	Family D (n=2)	Family E (n=2)
Inclination of SN/H	**P**	Normal	Upward (=2SD)	Upward (>3SD)	Upward (=3SD)	Upward (>7SD)
	F	Upward (=1SD)	Upward (>2SD)	Upward (>2SD)	Upward (>2SD)	Upward (>2SD)
Facial pattern	**P**	Normodivergent	Normodivergent	Normodivergent	Normodivergent	Hyperdivergent (>1SD)
	F	Normodivergent	Normodivergent	Normodivergent	Normodivergent	Hyperdivergent (=1SD)
Position of maxilla	**P**	Prognathic (=3SD)	Orthognathic	Prognathic (=2SD)	Orthognathic	Prognathic (>3SD)
	F	Prognathic (=3SD)	Orthognathic	Prognathic (>1SD)	Orthognathic	Prognathic (>5SD)
Position of mandible	**P**	Orthognathic	Retrognathic (=2SD)	Retrognathic (<1SD)	Orthognathic	Orthognathic
	F	Orthognathic	Retrognathic (<1SD)	Retrognathic (=1SD)	Orthognathic	Orthognathic
Inclination of maxillary incisors	**P**	Well-inclined	Proclined (=4SD)	Proclined (>7SD)	Retroclined (<4SD)	Retroclined (<3SD)
	F	Retroclined (<2SD)	Well-inclined	Proclined (=4SD)	Retroclined (<2SD)	Retroclined (<5SD)
Inclination of mandibular incisors	**P**	Proclined (>6SD)	Proclined(>10SD)	Proclined (>7SD)	Proclined (>4SD)	Proclined (>6SD)
	F	Proclined (>7SD)	Proclined (>9SD)	Proclined (>2SD)	Proclined (>6SD)	Proclined (=6SD)
Overjet	**P**	Positive	Positive	Positive	Positive	Positive
	F	Positive	Positive	Positive	Positive	Positive

P: Initial affected patient - **F:** All affected individuals of the family

We can state that out of the 5 selected families:

- 5 families have an upward inclination of SN to horizontal

- only 1 family has a hyperdivergent facial pattern and the remaining 5 were normodivergent

- 2 have an orthognathic mandible along with a prognathic maxilla, when 1 family has an orthognathic maxilla with a retrognathic mandible

- maxilla and mandible are both orthognathic in 1 family

- 5 show a dento-alveolar compensation involving either one arch (1 family) or both arches (4 families), along with a positive overjet in all of the families.

4. Mandibular length analysis: Z score.

Mandibular length measurements (Co-Pog) and Z scores of each family and its individuals are presented in Tables IV.10-14. Subjects are numbered by ascending order of generations, which means by descending order of age.

In family (A):

- Co-Pog value ranges between 95 and 103.7.
- Z score value ranges between -1.53 and -4.51
- The average Z score is equal to -3.01 (Table IV.10).

Table IV-16: Co-Pog values (mm) and Z scores of individuals that are part of the family (A).

Family (A)						
Subject number	**Age at x-ray time**	**Co-Pog value**	**Co-Pog mean**	**Co-Pog standard deviation**	**Z score**	**Average Z score**
1	73.1	100	116.7	3.7	-4.51	**-3.01**
5	45.1	101.5	116.7	3.7	-4.11	
8	43.9	103.7	116.7	3.7	-3.51	
6	42.1	102.3	106	2.7	-1.37	
10	10.5	95	100.5	3.6	-1.53	

Initial affected patient

In family (B):

- Co-Pog value ranges between 97 and 102.2.
- Z score value ranges between -2.45 and -3.92.
- The average Z score is equal to -3.18(Table IV.11).

Table IV-17: Co-Pog values (mm) and Z scores of individuals that are part of the family (B).

Family (B)						
Subject number	**Age at x-ray time**	**Co-Pog value**	**Co-Pog mean**	**Co-Pog standard deviation**	**Z score**	**Average Z score**
1	48.10	102.2	116.7	3.7	-3.92	**-3.18**
4	12.8	97	106.3	3.8	-2.45	

Initial affected patient

In family (C):

- Co-Pog value ranges between 95.6 and 109.9.
- Z score value ranges between -0.35 and -1.84.
- The average Z score is equal to -1.09 (Table IV.12).

Table IV-18: Co-Pog values (mm) and Z scores of individuals that are part of the family (C).

Family (C)						
Subject number	**Age at x-ray time**	**Co-Pog value**	**Co-Pog mean**	**Co-Pog standard deviation**	**Z score**	**Average Z score**
1	48.6	109.9	116.7	3.7	-1.84	**-1.09**
4	8.8	95.6	97.1	4.3	-0.35	

Initial affected patient

In family (D):

- Co-Pog value ranges between 94.4 and 94.7.
- Z score value ranges between -1.37 and -1.81.
- The average Z score is equal to -1.59 (Table IV.13).

Table IV-19: Co-Pog values (mm) and Z scores of individuals that are part of the family (D).

Family (D)						
Subject number	**Age at x-ray time**	**Co-Pog value**	**Co-Pog mean**	**Co-Pog standard deviation**	**Z score**	**Average Z score**
4	12.4	94.7	98.5	2.1	-1.81	**-1.59**
3	9.10	94.4	99.2	3.5	-1.37	

Initial affected patient

In family (E):

- Co-Pog value ranges between 94.8 and 109.3.
- Z score value ranges between -2.00 and -4.15.
- The average Z score is equal to -3.07 (Table IV.14).

Table IV-20: Co-Pog values (mm) and Z scores of individuals that are part of the family (E).

Family (E)						
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**	**Average Z score**
2	73.9	109.3	116.7	3.7	-2.00	**-3.07**
3	23	94.8	106	2.7	-4.15	

Initial affected patient

To sum up, mandibular length Co-Pog ranges between 94.4 (family E) and 109.9 (family C), Z score value ranges between -1.09 (family C) and -3.18 (family B) and average Z score corresponding to the 5 selected families is equal to -2.39, with the highest value corresponding to family B and the lowest value to family C. The average Z score, being less than 2 standard deviations below the norm, indicates a mandibular micrognathism.

Distribution of the Z score by family across the different generations shows that most of the available values correspond to individuals from the 2nd and 3rd generations (being the most accessible), reaching a maximum of -4.11 SD below the norm in the 2nd generation and -4.15 SD in the 3rd generation. The most severe scores correspond to the patient of the 1st and 2nd generation of family A and the initial patient of family E. The variation in severity of the Z score across individuals confirms the variable expressivity of MM. In most of the families, the highest values correspond to subjects from the 1st or 2nd generation (Figures IV.1,2).

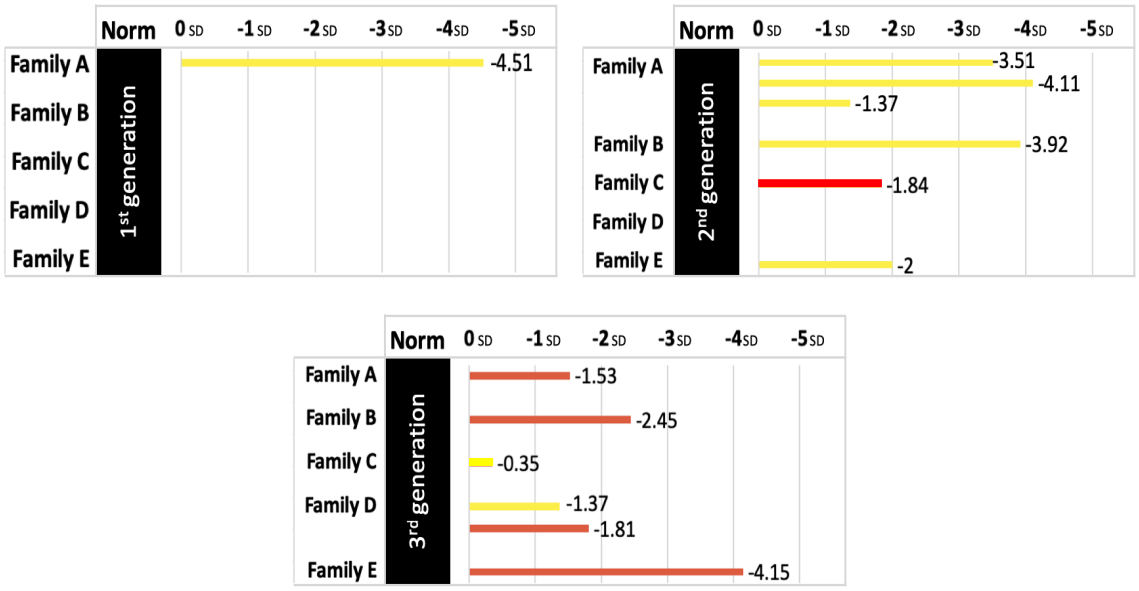


Figure IV-6: Distribution of the Z score by family across the different generations.

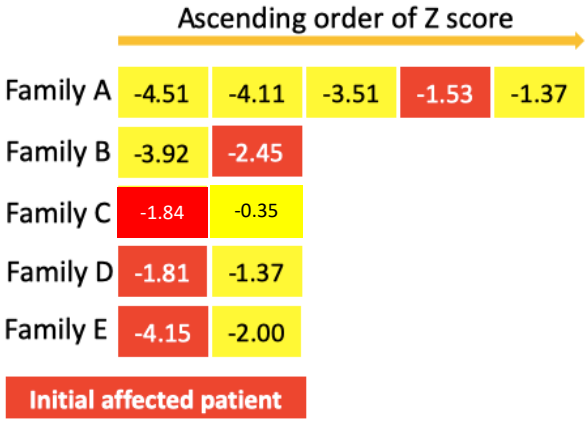


Figure IV-7: Order of severity of the Z score in each selected family.

Table IV-21: Summary of the family candidate genes in each individual and their corresponding z-score for mandibular length

Family	Gene Name	Chromosome	Family member	Z-score
A	*ADGRG4*	X	A1	-4.51
	GLUD2	X	A2	-
			A5	-4.11
			A8	-3.51
			A10	-1.53
	ARSH	X	A4	-
			A6	-1.37
B	*TGIF1*	18	B2	-3.92
			B4	-2.45
C	*FGFR3*	4	C1	-1.84
			C4	-0.35
D	*ZNF181*	19	D1	-
			D3	-1.37
			D4	-1.81
E	*INTS7*	1	E2	-2.00
			E3	-4.15
	WNT6	2	E2	-2.00
			E3	-4.15

Sdggfve: Most severe scores (above 3SD)

CHAPTER V
DISCUSSION

A. Summary

The consideration of factors influencing growth and development of the jaws has often led to the more seminal question about the impact of nature versus nurture on the orofacial phenotype. The complexity of the issue of growth and development is magnified by the fact that the individual response to environmental changes is influenced by genetic factors underscoring the concept of epigenetics. In a revisitation of the functional matrix hypothesis, Moss (Moss, 1997) emphasized the resolving synthesis of the relative roles of genomic and epigenetic processes and mechanisms that cause and control craniofacial growth and development. Neither genetic nor epigenetic factors alone are sufficient, and only their integrated (interactive) activities provide the necessary and sufficient causes of growth and development.

The genetic data on MM provides new routes to understanding the etiology of mandibular growth-related conditions, predicting a patient's risk to the trait or treatment response as well as improving personalized prevention and treatment. MM has been poorly explored and only one genome-wide family-based linkage has been done in a Hispanic population (Gutierrez et al., 2010). Other chromosomal regions or loci have been also identified that harbor susceptibility genes for Class II malocclusion but not for MM.

We investigated the association between genes and familial MM in an Eastern Mediterranean population, represented by 5 families where the phenotype has been seen to segregate over two to three generations. Both genetic and cephalometric aims of this

study were fulfilled. We identified 8 new candidate loci and genes (GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7 and WNT6) that may be responsible of the development and familial transmission of this condition in this specific population.

B. Major findings

1. Genetic findings

While most pedigree studies attribute to Class II/divison1 a polygenic mode of inheritance, the pedigree analysis of MM in our study suggest a Mendelian inheritance pattern for most of the families. The difference may be related to the sample definition, ClassII/ division1 with mandibular retrognathism versus ClassII/ division 1 with MM, although the latter may have been present in these studies. Since environmental factors and other genes may modify the clinical expression of a mendelian trait, but are not of crucial importance for its development, this potential increases confidence in finding a mutation or a series of mutations responsible of the phenotype. The pedigree analysis indicated also an equal number of reported generations per family (n=3), an equal average number of reported affected males and females (n=2.36) per family and an equal number of families with males and females predominance (n=4). The equal number of affected males and females suggests that in Eastern Mediterranean families in whom female predominance has been reported, the number of affected females is greater. The results of this study also support previous reports of no gender differences where no evidence of sex-linked or sex-influenced inheritance (Hughes, 1942) have been proposed for the Cl II, division 1. However, in one of the five families, we identified 3 genes located on chromosome X.

This study highlighted the complexity and the wide range of the etiology of the Class II/division 1, and specifically when featured with MM. It is a multifactorial, polygenic trait which most likely results from mutations in numerous genes as it did not uncover any common gene, loci or variant linked to previous studies in the literature on MM, Class II/division 1 or mandibular retrognathism in different ethnic populations. In other words, the family candidate genes identified in this study support both the polygenicity and locus heterogeneity of the condition whereby each candidate gene would partially be responsible for the etiology of this trait. Hence, screening for one universal gene in all affected individuals cannot be the appropriate approach.

We succeeded at finding family-candidate genes that may be associated with the phenotype in concern. In family A, GLUD2 gene was shared between 2 affected individuals and it segregated as an X-linked recessive trait (where the mother was a carrier, however non-affected). GLUD2 encodes for the protein hGDH2 (human Glutamate dehydrogenase 2) and is located on chromosome X at Xq24/ variant position: 120183030. While Glutamate dehydrogenase in almost all mammals is encoded by a single gene (GLUD1 in the human) that is expressed widely (housekeeping), humans have also acquired a GLUD2 gene with distinct tissue expression profile.

The GLUD2 gene arose via duplication in the hominoid ancestor (<25 million years ago) and, driven by positive selection, it evolved on the line that descended to the human. During this journey, the novel GLUD2 acquired several evolutionary amino acid substitutions that provided unique functional properties. Studies have shown that GLUD2 may have contributed to human brain development where hGDH2 evolution bestowed large human neurons with enhanced glutamate metabolizing capacity, thus strengthening cortical excitatory transmission (Spanaki, Kotzamani, Kleopa, &

Plaitakis, 2016). Moreover, both hGDH1 and hGDH2 are expressed in human steroidogenic tissues, where it was observed that hGDH2 expression may serve particular metabolic needs of steroidogenic organs and Steroid hormones interact with hGDH2 with a greater affinity than with hGDH1 (Spanaki, Kotzamani, Petraki, Drakos, & Plaitakis, 2015).

In the absence of a clear hit in family A, and specifically in subfamily1, we have hypothesized that GLUD2 may have a role in mandibular or craniofacial bone morphogenesis based on a genotype/phenotype correlation. As disclosed earlier, GLUD2 is exclusively a human evolutionary acquired gene and it is not expressed in mice, hence the difficulty to understand its role and any mutation that could segregate with the phenotype is plausible as causative.

ARSH (Arylsulfatase Family Member H) was shared among 2 other affected individuals in the same family and is also found on chromosome X at Xp22.33/ variant position 2928174. Its frequency is of 0.0008% only and it belongs to the family of sulfatases. The latter hydrolyzes sulfate esters from sulfated steroids, carbohydrates, proteoglycans, and glycolipids. They are involved in hormone biosynthesis, modulation of cell signaling, and degradation of macromolecules. ARSH is clustered with ARSE ((Arylsulfatase Family Member E) on the distal short arm of the X chromosome (Xp22.3) close to the human pseudo-autosomal region. The cluster is clearly the result of a series of recent duplications occurred during mammalian evolution. Sequence comparison and evolutionary analyses suggest that ARSE was the ancestor gene of the cluster. Importantly, mutations in ARSE are responsible for X-linked CDPX (chondrodysplasia punctata 1), a congenital disease characterized by abnormalities in cartilage and bone development with variable degrees of severity, whereas no mutation

leading to a disease has been reported so far for any of the other sulfatase genes of the cluster, suggesting that the function of these sulfatases may be redundant (Sardiello, Annunziata, Roma, & Ballabio, 2005).

Mutation in ADGRG4 gene was discovered in one affected individual of family A. it is also located on chromosome X at Xq26.3/ variant position: 135428968. Its frequency in the normal population is practically 0 and it was of interest as an antibody against the GPR112 protein (alias name for the human gene ADGRG4) shows the highest expression in the muscles of the jaws.

In family B, TGIF1 was considered potentially linked with MM. TGIF1 is located on chromosome 18 at 18p11.31/ variant position: 3452167. TGIF1 was identified as a novel regulator of bone remodeling (by impairing osteoblast differentiation and activity, leading to a reduced bone formation) and an essential component of the PTH anabolic action in a mouse model (Saito et al., 2019).

FGFR3 was selected for family C following the several steps of filtering. FGFR3 (Fibroblast growth factor receptor 3) resides at chromosome 4 at 4p16.3/ variation location: 1808029. It has been proven to be involved in Meckel's cartilage and mandibular bones morphogenesis, where FGFR3 signaling is required for the elongation of Meckel's cartilage and FGFR2 and FGFR3 have roles during intramembranous ossification of mandibular bones (Havens et al., 2008). Worth noting is that dominant gain-of-function mutations in FGFR2 account for the majority of the human craniosynostosis syndromes including Crouzon, Pfeiffer, Jackson-Weiss, Seathre Chotzen and Apert syndrome which are characterized by the premature fusion of cranial sutures before the completion of brain growth(Azoury, Reddy, Shukla, & Deng, 2017).

ZNF181 (Zinc finger protein 181) falls within the category of transcription factors (which are key cellular components that control gene expression) and it was selected as the candidate gene for family D. ZNF181 is located at 19q13.11/ variation location: 35232206. Their activity determines how cells function and respond to cellular environments and is ubiquitously expressed. Moreover, microdeletion of 19q13.1, where ZNF181 is located, has been associated with syndromes exhibiting dysmorphic facial features that entailed the mandible as well (Gana et al., 2012).

Two candidate genes were elected for family E: INTS7 and WNT6. INTS7 is located at 1q32.3/ variation location: 212154515. INTS7 (Integrator complex subunit 7 also known as C1orf73) encodes a subunit of the Integrator complex that mediates 3' end processing of small nuclear RNAs U1 and U2. The integrator complex is associated with the C-terminal domain of RNA pol II largest subunit (POLR2A). The associated diseases with INTS7 include gastric cancer and Obsessive-Compulsive Personality Disorder. In a zebrafish model, ints7 has been allied with abnormal mandibular arch skeleton shape (Golling et al., 2002).

Lastly, WNT6 gene is a member of WNT family, which consists of structurally related genes which encode secreted signaling proteins. It is found at 2q35/ variation location: 219724783. These proteins have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis (one example is the integumentary system). In a transgenic zebrafish model, Wnts were shown as upstream regulators of both Bmp and Edn1 signaling, thus regulating dorsal-ventral patterning and growth of the craniofacial skeleton where deficient embryos had lower jaw specific defects (Alexander, Piloto, Le Pabic, & Schilling, 2014). Furthermore, the precise sites of Wnt6 expression have been proven to

coincide with crucial changes in tissue architecture, namely epithelial remodeling and epithelial–mesenchymal transformation (EMT), as well as the expression of Wnt6 is closely associated with areas of Bmp signaling in a chick model (Schubert et al., 2002). In a study on the temporomandibular joint (TMJ) of a mouse model, Wnt6 was particularly enriched in the perichondrium of the condyle of the temporomandibular joint of a mouse model. Noteworthy is that members of Wnt family are regulated by Gli2. Gli2 functions mostly as a transcriptional activator and functional Gli2 is necessary for normal endochondral bone development. Mice deficient in Gli2 displayed aberrant TMJ development such that the condyle loses its growth-plate-like cellular organization and no disk is formed. In addition, the condyle was much smaller in size, and the cellular organization of the growth plate within the condyle was lost (Purcell et al., 2009). Strikingly, both affected patients of family E that had underwent the biospecimen collection had reported limitations in mouth opening (which is one of the pathognomonic signs of temporomandibular disorders). Little is known about the possible influence of disc disturbances on facial growth. However, the condyle is known to play a prominent role in normal mandibular growth and, consequently, facial development. Thus, categories of temporomandibular disorders that involve dysplasia of the condylar cartilage could be associated with aberrant facial growth and form (Dibbets & Carlson, 1995).

In summary, we have uncovered 8 genes among which 5 were recognized as having a direct link to mandibular and craniofacial morphogenesis. These 5 genes were either implicated in the endochondral (condyle) or the intramembranous (remodeling) ossification (Table V-1).

Table V-1: Summary on the 8 family candidate genes with their corresponding reported function and tissue expression

		Craniofacial Tissues	Other tissues	Functional processes
Family A	GLUD2		Human steroidogenic tissues	Human brain development (strengthening cortical excitatory transmission
	ARSH		Bone and cartilage	Hormone biosynthesis, modulation of cell signaling and degradation of macromolecules
	ADGRG4	Jaw muscles		
Family B	TGIF1			Bone remodeling (osteoblasts differentiation impairment)
Family C	FGRF3	Meckel's cartilage/ mandibular bones morphogenesis		• Elongation of Meckel's cartilage • Intramembranous ossification
Family D	ZNF181	Dysmorphic facial features/ mandible (syndromes)		Transcription factor (cell function)
Family E	INTS7	Abnormal mandibular arch skeleton	• Gastro-intestinal • OCPD	3' end processing of snRNAs (U1 and U2)
	WNT6	• Lower jaw specific defects • Condylar perichondrium • Aberrant TMJ development		• Oncogenesis • Cell fate and patterning regulation (embryogenesis) • Upstream regulators of Bmp and Edn1 signaling • Epithelial remodeling and epithelial-mesenchymal transformation

Bcsugfuw: Direct link to cranio-facial and mandibular morphogenesis
Fcsugfuw: Link to bone and cartilage

Previous genetic studies identified 8 susceptibility loci on various chromosomes. No loci from the present study were in proximity with those from previous studies which reinforces the polygenicity and heterogeneity of the trait (Table V-2). Therefore, no specific chromosome can be considered as potentially highly linked to MM.

Table V-2: Summary and number of the susceptibility loci reported on each chromosome by previous studies and the present study

Chromosome Number	Locus	Total number of loci reported on each chromosome
1	1q32.3	1
2	2q35	1
4	4p16.3	1
5	5p12, 5p13	2
10	10q26.13	1
11	11q13.2	1
12	12q24.11	1
16	16q24.2	1
17	17q22	1
18	18p11.31	1
19	19q13.11	1
X	Xq24, Xq26.3, Xp22.33	3

Bkabc: loci from present study

2. Cephalometric findings

As for the cephalometric analysis, the anatomical structures that have long gone contributed to a class II, division 1 malocclusion encompass both jaws and the cranial base. These components consist of: 1) Total mandibular length and position relative to the cranial base, 2) sagittal and vertical jaw relations, 3) flexure of the cranial base (Saddle angle), 4) mandibular dento-alveolar/skeletal relation, 5) Chin projection and its soft tissue thickness (Ghafari & Macari, 2014). Mandibular length (Co-Pog) value of the initial affected patient of each selected family confirmed the presence of a MM. The difference between the patients' values and the norm ranged between -1.5 and

-16.7, indicating a decreased mandibular length that is more than 1SD below the norm. The average Co-Pog value of the 5 initial affected patients was lesser than the norms by at least 1SD. Facial divergence angular measurements indicated a tendency toward a hyperdivergent facial pattern with an upward inclination of SN relative to the horizontal (N is higher than S).

Facial divergence was represented by the average MP/SN. The average value was skewed towards a hyperdivergent pattern due to the severe hyperdivergency that was noted in family E, while the remainder of the families had a normodivergent facial pattern. Reports in the literature on the facial divergence pattern are contradictory as they mainly state a steep mandibular plane angle with a decreased posterior facial height (Drelich, 1948; Sayin & Turkkahraman, 2005). Also, a correlation has been established between SNB and the gonial angle, where the larger the gonial angle was, the smaller SNB was predicted to be and this manifests in a downward backward rotation of the mandible. The gonial angle size tended to be inversely related to and significantly correlated with mandibular body length whereby an increase in gonial angle was accompanied by an increase in mandibular length (Ardani, Willyanti, & Narmada, 2018). As a matter of fact, the vertical facial pattern is not only related to bone development leading to the different malocclusions, but also to muscle development. The skeletal growth and form depend on many factors, and mechanical loading by muscle is one of the very important factors. Contraction of masticatory muscle generates mechanical load, which can affect skeletal growth in the adjoining region and dental eruption. This hyperdivergency that was described in Class II, division 1 patients have been largely attributed to impaired muscle activity as electromyographic (EMG) investigations on the temporal and masseter muscles in Class II, division 1

malocclusions revealed less EMG activity in both muscles compared to normal occlusion during maximum intercuspation, while only the masseter showed less EMG activity during mastication (Pancherz, 1980). More generally, the masseter muscle thickness varies among the three vertical dentofacial patterns, with the hypodivergent group having the maximum thickness followed by the normodivergent, and the hyperdivergent group having the minimum thickness. In other words, increase in the thickness of the masseter muscle increases the sagittal growth, while limiting the vertical growth of the jaws (Rohila, Sharma, Shrivastav, Nagar, & Singh, 2012).

Measurements related to the sagittal relationship of the jaws disclosed an orthognathic maxilla, a retrognathic mandible, denoting a skeletal Cl II. These findings were in agreement with other studies where on average, the maxilla of Class II division 1 patients was normally positioned, whereas their mandibles were smaller in size, posteriorly positioned, and rotated open when compared with Class I subjects (Hellman, 1922; (CRAIG, 1951); Blair, 1954; (McNamara, 1981; Sayin & Türkkahraman, 2005). When dissecting the sample, a high degree of variability can be seen as families A, C and E presented a prognathic maxilla with an orthognathic mandible (except for family B who had a retrognathic mandible). Such disparities may be best explained by the influence of anatomic variations in Nasion, as deviations in the anteroposterior position of Nasion affect significantly the sagittal relationship of both jaws (Hussels & Nanda, 1984); a backward positioned Nasion reflects in more obtuse SNB angle, thus, what seems a retrognathic mandible manifests in an orthognathic mandible and vice versa. This highlights the heterogeneity of Class II, division1 malocclusion and the importance of selecting stringent inclusion criteria, in relation to

the mandible specifically (micrognathia), focusing hence on a distinct phenotypic group.

This skeletal discrepancy was accompanied by dento-alveolar measurements, showing retroclined and retruded maxillary incisors versus proclined and protruded mandibular incisors as a dento-alveolar compensation of the skeletal Cl II malocclusion. A severe proclination of maxillary incisors have always been the solely characteristic of Class II, division1(Angle, 1899), which is in conflict with the results of this study. This is best interpreted by relating the retroclination of the maxillary incisors to a compensatory mechanism of the severe sagittal skeletal discrepancy between both jaws.

C. Strengths

Very few human genetic studies to identify genes conferring susceptibility to ClassII/ division1 malocclusion have been performed. More genetic studies have been conducted for Class III because the Class III phnotype is more readily observed within families, including historic ones (Habsburg family). Most genetic studies of mandibular hypoplasia have been associated with syndromes and craniofacial anomalies such as hemifacial microsomia or the Pierre Robin syndrome (Pirttiniemi, Peltomäki, Müller, & Luder, 2009). Hemifacial microsomia has been observed in a number of chromosome disorders. Some associations could have occurred by chance, but the repeated observation of deletion 5p (Ala-Mello et al., 2008), duplication 14q23.1 (Ballesta-Martínez et al., 2013; Zielinski et al., 2014), and abnormalities of chromosomes 18 (Clarren & Salk, 1983; Curran, al-Salihi, & Allderdice, 1970) and 22 (Quintero-Rivera & Martinez-Agosto, 2013; Torti, Braddock, Bernreuter, & Batanian,

2013) may represent causal associations. Thus, no reports are available relating the 8 novel candidate genes to craniofacial anomalies.

The most important strength of this study is its novelty as the first genetic study of MM conducted in 5 families, the greater number to date, and in the absence of previous genetic reports on MM worldwide, the present study contributes to better comprehend the genetic determinants of MM and the variation in the risk for MM in this population.

With recent technological advances that allowed the simultaneous characterization of entire genomes via high throughput genotyping of SNVs and Indels or sequencing of the genome to evaluate human genetic variation, gene and gene-environment studies of malocclusion could be performed on precisely defined phenotype, yielding valuable insights into the etio-pathogenesis underlying malocclusion. The results of the present study were possible through the application of the revolutionizing sequencing technique “Whole Exome Sequencing (WES)”. WES was preferred over Whole Genome sequencing (WGS) since, even though WES covers only 2% (around 180 000 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 has the capability to expand targeted content to include untranslated regions (UTRs) and microRNA for a more comprehensive view of gene regulation. No previous study includes the application of WES using NGS while exploring Cl II, mandibular retrognathism or MM. In contrast, 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 with craniofacial or mandibular morphology in the literature.

The genetic screening did not show any aberration in the reported genes linked to MM that were revealed in the earlier genetic studies. Therefore, 8 potentially novel genes (GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7 and WNT6) were recognized that segregate with the phenotype and could be implicated in mandibular development. Accordingly, if those genes are present in the genotype of an individual, the latter may express the trait.

A significant contribution of this study is also in its design, including subjects and families with MM, thus focusing on the underlying skeletal dysplasia and the heritability characteristic of the mandible. Such phenotypic characterization will represent data infrastructure to future large-scale genetic studies that should allow an in-depth analysis of the etiology of Class II/divison1 malocclusion. The identification of genetic influences in Class II/divison1 malocclusion would aid in the prevention and improve treatment modalities of maxillo-mandibular discrepancies.

Furthermore, the diagnosis of MM in this study was based on several cephalometric measurements: an ANB angle $\geq$ 4.5 degrees and/or a positive wits appraisal +2.0 mm accompanied with a reduced mandibular length (Co-Gn and Co-Go) below at least one standard deviation of the norm for their age (mandibular micrognathism) and a dental Cl II malocclusion at least on one side (with an increased OJ). Those traits translated in a significant convexity of the profile. The stringent selective criteria reinforce the credibility of this study, particularly in comparison with no previous studies, in which their definition of ClassII/division1 was mainly based on morphological and dental characteristics only, and not the severity of the underlying

skeleton. We allocated the severity of MM in relation to the norms of the corresponding age. The only study on MM in the literature referred to cephalometric measurements for diagnosis, but no mention was made whether the norms of corresponding age were used and the cephalometric data were not tabulated or reported (Gutierrez et al., 2010).

Our sample included several families 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. Except for the aforementioned (Gutierrez et al., 2010), other studies included non-related individuals (Yamaguchi et al., 2001) (Sasaki et al., 2009) (Zebrick et al., 2014) (Arun et al., 2016) (Balkhande et al., 2018) (da Fontoura et al., 2015). Also, our study was unique in involving non-affected individuals of each family that served as controls when comparing the genes and variants across individuals and families, as well as in cephalometric appraisals.

D. Clinical considerations

The question of whether environment or genetics exerts the greater influence in the etiology of malocclusion has been a matter of extensive discussion and controversy. The hereditary pattern is usually taken into consideration in the diagnosis and treatment of patients. The prior and present investigations confirm that Class II/division1 malocclusion, particularly when underlined by a skeletal discrepancy, shows familial tendency, while a probable polygenetic basis for this resemblance is probably polygenic. This complex malocclusion is continuous and multifactorial in nature, with a corresponding complex mode of inheritance.

Orthodontic treatment aims to and succeeds in achieving a normal Class I occlusion but does not, on average, change the phenotypic expression of the Class II skeletal discrepancy even with the assistance of long term dentofacial orthopedics, precisely because the deviant features within a malocclusion are greatly determined by genetics. In severe skeletal discrepancies, surgical treatment becomes the only option to modify the Class II phenotype. Accordingly, knowledge in the genetic role in the manifestation of particular malocclusion will help project such outcome and genetic screening could be integrated in our practice as a diagnostic and prognostic tool. The recent advances in sequencing (cost and time effective alternatives are emerging) and the rise of precision medicine, as preventive or therapeutic interventions might then be concentrated on those who will benefit, sparing expense and side effects for those who will not.

E. Research considerations

1. Limitations

It should be noted that there is no database in Lebanon or the region for results of WES for normal individuals. Therefore, we could not verify if the 8 candidate genes are present or not in the normal population of this region. In fact, we compared our results to the normal database of the whole globe, which includes 6000 healthy individuals for WES and 100000 healthy individuals for WGS and found that the 8 genes are not present in these databases.

2. Future Research

Because of the massive complexity of this skeletal jaw disharmony, a prospective association study would be warranted including subjects divided into various groups, covering the different phenotypes associated with Class II/division1. The present study laid the foundation for future studies to massively identify causative genes in this multifactorial problem and screen for the variants that were found in this study in other ethnic populations. As for the methodology, genetic testing may be expanded to WGS to include the entire genome and then subsequently to the epigenome, knowing that the final manifestation of the phenotype is the result of not only DNA sequences, but also heritable changes in gene regulatory information.

Future research should also focus on a larger number of pedigrees for more conclusive findings. The exploration of specific genes contributing to various phenotypes associated with Class II/division1 will help us understand the variation in mandibular and craniofacial growth along with potential prediction of the final manifestation of the growth pattern or the severity of the malocclusion conferred by a particular genotype.

The relative contribution of both genetics and environment to the etiology of malocclusion has always been a matter of controversy as, in any trait, both genes and environment are involved and collectively contribute to the ultimate phenotype. Well-designed twin studies (both mono and dizygotic twins) would contribute in quantifying the relative impact of genetic and environmental factors on Class II/division1 (when either featured with mandibular retro or micrognathism). Studying monozygotic twins alone does not solve our issue as not only they share similar genetic makeup, but also

relatively a similar environmental background. On the other hand, dizygotic twins could act as control, as they too share the same early environmental factors, but are not genetically alike; researchers may inspect for traits that show a great similarity in identical twins and compare it with dizygotic twins. Such great similarity must indicate a shared genetic basis for the trait.

CHAPTER VI
CONCLUSIONS

Class II/division1 is a complex trait, with both environmental and genetic influences interacting to create disharmony of the jaws and teeth. This interaction can create a distorted facial appearance and have significant effects on the quality of life, both psychosocially and functionally. The etiology of the malocclusion is not fully understood because of the large variability observed in the phenotype. This genetic study and the associated cephalometric data represent the first approach based on stringent inclusion criteria to highlight candidate genes related to non-syndromic mandibular micrognathism. The emphasis on mandibular micrognathism rather than simply retrognathism allowed the determination of genetic definitions heretofore not reported. The study is also the first family-based genetic investigation compared to other studies, which were based on non-related probands linkage analysis. The key conclusions are summarized:

1. The pedigree analysis of 11 Eastern Mediterranean families, comprising affected individuals over 2-3 generations, indicated an equal number of reported generations per family (n=3), an equal average number of reported affected males and females (n=2.36) per family and an equal number of families with males and females predominance (n=4). The data on the majority of the investigated families suggested an autosomal dominant mode of inheritance of mandibular micrognathia.
2. Through Whole Exome Sequencing (WES), 8 genetic variants were identified in exonic regions of the 28 subjects (15 affected and 13 non-affected) that are part of

the 5 enrolled families. These variants (GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7 and WNT6) were classified as family-candidate genes.

3. No specific chromosome was found to be suggestive of linkage to familial mandibular micrognathism in the Eastern Mediterranean population as the 8 genes were located on different chromosomes. In family A, the 3 (GLUD2, ARSH and ADGRG4) candidate genes were located on chromosome X. Two genes (INTS7 and WNT6) were identified in family E, where they may or may not segregate in a digenic pattern. WNT6 has been proven to be involved in the pathway of proper temporo-mandibular joint development and it was particularly enriched in the condylar perichondrium.
4. On average, cephalometric measurements of individuals with mandibular micrognathia disclosed a normal maxillary length, a tendency to a hyperdivergent facial pattern with posterior inferior inclination of SN relative to the horizontal (S lower than N), a skeletal Class II malocclusion underlined by an orthognathic maxilla and retrognathic mandible. Retroclined maxillary incisors and proclined mandibular incisors reflected typical dento-alveolar compensations of the ClassII/ division1 malocclusion.
5. Among different future research pathways, studies are warranted on linking clinical treatments with the familial genetic make-up of the patients. The significance of this research would be to determine the potential success of treatment with or without surgery in affected subjects.

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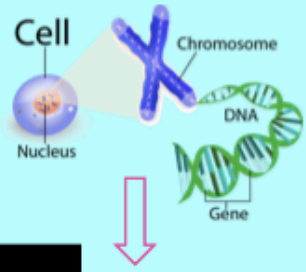
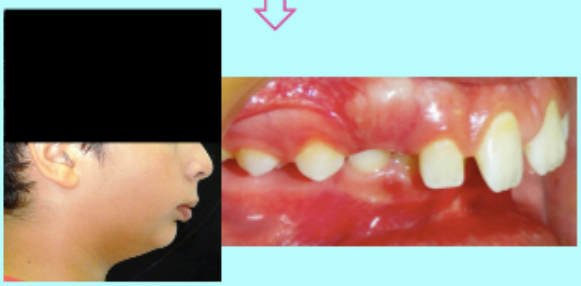
Appendix I

Invitation for participation in a research study

You are invited to participate in a research study entitled "**Association between genes and familial mandibular micrognathism in an eastern Mediterranean population**", conducted by Dr. Anthony Macari (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

- Explore whether the condition of mandibular micrognathism (reduced size of lower jaw) is inherited
- Help identify the genes involved in its development and familial transmission

Inclusion

- Families known to have subjects affected by mandibular micrognathism (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

- You will not receive payment for participation in this study.
- 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 for mandibular micrognathism in our population and the genetic mechanisms that may influence the response of the lower jaw to orthodontic and orthopedic treatment.
- Early prediction of the condition would also lead also to variation in the approach of treatment

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. Anthony Macari at (01)350 000 ext 5702.

Commitment Required

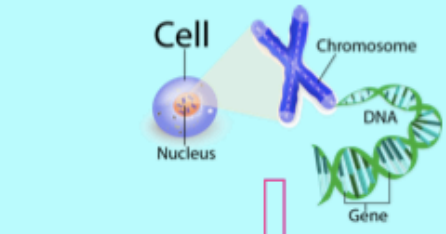
This will require you to pass by AUBMC for one (45mins) or two (30mins + 15mins) visits: the first one to explain the project in detail, sign this consent form, have a clinical examination and a lateral cephalometric x-ray, and the second visit to have 5cc of your blood collected.

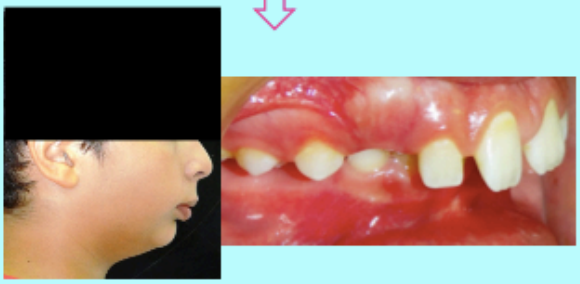
Location of Research

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 و تراجع و صغر حجم الفك السفلي العائلي لدى سكان منطقة شرق البحر المتوسط , يجريها الدكتور أنطوني مكاري. ستجرى الدراسة في الجامعة الأميركية في بيروت (قسم تقويم الأسنان وتأهيل الفكين). تسير مجريات الدراسة وفقا" للشروط والأحكام الموافق عليها من قبل لجنة الأخلاقيات.





أهداف الدراسة :

استكشاف ما إذا كان يتم توريث حالة صغر حجم و تراجع الفك السفلي.
المساعدة في تحديد الجينات المسؤولة عن تطوير وانتقال هذه الحالة وراثياً.

التضمين:

الأسر المعروف أن لديها أفراد مصابين ببروز الفك السفلي (بسبب علاج سابق أو جاري لأحد منهم في قسم تقويم الأسنان وتأهيل الفكين في الجامعة الأميركية في بيروت). سيتم ادراج الأعضاء المصابين وغير المصابين في الدراسة.

الفوائد الناتجة عن المشاركة في الدراسة

لن تحصل على أي مردود مالي مقابل مشاركتك في الدراسة.
انها تقدم بعض الفوائد للمجتمع والطب/ العلم ككل لأن هذه الدراسة ستسمح لنا بأن نفهم أكثر الجينات المعنية التي تساهم في تفاوت خطر بروز الفك السفلي لدى شعبناوالأليات الوراثية التي تؤثر على تفاعل الفك السفلي مع المعالجة التقويمية والعظمية.
كماوقد يؤدي التنبؤ المبكر الى تغير في نهج العلاج.

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 أنطوني مكاري على الرقم 01350000 مقسم 5702.

الالتزام المطلوب:

سيتطلب هذا الأمر منك أن تقوم بزيارة AUBMC مَرّة(45 دقيقة) أو مَرّتين (30 دقيقة +15 دقيقة): الزيارة الأول لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء استمارة الموافقة هذه، ولمعاينة أسنانك وأخذ صورة بالاشعة السينية جانبية لقياسات الرأس. الزيارة الثانية لنأخذ 5 سنتيمتر مكّعَب من دمك.

موقع الدراسة :

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

Appendix II

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: ***ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR MICROGNATHISM IN AN EASTERN MEDITERRANEAN POPULATION***

Protocol Number	
Principal Investigator(s)	Dr Anthony MACARI
Address	American University of Beirut Medical Center
Phone	(01) 350 000 ext: 5702
Site where the study will be conducted	Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

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 the genes involved in the development and familial transmission of your lower jaw because it is placed backward and small in size, which positions your lower teeth more backward relative to the upper teeth. You are being asked to join the study:

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

1. WHAT IS INVOLVED IN THE STUDY?

If you decide that you want to be in this study, this is what will happen:

Step	Procedure
1	- Medical history will be filled out: gender, date of birth, age, family origin, health status, affection status of other individuals in the family - The orthodontist will look at your teeth and will take one x-ray of your profile - You will be positioned in the x-ray machine, which will move next to your face, for about 2 minutes. - An image of your profile will appear on the screen, showing your teeth and face. - This is done to find out if your lower jaw is backward or not. - 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.
2	- A nurse or doctor will take 5cc of blood from your arm, using a clean needle. This is done to find out the genes that are causing your lower jaw to be small in size and backward

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 lateral cephalometric x-ray, and the second visit to have 5cc of your blood collected.

2. 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 (pain, bruising, slight possibility of fainting). There is also radiation risk from the X-ray, however there are no proven harmful effects from irradiation levels that you will be exposed to during the study. Please note that there may be unforeseeable risks.

3. CAN ANYTHING GOOD HAPPEN TO ME?

If we are able through treatment with braces to fix your problem, which will be most good we do for you. By studying your lower jaw problem and that in other members of your family, we will be adding to our knowledge of the problem, so that in the future, people with the same problem may benefit from this study, including perhaps your children or those of your brother(s), sister(s) or cousins.

4. DO I HAVE OTHER CHOICES?

You can choose not to be in this study.

5. WILL ANYONE KNOW I'M 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.

6. WHAT HAPPENS IF I GET HURT?

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 that this risk is minimal.

7. WHAT ARE THE COSTS

There are no costs associated with participation. The study will cover the costs of the procedures done for its purpose: the blood withdrawal, genetic analysis and the X-ray that is taken on relatives where features of the condition are noted clinically. All other costs will not be covered.

Before you say yes to be in this study, be sure to ask Dr Anthony MACARI to tell you more about anything that you don't understand.

8. WHAT IF I DON'T 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. Also, the investigator may end your participation at any time. 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 # , Date
Protocol # BIO-2018-0289

الأحرف الأولى لاسم القاصر:
لجنة الأخلاقيات
الجامعة الأميركية في بيروت كلية الطب
شارع بلسبيروت، لبنان
هاتف: 350000-(10) مقسم: 5445

الموافقة للإشتراك في البحث العلمي للمشترك القاصر

قاصرون تتراوح أعمارهم بين 7 سنوات و 12 سنة

الارتباط بين الجينات تراجع و صغر حجمالفك السفلي العائلي لدى سكان منطقة شرق البحر المتوسط	إسم المشترك:
الموقع حيث ستجرى الدراسة: **قسم تقويم الأسنان وتأهيل الفكّين، الطابق السادس، المركز الطبي في الجامعة الأميركية في بيروت**	الباحث الرئيسي: الدكتور أنطوني مكاري المركز الطبي في الجامعة الأميركية شارع الحمرا – بيروت – لبنان الهاتف: 01350000 مقسم:5702
نريد ان نخبرك عن دراسة نقوم بها نحن. **إن البحث هو عبارة عن طريقة خاصة تستخدم لمعرفة حقائق حول شيىء ما.**	

إننا نحاول معرفة المزيد حول الجينات المعنية بتطوير الفك السفليّ الخاص بك لأنه صغير الحجم ومتراجع إلى الخلف (مما يجعل أسنانك العلوية متقدمة أكثر من الأسنان السفلية). أنت مدعو للانضمام إلى الدراسة:

- إذا كنت مريضاً تتلقى العلاج في قسمنا: خلال موعدك الطبي، سيشرح لك طبيبك المعالج أهداف الدراسة، إجراءاتها، وفوائدها / مخاطرها، وستسألك إذا كنت ترغب في المشاركة فيها. اذا وافقت، سيعرفك طبيبك على منسقة الدراسة (د. ميشيل شقية) و سيُطلب منك الامضاء على استمارة الموافقة هذه. بعد امضائها، ستسأل عن تاريخ عائلتك، بما في ذلك حالة صغر حجم و تراجع الفكّ السفليّ لأفراد آخرين من عائلتك. كما وسيتم اعطاؤك نشرات لتوزّعها على أقربائك المقربين الذين قد يستفيدون، بحسب اعتقادك، من هذه الدراسة.
كما وسيتم اعطاؤك نشرات لتوزّعها على أقربائك المقربين الذين قد يستفيدون، بحسب اعتقادك، من هذه الدراسة.
- أو، اذا كنت من أقرباء مريض يتلقى العلاج في قسمنا، وكنت قد أخذت النشرة منه وقرأتها.

1. ماذا تشمل هذه الدّراسة؟

سيتم تعيين 30 شخص أ للمشاركة في الدراسة. إذا قررت الإشتراك في الدراسة، إليك ما سيحصل:

الخطوة	الإجراءات
1	- تعبئة تاريخ طبي حول الأصل العائلي و تاريخ عائلتك ومدى تأثر أفراد آخرين في الأسرة - سينظر طبيب تقويم الأسنان إلى أسنانك وسيأخذ صورة بالأشعة السينية (في حال وجود علامات الحالة عند معاينة اسنانك). - سيتم وضعك في جهاز الفحص بالأشعة السينية الذي سيتحرك حول وجهك لمدة دقيقتيْن. - ستظهر على الشاشة صورة جانبية تظهر فيها أسنانك ووجهك. - سيتم ذلك لمعرفة ما إذا كان فكك السفلي صغير الحجم و متراجع أم لا. - حتى إذا كنت لا ترغب في المشاركة في الدراسة البحثية، إلا أن الأشعة السينية هذه قد تلزمك في حال قررت الخضوع لتقييم وعلاج تقويم الأسنان. في حال وجود إكتشاف غير طبيعي على الأشعة السينية، سيتم إجراء المزيد من التحقيقات وفقاً لذلك. ستستفيد من تشخيص مجاني وسيتم إقتراح العلاج المناسب. **إذا كان(ت) مريضاً/(ة) في قسمنا، فلدينا السجلات المذكورة أعلاه، ولن نقوم بتكرارها إلا إذا قد مضى وقت طويل مما يتطلب تحديثها.**
2	ستسحب ممرضة أو طبيب 5 سنتيمتر مكعّب من الدم من ذراعك باستخدام إبرة نظيفة. سيتم ذلك لمعرفة ما هي الجينات التي تتسبب بصغر حجم و تراجع الفكّ السفليّ.

سيتطلب هذا الأمر منك أن تقوم بزيارة AUBMCمَرّة(45دقيقة) أو مَرّتين (30 دقيقة 15+ دقيقة(: الزيارة الأول لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء استمارة الموافقة هذه، ولمعاينة أسنانك وأخذ صورة بالاشعة السينية جانبية لقياسات الرأس . الزيارة الثانية لنأخذ 5 سنتيمتر مكّعب من دمك.

2. هل ممكن أن يصيبني أي ضرر؟

نريد أن نخبرك عن بعض الأشياء التي قد تؤلمك أو تزعجك اذا شاركت في البحث. قد تؤلمك الإبرة التي نستخدمها لسحب الدم. وقد تحصل على كدمة على ذراعك مع احتمال ضئيل أن يغمى عليك. كما وهناك خطر الإشعاع، إلا أنه ليس هناك أي آثار ضارة مُثبتة تنتج عن مستويات الإشعاع التي ستتعرض لها أثناء هذه الدراسة. يرجى ملاحظة أنه قد تكون هناك مخاطر غير متوقعة.

3. هل ممكن أن يحصل لي أي شيء جيد ؟

اذا تمكنا من حَلّ مشكلتك من خلال العلاج بمشابك الأسنان، فسيكون هذا أفضل ما نقدمها لك. من خلال دراسة مشكلة فكك السفلي ومشكلة أفراد آخرين من عائلتك، ستزداد معلوماتنا فيما يخص المشكلة. وقد يسمح ذلك في المستقبل للأشخاص الذين يعانون المشكلة ذاتها، بما فيهم ربما أولادك أو أولاد أشقائك وشقيقاتك وأقاربك، من الاستفادة من هذه الدراسة.

4. هل لي خيار آخر؟

يمكنك أن تختار عدم المشاركة في هذه الدراسة.

5. هل يمكن لأحد أن يعلم أنني مشترك في الدراسة؟

والداك ومن يقوم بالبحث وحسب. لن نخبر أحداً أنك شاركت في الدراسة. عندما ننتهي من الدراسة، سنكتب تقريراً عمّا وجدنا. ولن نستعمل إسمك في التقرير.

6. ماذا يحصل لو أصابني أذى؟

إن المركز الطبي في الجامعة الأمريكية في بيروت سوف يغطي تكاليف العلاج في المركز للعوارض الطبية السلبية الناجمة مباشرة عن الأدوية و/أو الإجراءات الطبية الخاصة بهذه الدراسة البحثية. فيما عدا ذلك، لن يقوم المركز الطبي بتغطية تكاليف العناية الطبية لأية حالة أو مشكلة مرضية. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جداً.

7. ما هي التكاليف؟

ليس ثمة أي تكاليف مرتبطة بالمشاركة. ستغطي الدراسة تكاليف الاجراءات المطلوبة لتحقيق اهدافها: سحب الدم، التحليل الجيني والأشعة السينية التي ستؤخذ للأقارب الذين لوحظت عندهم ملامح الحالة سريرياً. لن تتم تغطية أي تكاليف أخرى.

8. ماذا لو لم أرد أن أفعل ذلك؟

لست مجبراً على أن تشارك في هذه الدراسة. فإنه إختيارك. إذا قلت نعم الآن لكن غيرت رأيك لاحقاً، لا بأس بذلك أيضاً. كل ما عليك فعله هو أن تخبرنا أنك غيرت رأيك .كما وأنه يحق للباحث الرئيسي إنهاء مشاركتكم فالدراسة في أي وقت. إذا أردت الإشتراك في هذه الدراسة، الرجاء كتابة أو إمضاء اسمك.

☐ نعم أريد الإشتراك في البحث ☐ لا أريد الإشتراك في البحث

_____	_____
اسم المشترك القاصر	توقيع المشترك القاصر
_____	_____
اسم الشخص الحاصل على الموافقة	التوقيع

_____

التاريخ و الساعة

لجنة الأخلاقيات

تاريخ النسخة:

بروتوكول#: BIO-2018-0289

Appendix III

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)

Project Title: ***ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR MICROGNATHISM IN AN EASTERN MEDITERRANEAN POPULATION***

Protocol Number	
Principal Investigator(s)	Dr Anthony MACARI
Address	American University of Beirut Medical Center
Phone	(01) 350 000 ext: 5702
Site where the study will be conducted	Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

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 the genes involved in the development and familial transmission of mandibular micrognathism (deficient lower jaw in size) in the Mediterranean population. You are being asked to join the study:

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

1. WHAT IS INVOLVED IN THE STUDY?

This is what will happen if you are in this study:

Step	Procedure
1	- Medical history will be filled out: demographics (gender, date of birth, age, family origin), health status, genetics (consanguinity, affection status of other individuals in the family) - The orthodontist will look at your teeth and will take one x-ray of your profile - You will be positioned in the x-ray machine, which will move next to your face, for about 2 minutes. - An image of your profile will appear on the screen, showing your teeth and face. - This is done to find out if your lower jaw is backward or not.

	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. ***"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"***
2	- A nurse or doctor will take 5cc of blood from your arm, using a clean needle. - This is done to find out the genes that are causing your lower jaw to be deficient in size and backward

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 lateral cephalometric x-ray, and the second visit to have 5cc of your blood collected.

2. WHAT ARE THE RISKS OF THE STUDY?

Risk	Procedure
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 cephalogram is only 1.7mrem, 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.

3. 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 mandibular micrognathism in our population. 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 response of the lower jaw to orthodontic and orthopedic treatment. Early prediction of the condition would lead to:

1. Earlier treatment that might reduce the severity of the condition and possibly avoidance of surgery OR
2. More importantly, forego earlier interventions in favor of a later orthognathic surgery when mandibular micrognathism is genetically determined as a "certainty".

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

Minor's Initials: _____

4. WHAT OTHER OPTIONS ARE THERE?

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

5. 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.

6. WHAT ABOUT CONFIDENTIALITY?

Every reasonable effort will be made to keep your records confidential. 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 (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.
However, while you are in this study we do have to let some people look at your records. These people will be the study doctor and designee, the ethics committee and inspectors from governmental agencies. **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: September 2019).

- ☐ I want to be informed of the results of the study
- ☐ I don't want to be informed of the results of the study

The study doctor can use the study results as long as you cannot be identified.

7. 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.

8. WHAT ARE THE COSTS?

There are no costs associated with participation. The study will cover the costs of the procedures done for its purpose: the blood withdrawal, genetic analysis and the X-ray that is taken on relatives where features of the condition are noted clinically. All other costs will not be covered.

9. WILL YOU GET PAID TO BE IN THIS STUDY?

You will not be paid to participate in this study.

10. 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.

Minor's Initials: _____

AGREEMENT TO BE IN THE STUDY

Your signature below means that you have read the above information about the Association between genes and familial mandibular micrognathism in the Mediterranean population study and have had a chance to ask questions to help you understand what you will do in this study. Your signature also means that you have been told that you can change your mind later if you want to without loss of benefits. You will be given a copy of this assent form. By signing this assent form you are not giving up any of your legal rights.

_____	_____
NAME OF SUBJECT	**AGE**
_____	_____
SIGNATURE OF SUBJECT (13 YRS - 17 YRS)	**DATE & Time**

_____	_____	_____
PERSON OBTAINING ASSENT	**SIGNATURE**	**DATE & Time**

الأحرف الأولى لاسم القاصر:
لجنة الأخلاقيات
الجامعة الأميركية في بيروت كلية الطب
شارع بلسبيروت، لبنان
هاتف: (10)-350000 مقسم: 5445

الموافقة للإشتراك في البحث العلمي للمشترك القاصر

مراهقون تتراوح أعمارهم بين 13-17 سنة

الارتباط بين الجينات تراجع و صغر حجم الفك السفلي العائلي لدى سكان منطقة شرق البحر المتوسط	إسم المشترك:
الموقع حيث ستجرى الدراسة: **قسم تقويم الأسنان وتأهيل الفكّين، الطابق السادس، المركز الطبي في الجامعة الأميركية في بيروت**	الباحث الرئيسي: الدكتور أنطوني مكاري المركز الطبي في الجامعة الأميركية شارع الحمرا – بيروت – لبنان الهاتف: 01350000 مقسم:5702
نريد ان نخبرك عن دراسة نقوم بها نحن. **إن البحث هو عبارة عن طريقة خاصة تستخدم لمعرفة حقائق حول شيىء ما.**	

إننا نحاول معرفة المزيد حول الجينات المعنية بتطوير الفك السفليّ الخاص بك لأنه صغير الحجم ومتراجع إلى الخلف (مما يجعل أسنانك العلوية متقدمة أكثر من الأسنان السفلية). أنت مدعو للانضمام إلى الدراسة:

- إذا كنت مريضاً تتلقى العلاج في قسمنا: خلال موعدك الطبي، سيشرح لك طبيبك المعالج أهداف الدراسة، إجراءاتها، وفوائدها / مخاطرها، وستسألك إذا كنت ترغب في المشاركة فيها. اذا وافقت، سيعرفك طبيبك على منسقة الدراسة (د. ميشيل شقية) و سيُطلب منك الامضاء على استمارة الموافقة هذه. بعد امضائها، ستسُأل عن تاريخ عائلتك، بما في ذلك حالة صغر حجم و تراجع الفكّ السفليّ لأفراد آخرين من عائلتك. كما وسيتم اعطاؤك نشرات لتوزّعها على أقربائك المقربين الذين قد يستفيدون، بحسب اعتقادك، من هذه الدراسة.
- أو، اذا كنت من أقرباء مريض يتلقى العلاج في قسمنا، وكنت قد أخذت النشرة منه وقرأتها.

1. ماذا تشمل هذه الدّراسة؟

سيتم تعيين 30 شخصاً للمشاركة في الدراسة. إذا قررت الإشتراك في الدراسة، إليك ما سيحصل:

الخطوة	الإجراءات
1	- تعبئة تاريخ طبي حول الأصل العائلي و تاريخ عائلتك ومدى تأثّر أفراد آخرين في الأسرة - سينظر طبيب تقويم الأسنان إلى أسنانك وسيأخذ صورة بالأشعة السينية (في حال وجود علامات الحالة عند معاينة اسنانك). - سيتم وضعك في جهاز الفحص بالأشعة السينية الذي سيتحرك حول وجهك لمدة دقيقتيْن. - ستظهر على الشاشة صورة جانبية تظهر فيها أسنانك ووجهك. - سيتم ذلك لمعرفة ما اذا كان فكك السفلي صغير الحجم و متراجع أم لا. - حتى إذا كنت لا ترغب في المشاركة في الدراسة البحثية، إلا أن الأشعة السينية هذه قد تلزمك في حال قررت الخضوع لتقييم وعلاج تقويم الأسنان. في حال وجود إكتشاف غير طبيعي على الأشعة السينية، سيتم إجراء المزيد من التحقيقات وفقاً لذلك. ستستفيد من تشخيص مجاني وسيتم إقتراح العلاج المناسب. **إذا كان(ت) مريضاً/(ة) في قسمنا، فلدينا السجلات المذكورة أعلاه، ولن نقوم بتكرارها إلا إذا قد مضى وقت طويل مما يتطلب تحديثها.** **"لا تستطيعين المشاركة في هذه الدراسة إذا كنت حاملاً. إذا كنت قادرة على الحمل، سيتم إجراء اختبار الحمل قبل أن تتعرضي لأي إشعاع. ينبغي أن تخبرينا إذا ثمة احتمال أن تكوني قد حملت في غضون الأيام الأربعة عشرة الماضية، وذلك لأنه لا يمكن الاعتماد على اختبار الحمل خلال تلك الفترة".**
2	ستسحب ممرضة أو طبيب 5 سنتيمتر مكعّب من الدم من ذراعك باستخدام إبرة نظيفة. سيتم ذلك لمعرفة ما هي الجينات التي تتسبب تراجع و صغر حجم الفك السفلي.

سيتطلب هذا الأمر منك أن تقوم بزيارة AUBMC مَرّة(45 دقيقة) أو مَرّتين (30 دقيقة +15 دقيقة) : الزيارة الأول لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء استمارة الموافقة هذه، ولمعاينة أسنانك وأخذ صورة بالاشعة السينية جانبية لقياسات الرأس. الزيارة الثانية لنأخذ 5 سنتيمتر مكّعّب من دمك.

2. ما هي مخاطر هذه الدراسة ؟

المخاطر	الإجراءات
خطر الإشعاع	على الرغم من أنه ليس هناك أي آثار ضارة مُثبتة تنتج عن مستويات الإشعاع التي ستتعرض لها أثناء هذه الدراسة، إلا أنه لا يمكن استبعاد آثار طويلة الأمد على صحتك. الجرعة الفعالة لصورة واحدة لقياسات الرأس هي 7.1 ميلي ريم فقط، وهي نسبة ضئيلة مقارنةً بمعدّل الجرعة السنوية التي يتلقاها الشخص من الإشعاع البيئي (ما يقارب 300 ميلي ريم).
المرتبطة بسحب الدم	قد تشمل هذه المخاطر الكدمات، ألم موضعي، ورم دموي، واحتمال ضئيل للإلتهاب أو الإغماء. سيتم سحب الدم من قبل ممرضة متخصصة أو طبيب في المستشفى (AUBMC)، وذلك باستخدام إبرة نظيفة. وبالتالي، هذا الخطر ضئيل.

للمزيد من المعلومات حول هذه المخاطر، إسأل الطبيب المسؤل عن الدراسة. يرجى ملاحظة أنه قد تكون هناك مخاطر غير متوقعة.

3. هل هناك فوائد من المشاركة في الدراسة؟

العلم ككل لأن هذه الدراسة ستسمح لنا بأن نفهم أكثر الجينات المعنية التي تساهم في تفاوت خطر صغر حجم و تراجع الفك السفلي لدى شعبنا. ويمكن أن تؤدي هذه المعرفة إلى تقدير أفضل لقابلية حصول هذه الحالة وكذلك إلى فهم أفضل للآليات الوراثية التي تؤثر على تفاعل الفكّ السفليّ مع المعالجة التقويمية و العظمية. وقد يؤدي التنبؤ المبكر للحالة إلى التالي:

1. العلاج المبكر الذي يمكن أن يقلل من وخامة الحالة، وربما تجنب عملية جراحية في وقت لاحق أو
2. الأهم، الامتناع عن القيام بأي تدخلات مبكرة من أجل القيام بجراحة فكّ في وقت لاحق حينما يكون صغر حجم و تراجع الفك السفلي محدد وراثياً على أنه حتميّ.

4. ما هي الخيرات الاخرى؟

هذه ليست دراسة مخصصة للعلاج، فالوسيلة الوحيدة البديلة هي عدم مشاركتك في هذه الدراسة.

5. هل يمكن اخرجك من الدراسة بدون موافقتك؟

لن يتم اخرجك من الدراسة من قبل الفريق المختص بالدراسة من دون موافقتك. غير أنه يحق للباحث الرئيسي إنهاء مشاركتكم فالدراسة في أي وقت.

6. ماذا عن السرية؟

إذا وافقت على المشاركة في الدراسة، ستحفظ المعلومات **بسريةٍ**. لن يكون لأي شخص، ما لم ينص القانون على ذلك، حق الاطلاع مباشرةً على سجلاتك الطبية باستثناء الطبيب المسؤول عن البحث ومن ينوب عنه، لجنة الأخلاقيات ومفتشين من الادارات الحكومية.

ستؤخذ الصورة بالأشعة في قسم تقويم الأسنان وتأهيل الفكّين في المركز الطبي في الجامعة الأميركية في بيروت وتحفظ في بنك الصور الشعاعية الذي تم احداثها وضمها في البرنامج الشعاعي المطابق (CLINIVIEW). سيتم وضعها في ملفّ رقميّ منفصل على كومبيوتر في قسمنا، وسيتم تطبيق رموز بحيث لا يمكن لأحد الولوج إلى الملف إلا أعضاء فريق البحث **وحسب**.	سيتم ترقيم عينات الدم وترميزها وتخزينها في مكان آمن حتى نهاية الدراسة. سيتم الاحتفاظ بجميع عينات الدم ومشتقاتها إذا أردت المشاركة في دراسات جينية أخرى. وإلا، سيتم التخلصّ منها في نهاية الدراسة.

بناءً على طلبك، سيتم الكشف عن النتائج الشخصية لك ولأفراد عائلتك بمجرد الانتهاء من الدراسة (التاريخ المتوقع: أيلول 2019).

☐ **أريد أن أبلّغ بنتائج الدراسة.**

☐ **لا أريد أن أبلّغ بنتائج الدراسة.**

7. ماذا يحصل لو أصابك أذى خلال الدراسة؟

إن المركز الطبي في الجامعة الأمريكية في بيروت سوف يغطي تكاليف العلاج في المركز للعوارض الطبية السلبية الناجمة مباشرة عن الأدوية و/أو الإجراءات الطبية الخاصة بهذه الدراسة البحثية. فيما عدا ذلك، لن يقوم المركز الطبي بتغطية تكاليف العناية الطبية لأية حالة أو مشكلة مرضية. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جداً.

8. ما هي التكاليف؟

ليس ثمة أي تكاليف مرتبطة بالمشاركة. ستغطي الدراسة تكاليف الاجراءات المطلوبة لتحقيق اهدافها: سحب الدم، التحليل الجيني والأشعة السينية التي ستؤخذ للأقارب الذين لوحظت عندهم ملامح الحالة سريرياً. لن تتم تغطية أي تكاليف أخرى.

9. هل ستتقاضى أي مردود مالي مقابل المشاركة في الدراسة؟

لن تحصل على أي مردود مالي مقابل مشاركتك في الدراسة.

10. ما هي حقوقك كموضوع للبحث؟

مشاركتك في الدراسة أمرٌ طوعي. لستة مجبراً على المشاركة في الدراسة إذا لم تريد ذلك، كما أنه يمكنك الإنسحاب من الدراسة في أي وقت. قرارك هذا لن يؤدي إلى أي عقوبة أو خسارة المزايا التي تملكها الأن. إذا لديك اسئلة تتعلق بحقوقك، يمكنك الاتصال ب:

لجنة الأخلاقيات على 01/350000 مقسم 5445

سيتم اعلامك عن أي معلومات جديدة قد تؤثر على صحتك أو رغبتك في البقاء في هذه الدراسة.

الموافقة على المشاركة في البحث

توقيعك أدناه يعني أنك قد قرأت المعلومات الواردة أعلاه حول دراسة "الارتباط بين الجينات و تراجع و صغر حجم الفك السفلي العائلي لدى سكان منطقة شرق البحر المتوسط" وأنه قد تسنت لك فرصة طرح الأسئلة لتساعدك على فهم ما ستقوم به في هذه الدراسة. كما أن توقيعك يعني أنه قيل لك بأنه يمكنك تغير رأيك إذا شئت. سيتم اعطائك نسخة من نموذج الموافقة هذه. وانك، من خلال توقيع نموذج الموافقة هذا، لا تتخلى عن أي من حقوقك القانونية

اسم المشترك		السن
توقيع المشترك (17- 13 سنة)		التاريخ والساعة
اسم الشخص الحاصل على الموافقة	التوقيع	التاريخ والساعة

Appendix IV

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

ADULT PARTICIPANT CONSENT FORM

Project Title: ***ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR MICROGNATHISM IN AN EASTERN MEDITERRANEAN POPULATION***

Protocol Number	
Principal Investigator(s)	Dr Anthony MACARI
Address	American University of Beirut Medical Center
Phone	(01) 350 000 ext: 5702
Site where the study will be conducted	Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

You are being asked to join the study:

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

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.

1. AIM OF THE STUDY

The aims of the study are to explore whether the condition of mandibular micrognathism (reduced size of lower jaw and retruded position) is inherited and help identify the genes involved in its development and familial transmission. Because many participants are expected to be in Lebanon and the region, we have labeled these participants as representing a Mediterranean population.
The study will include families known to have subjects affected by mandibular micrognathism. 30 subjects will be recruited. **Your enrolment is not obligatory**. If you participate, you will commit to go through the following steps:

Step	Procedure
1	Medical history will be filled out: demographics (gender, date of birth, age, family origin), health status, genetics (consanguinity, affection status of other individuals in the family)
2	Have a clinical examination to determine whether your bite corresponds to the condition.

3	Have an X-ray of the face (lateral cephalometric X-ray) taken in our clinics to help us confirm that you are affected by mandibular micrognathism. If you are a patient in our division, we already have the above records and will not repeat them unless a long time has elapsed that would require updating them. 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. ***"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"***
4	A pedigree (a form of family tree) will be drawn to find out who are the subjects in the family affected by mandibular micrognathism
5	5cc of blood will be collected to isolate DNA from whole blood cells. Your DNA will be stored in coded tubes at -80 degrees at the Core Facilities at the American Univeristy of Beirut. Part of your coded DNA will be then shipped to Macrogen-Korea, where Next Generation Sequencing will be performed. The data will be collected at AUB and further analyzed to detect any variations that could cause your disease

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 lateral cephalometric x-ray, and the second visit to have 5cc of your blood collected.

2. RISKS ASSOCIATED WITH PARTICIPATING IN THE STUDY

Risk	Procedure
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 cephalogram is only 1.7mrem, 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.

3. BENEFITS OF 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 mandibular micrognathism in our population. 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 response of the lower jaw to orthodontic and orthopedic treatment. Early prediction of the condition would lead to:

1. Earlier treatment that might reduce the severity of the condition and possibly avoidance of surgery OR
2. More importantly, forego earlier interventions in favor of a later orthognathic surgery when mandibular micrognathism is genetically determined as a "certainty"

We believe this study would be a major advance in the clinical and research fields of orthodontics and dentofacial orthopedics and in oral and maxillofacial surgery. Please note that refusal of participation will not lead to a loss of benefits.

4. WHAT OTHER OPTIONS ARE THERE?

This is not a treatment study so the only alternative is not to participate in the study. However, the investigator may end your participation at any time.

5. CONFIDENTIALITY

Every reasonable effort will be made to keep your records confidential. 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 (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.
However, while you are in this study we do have to let some people look at your records. These people will be the study doctor and designee, the ethics committee and inspectors from governmental agencies. **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: September 2019).

- ☐ I want to be informed of the results of the study
- ☐ I don't want to be informed of the results of the study

The study doctor can use the study results as long as you cannot be identified.

6. 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.

7. WHAT ARE THE COSTS?

There are no costs associated with participation. The study will cover the costs of the procedures done for its purpose: the blood withdrawal, genetic analysis and the X-ray that is taken on relatives where features of the condition are noted clinically. All other costs will not be covered.

8. WILL YOU GET PAID TO BE IN THIS STUDY?

You will not be paid to participate in this study.

9. AGREEMENT FO THE USE OF SAMPLES FOR GENETIC TESTING

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

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

SIGNATURE SECTION

Investigator's Statement

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

Patient's Participation

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. Anthony MACARI at (01) 350 000 ext. 5702 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 patient **Signature**

_____

Date & Time

_____ _____

Witness's Name **Signature**

لجنة الأخلاقيات
الجامعة الأميريكية في بيروت
كلية الطب
شارع بلس بيروت، لبنان
هاتف: 350000 -(01)مقسم: 5445

موافقة الراشدين على المشاركة في دراسة بحثية وراثية	إسم المشترك:
الإرتباط بين الجينات و تراجع وصغر حجم الفك السفلي العائلي لدى سكان منطقة شرق البحر المتوسط	الباحث الرئيسي: الدكتور أنطوني مكاري المركز الطبي في الجامعة الأميركية شارع الحمرا – بيروت – لبنان الهاتف: 01350000 مقسم: 5702

أنت مدعو للمشاركة في دراسة بحثية سريرية ستجرى في الجامعة الأميركية في بيروت (قسم تقويم الأسنان وتأهيل الفكّين):

- إذا كنت مريضاً تتلقى العلاج في قسمنا: خلال موعدك الطبي، سيشرح لك طبيبك المعالج أهداف الدراسة، إجراءاتها، وفوائدها / مخاطرها، وستسألك إذا كنت ترغب في المشاركة فيها. اذا وافقت، سيعرفك طبيبك على منسقة الدراسة (د. ميشيل شقية) و سيُطلب منك الامضاء على استمارة الموافقة هذه. بعد امضائها، ستسأل عن تاريخ عائلتك، بما في ذلك حالة صغر حجم و تراجع الفكّ السفليّ لأفراد آخرين من عائلتك. كما وسيتم اعطاؤك نشرات لتوزّعها على أقربائك المقربين الذين قد يستفيدون، بحسب اعتقادك، من هذه الدراسة.
- أو، إذا كنت من أقرباء مريض يتلقى العلاج في قسمنا، وكنت قد أخذت النشرة منه وقرأتها.

يرجى: 1) أخذ الوقت لقراءة المعلومات التالية بعناية **قبل أن تقرر ما إذا كنت تريد المشاركة في هذه الدراسة أم لا.**

2) عدم التردد في سؤال **الطبيب اذا كنت بحاجة الى مزيد من المعلومات أو توضيح** حول ما ورد في هذا النموذج وحول الدراسة ككل.

1. أهداف الدراسة

أهداف الدراسة هي استكشاف ما إذا كان يتم توريث حالة صغر حجم و تراجع الفك السفلي والمساعدة في تحديد الجينات المسؤولة عن تطوير وانتقال هذه الحالة وراثياً. بما أنه من المتوقع أن يكون في لبنان والمنطقة العديد من المشاركين، لقد صنفّنا هؤلاء المشاركين كممثّلي سكان منطقة البحر المتوسط.

ستشمل الدراسة الأسر المعروف أن لديها أفراد مصابين بصغر حجم و تراجع الفك السفلي. سيتم تعيين 30 شخصاً. **التحاقك ليس إلزامياً**. إذا قمت بالمشاركة، ستلتزم بالقيام بالخطوات التالية:

الخطوة	الإجراءات
1	تعبئة تاريخ طبي حول الأصل العائلي و تاريخ عائلتك ومدى تأثّر أفراد آخرين في الأسرة.
2	تخضع للفحص السريري لتحديد ما إذا كانت العضة الخاصة بك تتطابق مع الحالة.
3	القيام بصورة بالأشعة السينية للوجه (صورة جانبية بالأشعة السينية لقياسات الرأس) في عيادتنا لمساعدتنا على التأكد من أنك مصاب(ة) بصغر حجم و تراجع الفك السفل. في حال وجود إكتشاف غير طبيعي على الأشعة السينية، سيتم إجراء المزيد من التحقيقات وفقاً لذلك. ستستفيد من تشخيص مجاني وسيتم إقتراح العلاج المناسب. **إذا كان(ت) مريضاً/(ة) في قسمنا، فلدينا السجلات المذكورة أعلاه، ولن نقوم بتكرارها إلا إذا قد مضى وقت طويل مما يتطلب تحديثها.** **" لا تستطيع ابنتكم المشاركة في هذه الدراسة إذا كانت حاملاً. إذا كانت قادرة على الحمل، سيتم إجراء اختبار الحمل قبل أن تتعرض لأي إشعاع. ينبغي أن تخبرنا إذا ثمة احتمال أن تكون قد حملت في غضون الأيام الأربعة عشرة الماضية، وذلك لأنه لا يمكن الاعتماد على اختبار الحمل خلال تلك الفترة".**
4	عقب تحليل الأشعة السينية، سيتم رسم شجرة النسب (شكل من أشكال شجرة العائلة) لمعرفة من هم الأفراد في الأسرة المصابين بصغر حجم و تراجع الفك السفلي.

5	سيتم أخذ 5 سنتيمتر مكّعّب من دمك، ما سيمسح لنا بعزل الحمض النووي من خلايا الدم بأكملها. سيتم تخزين الحمض النووي الخاص بك في أنابيب مشفرة بدرجة حرارة مئوية 80 تحت الصفر في المنشآت الأساسية في الجامعة الأميركية في بيروت. ثم سيشحن جزء من الحمض النووي المشفر الخاص بك إلى ماكروغن كوريا، حيث سيتم تنفيذ الجيل القادم من تكنولوجيات ترتيب الحمض النووي. سيتم جمع البيانات في الجامعة الأميركية في بيروت وتحليلها بعد ذلك بغية اكتشاف أي تغيّرات التي يمكن أن تسبب حالتك.

ستتم جميع الإجراءات المذكورة أعلاه، بما في ذلك الدراسة الوراثية، في عيادتنا والمنشآت ضمن المركز الطبي في الجامعة الأميركية في بيروت. 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 جانبية لقياسات الرأس. الزيارة الثانية لنأخذ 5 سنتيمتر مكّعّب من دمك.

2. المخاطر الناتجة عن المشاركة في الدراسة

المخاطر	الإجراءات
خطر الإشعاع	على الرغم من أنه ليس هناك أي آثار ضارة مُثبتة تنتج عن مستويات الإشعاع التي ستتعرض لها أثناء هذه الدراسة، إلا أنه لا يمكن استبعاد آثار طويلة الأمد على صحتك. الجرعة الفعالة لصورة واحدة لقياسات الرأس هي 7.1 ميلي ريم فقط وهي نسبة ضئيلة مقارنةً بمعدّل الجرعة السنوية التي يتلقاها الشخص من الإشعاع البيني (ما يقارب 300 ميلي ريم).
المرتبطة بسحب الدم	قد تشمل هذه المخاطر الكدمات، ألم موضعي، ورم دموي، واحتمال ضئيل للإلتهاب أو الإغماء. سيتم سحب الدم من قبل ممرضة متخصصة أو طبيب في المستشفى (AUBMC)، وذلك باستخدام إبرة نظيفة. وبالتالي، هذا الخطر ضئيل.

يرجى ملاحظة أنه قد تكون هناك مخاطر غير متوقعة.

3. الفوائد الناتجة عن المشاركة في الدراسة

ليس ثمة أي فوائد مباشرة لك من هذه الدراسة. إلا أنها تقدم بعض الفوائد للفئة التي تنتمي اليها وكذلك للمجتمع والطب/العلم ككل لأن هذه الدراسة ستسمح لنا بأن نفهم أكثر الجينات المعنية التي تساهم في تفاوت خطر صغر حجم و تراجع الفك السفلي لدى شعبنا. ويمكن أن تؤدي هذه المعرفة إلى تقدير أفضل لقابلية حصول هذه الحالة وكذلك إلى فهم أفضل للآليات الوراثية التي تؤثر على تفاعل الفكّ السفليّ مع المعالجة التقويمية و العظمية. وقد يؤدي التنبؤ المبكر للحالة إلى التالي:

1. العلاج المبكر الذي يمكن أن يقلل من وخامة الحالة، وربما تجنب عملية جراحية في وقت لاحق أو
2. الأهم، الامتناع عن القيام بأي تدخلات مبكرة من أجل القيام بجراحة فكّ في وقت لاحق حينما يكون صغر حجم و تراجع الفك السفلي محدد وراثياً على أنه حتميّ.

إننا نؤمن بأن هذه الدراسة ستحرز تقدم أ كبيراً في المجالات السريرية والبحثية في تقويم الأسنان وتأهيل الفكّين.

4. ما هي الخيارات الأخرى؟

هذه ليست دراسة مخصصة للعلاج، فالوسيلة الوحيدة البديلة هي عدم مشاركتك في هذه الدراسة.

5. السرية

إذا وافقت على المشاركة في الدراسة، ستحفظ المعلومات **بسريةً**. لن يكون لأي شخص، ما لم ينص القانون على ذلك، حق الاطلاع مباشرةً على سجلاتك الطبية باستثناء الطبيب المسؤول عن البحث ومن ينوب عنه، لجنة الأخلاقيات ومفتشين من الادارات الحكومية.

ستؤخذ الصورة بالأشعة في قسم تقويم الأسنان وتأهيل الفكّين في المركز الطبي في الجامعة الأميركية في بيروت وتحُفظ في بنك الصور الشعاعية الذي تم احداثها وضمها في البرنامج الشعاعي المطابق (CLINIVIEW). سيتم وضعها في ملف رقميّ منفصل على كومبيوتر في قسمنا، وسيتم تطبيق رموز بحيث لا يمكن لأحد الولوج إلى الملف إلا أعضاء فريق البحث **وحسب**.	سيتم ترقيم عينات الدم وترميزها وتخزينها في مكان آمن حتى نهاية الدراسة. سيتم الاحتفاظ بجميع عينات الدم ومشتقاتها إذا أردت المشاركة في دراسات جينية أخرى. وإلا، سيتم التخلصّ منها في نهاية الدراسة.

بناءً على طلبك، سيتم الكشف عن النتائج الشخصية لك ولأفراد عائلتك بمجرد الانتهاء من الدراسة (التاريخ المتوقع: أيلول 2019).

☐ **أريد أن أبلّغ بنتائج الدراسة.**

☐ **لا أريد أن أبلّغ بنتائج الدراسة**

6. ماذا يحصل لو أصابك أذى خلال الدراسة؟

إن المركز الطبي في الجامعة الأمريكية في بيروت سوف يغطي تكاليف العلاج في المركز للعوارض الطبية السلبية الناجمة مباشرة عن الأدوية و/أو الإجراءات الطبية الخاصة بهذه الدراسة البحثية. فيما عدا ذلك، لن يقوم المركز الطبي بتغطية تكاليف العناية الطبية لأية حالة أو مشكلة مرضية. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جداً.

7. ما هي التكاليف؟

ليس ثمة أي تكاليف مرتبطة بالمشاركة. ستغطي الدراسة تكاليف الاجراءات المطلوبة لتحقيق اهدافها: سحب الدم، التحليل الجيني والأشعة السينية التي ستؤخذ للأقارب الذين لوحظت عندهم ملامح الحالة سريرياً. لن تتم تغطية أي تكاليف أخرى.

8. هل ستتقاضى أي مردود مالي مقابل المشاركة في الدراسة؟

لن تحصل على أي مردود مالي مقابل مشاركتك في الدراسة.

9. موافقة على استخدام العينات للفحص الجيني

مشاركتك اختيارية ويمكنك الانسحاب في أي وقت تشاء. ولن تؤثر عليك أي **عواقب سلبية** على الإطلاق إذا قررت عدم المشاركة. غير أنه يحق للباحث الرئيسي إنهاء مشاركتكم فالدراسة في أي وقت. إذا وافقت على المشاركة، يرجى ملء في ما يلي:

أسمح بالاستخدام المشفّر للمواد البيولوجية الخاصة بي (الدم) للدراسة المقترحة.
إني أحدد استخدام العينات بالطريقة التالية (ضع إشارة على احدى الخانات التالية وحسب):

☐ أسمح للفريق بالتواصل معي لاحق اً للحصول على إذن للقيام بالمزيد من الدراسات على العينات. أو
☐ لا أسمح باستخدام العينات البيولوجية الخاصة بي في دراسات لاحقة. أو
☐ أسمح بالاستخدام المُغفل للعينات البيولوجية الخاصة بي (عينات لا يمكن ربطها بالمشترك) في دراسات أخرى بدون التواصل معي.

قسم التوقيع

بيان المحقق

لقد استعرضت، بالتفصيل، وثيقة الموافقة المسبقة لهذه الدراسة البحثية مع

_____

اسم المريض

الغرض من الدراسة، مخاطرها وفوائدها.
لقد أجبت على كل أسئلة المريض بوضوح. سأبلغ المشترك في حال طرأت أي تغييرات على البحث.

_____ _____

اسم المحقق أو من ينوب عنه **التوقيع**

_____

التاريخ والساعة

مشاركة المريض

لقد قرأت وفهمت كل جوانب الدراسة البحثية وقد تمت الإجابة على جميع أسئلتي. وأنا أوافق طوعاً على المشاركة في هذه الدراسة البحثية وأعلم أنه يمكنني الاتصال بالدكتور أنطوني مكاري على الرقم 01/350000 مقسم 5702 أو بمن ينوب عنه في الدراسة في حال لدَيّ أي سؤال.

إذا شعرت أنه لم يتم الاجابة على أسئلتي، يمكنني الاتصال بلجنة الأخلاقيات لحقوق الانسان على الرقم 01/350000 مقسم 5445.

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 يؤثر على رعايتي.

أعلم أنني سأحصل على نسخة من هذه الموافقة الموقعة.

_____ _____

اسم المريض **التوقيع**

_____

التاريخ والساعة

_____ _____

اسم الشاهد **التوقيع**

_____

التاريخ والساعة

Appendix V

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

Project Title: ***ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR MICROGNATHISM IN AN EASTERN MEDITERRANEAN POPULATION***

Parental consent to participate in a genetic research study	**Participant name:**
Principal Investigator(s)	Dr Anthony MACARI
Address	American University of Beirut Medical Center
Phone	(01) 350 000 ext: 5702
Site where the study will be conducted	Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC

Your child is being asked to join the study:

- If your child is a patient treated in our division: during your orthodontic appointment, you will be approached first by their primary physician. He/she will explain in detail and ask you if you are willing to allow your child to participate in the research project. If you agree, your child and yourself will be introduced to the research coordinator (Dr. Michelle El-Chekie) to sign the consent/ assent forms. 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.
- Or if you are a relative of a patient treated in our division who read flyer handed to you by him/her.

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.

1. AIM OF THE STUDY

The aims of the study are to explore whether the condition of mandibular micrognathism (reduced size of lower jaw and retruded position) is inherited and help identify the genes involved in its development and familial transmission. Because many participants are expected to be in Lebanon and the region, we have labeled these participants as representing a Mediterranean population.

The study will include families known to have subjects affected by mandibular micrognathism. 30 subjects will be recruited. **His/her enrolment is not obligatory**. If he/she participates, he/she will commit to go through the following steps:

Step	Procedure
1	Medical history will be filled out: demographics (gender, date of birth, age, family origin), health status, genetics (consanguinity, affection status of other individuals in the family)
2	Have a clinical examination to determine whether your bite corresponds to the condition.

3	Have an X-ray of the face (lateral cephalometric X-ray) taken in our clinics to help us confirm that you are affected by mandibular Micrognathism. If he/she is a patient in our division, we already have the above records and will not repeat them unless a long time has elapsed that would require updating them. In case an abnormal finding was present on the X-ray, further investigation will be performed accordingly. He/she will benefit from free diagnosis and treatment will be suggested. ***"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"***
4	A pedigree (a form of family tree) will be drawn to find out who are the subjects in the family affected by mandibular micrognathism
5	5cc of blood will be collected to isolate DNA from whole blood cells. His/ her DNA will be stored in coded tubes at -80 degrees at the Core Facilities at the American Univeristy of Beirut. Part of the coded DNA will be then shipped to Macrogen-Korea, where Next Generation Sequencing will be performed. The data will be collected at AUB and further analyzed to detect any variations that could cause your disease

This will require 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 lateral cephalometric x-ray, and the second visit to have 5cc of your blood collected.

2. RISKS ASSOCIATED WITH PARTICIPATING IN THE STUDY

Risk	Procedure
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 cephalogram is only 1.7mrem, 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.

3. BENEFITS OF TAKING PART IN THE STUDY

There is no direct benefit to them from this study. However, it has some benefits to the class to which he/ she 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 mandibular micrognathism in our population. 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 response of the lower jaw to orthodontic and orthopedic treatment. Early prediction of the condition would lead to:

1. Earlier treatment that might reduce the severity of the condition and possibly avoidance of surgery OR
2. More importantly, forego earlier interventions in favor of a later orthognathic surgery when mandibular micrognathism is genetically determined as a "certainty"

We believe this study would be a major advance in the clinical and research fields of orthodontics and dentofacial orthopedics and in oral and maxillofacial surgery. Please note that refusal of participation will not lead to a loss of benefits.

4. WHAT OTHER OPTIONS ARE THERE?

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

5. CONFIDENTIALITY

Every reasonable effort will be made to keep your records **confidentia**l. 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 (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.
However, while he/ she is in this study we do have to let some people look at their records. These people will be the study doctor and designee, the ethics committee and inspectors from governmental agencies. **We will keep the 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: September 2019).

- ☐ I want to be informed of the results of the study
- ☐ I don't want to be informed of the results of the study

The study doctor can use the study results as long as you cannot be identified.

6. WHAT IF HE/ SHE 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 that this risk is minimal.

7. WHAT ARE THE COSTS?

There are no costs associated with participation. The study will cover the costs of the procedures done for its purpose: the blood withdrawal, genetic analysis and the X-ray that is taken on relatives where features of the condition are noted clinically. All other costs will not be covered.

8. WILL HE/ SHE GET PAID TO BE IN THIS STUDY?

He/ she will not be paid to participate in this study.

9. AGREEMENT FO THE USE OF SAMPLES FOR GENETIC TESTING

I permit coded use of his/ her biological materials (blood) for the proposed study.
I specify the use of the samples in the following manner (please check only one of the following):

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

SIGNATURE SECTION

Investigator's Statement

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

Patient's Participation

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree for my child to be a part of this research study and I know that I can contact Dr. Anthony MACARI at (01) 350 000 ext. 5702 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. Also, the investigator may end their participation at any time.

_____ _____

Name of **Signature**

☐ Patient, ☐ Legal Representative or ☐ Parent/Guardian

_____

Date & Time

_____ _____

Witness's Name **Signature**

_____

Date & Time

لجنة الأخلاقيات
الجامعة الأميركية في بيروت
كلية الطب
شارع بلس بيروت، لبنان
هاتف: 350000 -(01)مقسم: 5445

موافقة الراشدين على المشاركة في دراسة بحثية وراثية	إسم المشترك:
الإرتباط بين الجينات و تراجع وصغر حجم الفك السفلي العائلي لدى سكان منطقة شرق البحر المتوسط	الباحث الرئيسي: الدكتور أنطوني مكاري المركز الطبي في الجامعة الأميركية شارع الحمرا – بيروت – لبنان الهاتف: 01350000 مقسم: 5702

ابنك/ابنتك مدعو(ة) للمشاركة في دراسة بحثية سريرية ستجرى في الجامعة الأميركية في بيروت (قسم تقويم الأسنان وتأهيل الفكين):

- اذا هو/هي يتلقون العلاج في قسمنا: خلال الموعد الطبّيّ ، سيشرح لك طبيبك المعالج أهداف الدراسة ، إجراءاتها، فوائدها ومخاطرها، وستسألكم اذا كنتم ترغبون مشاركة ولدكم فيها. إذا وافقتم، سيعرفك طبيبك على منسقة الدراسة (د. ميشيل شقية) وسيُطلب منكم الامضاء على استمارة الموافقة هذه. بعد امضائها، ستسُألون عن تاريخ عائلتكم، بما في ذلك حالة صغر حجم و تراجع الفك السفليّ لأفراد آخرين من العائلة. كما سيتم اعطاؤكم نشرات لتوزيعها على أقربائكم الذين قد يستفيدون، بحسب اعتقادكم، من هذه الدراسة.

- أو، اذا أنتم من أقرباء مريض يتلقى العلاج في قسمنا، وكنتم قد أخذتم النشرة منه وقرأتموها.

يرجى: 1) أخذ الوقت لقراءة المعلومات التالية بعناية **قبل أن تقرروا الموافقة على مشاركة ابنكم/ابنتكم في هذه الدراسة أم لا.**
2) عدم التردد في سؤال **الطبيب اذا كنتم بحاجة الى مزيد من المعلومات أو توضيح** حول ما ورد في هذا النموذج وحول الدراسة ككل.

1. أهداف الدراسة

أهداف الدراسة هي استكشاف ما إذا كان يتم توريث حالة صغر حجم و تراجع الفك السفلي والمساعدة في تحديد الجينات المسؤولة عن تطوير وانتقال هذه الحالة وراثياً. بما أنه من المتوقع أن يكون في لبنان والمنطقة العديد من المشاركين، لقد صنفّنا هؤلاء المشاركين كممثّلي سكان منطقة البحر المتوسط.

ستشمل الدراسة الأسر المعروف أن لديها أفراد مصابين بصغر حجم و تراجع الفك السفلي. سيتم تعيين 30 شخصاً. **التحاق ابنكم/ابنتكم ليس إلزامياً.** في حال موافقتكم، سيلتزم ابنكم/ابنتكم بالقيام بالخطوات التالية:

الخطوة	الإجراءات
1	تعبئة تاريخ طبي حول الأصل العائلي و تاريخ عائلتك ومدى تأ ثرّ أفراد آخرين في الأسرة.
2	الخضوع للفحص السريري لتحديد ما إذا كانت العضة الخاصة بها/بها تتطابق مع الحالة.
3	القيام بصورة بالأشعة السينية للوجه (صورة جانبية بالأشعة السينية لقياسات الرأس) في عيادتنا لمساعدتنا على التأكد من أنها/أنها مصاب(ة) بصغر حجم و تراجع الفك السفل. في حال وجود إكتشاف غير طبيعي على الأشعة السينية، سيتم إجراء المزيد من التحقيقات وفقاً لذلك. ستستفيد من تشخيص مجاني وسيتم إقتراح العلاج المناسب. **إذا كان(ت) مريضاً/(ة) في قسمنا، فلدينا السجلات المذكورة أعلاه، ولن نقوم بتكرارها إلا إذا قد مضى وقت طويل مما يتطلب تحديثها.** **"لا تستطيع ابنتكم المشاركة في هذه الدراسة إذا كانت حاملاً. إذا كانت قادرة على الحمل، سيتم إجراء اختبار الحمل قبل أن تتعرض لأي إشعاع. ينبغي أن تخبرنا إذا ثمة احتمال أن تكون قد حملت في غضون الأيام الأربعة عشرة الماضية، وذلك لأنه لا يمكن الاعتماد على اختبار الحمل خلال تلك الفترة".**
4	عقب تحليل الأشعة السينية، سيتم رسم شجرة النسب (شكل من أشكال شجرة العائلة) لمعرفة من هم الأفراد في الأسرة المصابين بصغر حجم و تراجع الفك السفلي.

5	سيتم أخذ 5 سنتيمتر مكّعّب من دمه(ها)، ما سيسمح لنا بعزل الحمض النووي من خلايا الدم بأكملها. سيتم تخزين الحمض النووي الخاص بابنكم/ابنتكم في أنابيب مشفرة بدرجة حرارة مئوية 80 تحت الصفر في المنشآت الأساسية في الجامعة الأميركية في بيروت. ثم سيشحن جزء من الحمض النووي المشفر الخاص بابنكم/ابنتكم إلى ماكروغن كوريا، حيث سيتم تنفيذ الجيل القادم من تكنولوجيات ترتيب الحمض النووي. سيتم جمع البيانات في الجامعة الأميركية في بيروت وتحليلها بعد ذلك بغية اكتشاف أي تغيّرات التي يمكن أن تسبب حالته(ها).

ستتم جميع الإجراءات المذكورة أعلاه، بما في ذلك الدراسة الوراثية، في عيادتنا والمنشآت ضمن المركز الطبي في الجامعة الأميركية في بيروت. سيتطلب هذا الأمر من ابنكم/ابنتكم القيام بزيارة AUBMC مَرّة(45 دقيقة) أو مَرّتين (30 دقيقة +15 دقيقة): الزيارة الأول لنشرح لكم المشروع بالتفصيل، لإمضاء استمارة الموافقة هذه، لخضوعه(ها) لفحص سريري ولصورة أشعة سينية جانبية لقياسات الرأس .الزيارة الثانية لنأخذ 5 سنتيمتر مكّعّب من دمه (ها).

2. المخاطر الناتجة عن المشاركة في الدراسة

المخاطر	الإجراءات
خطر الإشعاع	على الرغم من أنه ليس هناك أي آثار ضارة مُثبتة تنتج عن مستويات الإشعاع التي سيتعرض لها ابنكم/ابنتكم أثناء هذه الدراسة، إلا أنه لا يمكن استبعاد آثار طويلة الأمد على صحته(ها). الجرعة الفعالة لصورة واحدة لقياسات الرأس هي 7.1 ميلي ريم فقط، وهي نسبة ضئيلة مقارنةً بمعدّل الجرعة السنوية التي يتلقاها الشخص من الإشعاع البيئي (ما يقارب 300 ميلي ريم).
المرتبطة بسحب الدم	قد تشمل هذه المخاطر الكدمات، ألم موضعي، ورم دموي، واحتمال ضئيل للإلتهاب أو الإغماء. سيتم سحب الدم من قبل ممرضة متخصصة أو طبيب في المستشفى (AUBMC)، وذلك باستخدام إبرة نظيفة. وبالتالي، هذا الخطر ضئيل.

يرجى ملاحظة أنه قد تكون هناك مخاطر غير متوقعة.

3. الفوائد الناتجة عن المشاركة في الدراسة

ليس ثمة أي فوائد مباشرة له/لها من هذه الدراسة. إلا أنها تقدم بعض الفوائد للفئة التي تنتمون اليها وكذلك للمجتمع والطب والعلم ككل لأن هذه الدراسة ستسمح لنا بأن نفهم أكثر الجينات المعنية التي تساهم في تفاوت خطر صغر حجم و تراجع الفك السفلي لدى شعبنا. ويمكن أن تؤدي هذه المعرفة إلى تقدير أفضل لقابلية حصول هذه الحالة وكذلك إلى فهم أفضل للآليات الوراثية التي تؤثر على تفاعل الفكّ السفليّ مع المعالجة التقويمية و العظمية. وقد يؤدي التنبؤ المبكر للحالة إلى التالي:

1. العلاج المبكر الذي يمكن أن يقلل من وخامة الحالة، وربما تجنب عملية جراحية في وقت لاحق أو
2. الأهم، الامتناع عن القيام بأي تدخلات مبكرة من أجل القيام بجراحة فكّ في وقت لاحق حينما يكون صغر حجم و تراجع الفك السفلي محدد وراثياً على أنه حتميّ.

إننا نؤمن بأن هذه الدراسة ستحرز تقدماً كبيراً في المجالات السريرية والبحثية في تقويم الأسنان وتأهيل الفكّين.

4. ما هي الخيارات الأخرى؟

هذه ليست دراسة مخصصة للعلاج، فالوسيلة الوحيدة البديلة هي عدم مشاركة ابنكم/ابنتكم في هذه الدراسة.

5. السرية

إذا وافقتم على مشاركة ابنكم/ابنتكم في الدراسة، ستحفظ المعلومات **بسريةٍ**. لن يكون لأي شخص، ما لم ينص القانون على ذلك، حق الاطلاع مباشرةً على سجلاته(ها) الطبية باستثناء الطبيب المسؤول عن البحث ومن ينوب عنه، لجنة الأخلاقيات ومفتشين من الادارات الحكومية.

ستؤخذ الصورة بالأشعة في قسم تقويم الأسنان وتأهيل الفكّين في المركز الطبي في الجامعة الأميركية في بيروت وتحفظ في بنك الصور الشعاعية الذي تم احداثها وضمها في البرنامج الشعاعي المطابق (CLINIVIEW). سيتم وضعها في ملفّ رقميّ منفصل على كومبيوتر في قسمنا، وسيتم تطبيق رموز بحيث لا يمكن لأحد الولوج إلى الملف إلا أعضاء فريق البحث **وحسب**.	سيتم ترقيم عينات الدم وترميزها وتخزينها في مكان آمن حتى نهاية الدراسة. سيتم الاحتفاظ بجميع عينات الدم ومشتقاتها في حال تختارون المشاركة في دراسات جينية أخرى. وإلا، سيتم التخلصّ منها في نهاية الدراسة.

بناءً على طلبكم، سيتم الكشف عن النتائج الشخصية لكم ولأفراد عائلتكم بمجرد الانتهاء من الدراسة (التاريخ المتوقع: أيلول 2019)

☐ **أريد أن أبلّغ بنتائج الدراسة.** ☐ **لا أريد أن أبلّغ بنتائج الدراسة.**

6. ماذا يحصل لو أصابني أذى؟

إن المركز الطبي في الجامعة الأمريكية في بيروت سوف يغطي تكاليف العلاج في المركز للعوارض الطبية السلبية الناجمة مباشرة عن الأدوية و/أو الإجراءات الطبية الخاصة بهذه الدراسة البحثية. فيما عدا ذلك، لن يقوم المركز الطبي بتغطية تكاليف العناية الطبية لأية حالة أو مشكلة مرضية. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جداً.

7. ما هي التكاليف؟

ليس ثمة أي تكاليف مرتبطة بالمشاركة. ستغطي الدراسة تكاليف الاجراءات المطلوبة لتحقيق اهدافها: سحب الدم، التحليل الجيني والأشعة السينية التي ستؤخذ للأقارب الذين لوحظت عندهم ملامح الحالة سريرياً. لن تتم تغطية أي تكاليف أخرى.

8. هل ستتقاضون أي مردود مالي مقابل مشاركة ابنكم/ابنتكم في الدراسة؟

لن تحصلون على أي مردود مالي مقابل مشاركة ابنكم/ابنتكم في الدراسة.

9. موافقة على استخدام العينات للفحص الجيني

مشاركة ابنكم/ابنتكم اختيارية ويمكنه(ها) الانسحاب في أي وقت. ولن تؤثر عليه(ها) أي **عواقب سلبية** على الإطلاق إذا قررتم عدم السماح له(ها) بالمشاركة. إذا وافقتم على المشاركة، يرجى ملء في ما يلي:

نسمح بالاستخدام المشفّر للمواد البيولوجية الخاصة بابننا/ابنتنا (الدم) للدراسة المقترحة.

إننا نحدد استخدام العينات بالطريقة التالية (ضع إشارة على احدى الخانات التالية وحسب):

☐ نسمح للفريق بالتواصل معنا لاحقاً للحصول على إذن للقيام بالمزيد من الدراسات على العينات.

أو

☐ لا نسمح باستخدام العينات البيولوجية الخاصة بابننا/ابنتنا في دراسات لاحقة.

أو

☐ نسمح بالاستخدام المُغفل للعينات البيولوجية الخاصة بابننا/ابنتنا (عينات لا يمكن ربطها بالمشترك) في دراسات أخرى بدون التواصل معنا.

قسم التوقيع

بيان المحقق

لقد استعرضت، بالتفصيل، وثيقة الموافقة المسبقة لهذه الدراسة البحثية مع

_____

(اسم ☐ المريض، ☐ الممثل القانوني، أو ☐ والد، والدة / وصي)، الغرض من الدراسة، مخاطرها وفوائدها.

لقد أجبت على كل أسئلة المريض بوضوح. سأبلغ المشارك في حال طرأت أي تغييرات على البحث.

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اسم المحقق أو من ينوب عنه **التوقيع**

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التاريخ والساعة

مشاركة المريض

لقد قرأنا وفهمنا كل جوانب الدراسة البحثية وقد تمّت الإجابة على جميع أسئلتنا. ونحن نوافق طوعاً على السماح لابننا/ابنتنا بالمشاركة في هذه الدراسة البحثية ونعلم أنه يمكننا الاتصال بالدكتور أنطوني مكاري على الرقم 01350000 مقسم 5702 أو بمن ينوب عنه في الدراسة في حال لدينا أي سؤال.

إذا شعرنا أنه لم يتم الاجابة على أسئلتنا، يمكننا الاتصال بلجنة الأخلاقيات لحقوق الانسان على الرقم 01350000 مقسم 5445.

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 يؤثر على رعاية ابننا/ابنتنا .كما وأنه يحق للباحث الرئيسي إنهاء مشاركة ابننا/ابنتنا فالدراسة في أي وقت. نعلم أننا سنحصل على نسخة من هذه الموافقة الموقعة.

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اسم ☐ الممثل القانوني، أو ☐ والد/ وصي **التوقيع**

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التاريخ والساعة

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اسم ☐ الممثل القانوني، أو ☐ والدة / وصي **التوقيع**

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التاريخ والساعة

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اسم الشاهد **التوقيع**

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التاريخ والساعة