AMERICAN UNIVERSITY OF BEIRUT

ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR PROGNATHISM IN THE MEDITERRANEAN POPULATION

by
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Appreciation can make a day – even change a life. Your willingness to put it into words is all that is necessary.
Margaret Cousins

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AN ABSTRACT OF THE THESIS OF

Pamela Georges Genno for Master of Science
Major: Orthodontics

Title: Association between genes and familial mandibular prognathism in the Mediterranean population.

Background:

Mandibular prognathism (MP) is a dentofacial phenotype characterized by overgrowth and/or prognathism of the mandible with or without maxillary retrognathism, leading to esthetic and functional impairments. While MP segregates within families in an autosomal-dominant manner with incomplete penetrance, environmental factors remain involved in its etiology. Only a few genome-wide family-based linkages have been done in different ethnic populations to identify the gene(s) involved in the trait, showing that the genetic determinants of MP remain unclear.

Aims:

1. Explore the inheritance pattern and identify the candidate genes and loci involved in the development and familial transmission of MP in Mediterranean families; 2. Evaluate the skeletal and dento-alveolar cephalometric characteristics of affected individuals.

Methods:

Pedigrees of 51 Mediterranean families known to include individuals affected by MP were drawn. 14 of them, including several affected subjects, underwent a detailed data and biospecimen collection procedure. This consisted of a clinical examination followed by a lateral cephalometric radiograph on subjects where features of a Class III malocclusion were noted clinically and 5cc of blood collection from the affected and some non-affected relatives. Genetic screening was performed on 8 families (7 Lebanese; 1 Lebanese/Syrian), including the largest number of affected individuals over many generations and the most severe conditions, using Next Generation Sequencing (NGS) technology by application of a Whole Exome Sequencing (WES).

Results:

Most of the pedigrees suggest a Mendelian inheritance pattern and segregate in an autosomal-dominant manner. Pedigree analysis showed an average of 3 reported generations per family, equal number of reported affected males and females (n=2) and more families with male predominance. Cephalometric analysis on affected individuals denoted long mandibular length (mandibular macrognathism), normal maxillary length, tendency to a hyperdivergent facial pattern, skeletal Class III malocclusion underlined by an orthognathic maxilla and a prognathic mandible, along with a dento-alveolar

compensation and anterior crossbite. Genetic screening didn't show any aberration in the previously reported genes linked to MP, but did point out to 3 potentially novel genes (*C1orf167, NBPF8* and *NBPF9*) located on chromosome 1, that could be implicated in mandibular development and lead mainly to mandibular macrognathism.

Conclusion:

This is the first genetic study on large families with MP worldwide using NGS to better understand the variations and risks for MP. *C1orf167, NBPF8* and *NBPF9* are novel genes discovered to be associated with familial MP in the Mediterranean population. *NBPF8* and *NBPF9* genes contributed also to the evolution of primates, including man. Chromosome 1, that harbors the 3 novel genes, is potentially highly linked to MP. Such discovery will help better estimate the genetic susceptibility to this condition in families with affected individuals and know better the molecular mechanisms of jaw development and how they influence the response to orthodontic/orthopedic treatment.

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ABBREVIATIONS

MP	Mandibular Prognathism
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
F	Inbreeding coefficient
GC	Guanine-Cytosine
HGP	Human Genome Project
GWAS	Genome Wide Association Study
IHH	Indian Hedgehog Homolog
PTHrP	Parathyroid Hormone-Related Protein
GHR	Growth Hormone Receptor
CBC	Capillary Blood Collection
AW	Wash Buffer
TGF-β	Transforming Growth Factor Beta
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
Hiseq	High troughput sequencer
VCF	Variant Call Format
Bp	Base pairs
Mbp	Mega base pairs
ORF	Open Reading Frame

CHAPTER I

INTRODUCTION

A. Background

Mandibular prognathism (MP) is one of the most severe maxillofacial deformities. It is a dentofacial phenotype/skeletal disproportion characterized by overgrowth and/or prognathism of the mandible, with (50-60%) or without (20%) undergrowth of the maxilla, leading to a more prominent lower jaw/chin, a concave profile and a negative overjet (anterior crossbite), which represent the characteristics of Class III malocclusion, or mesiocclusion (Figure I.1) (Proffit, Phillips, & Dann, 1990; VanVuuren, 1991).

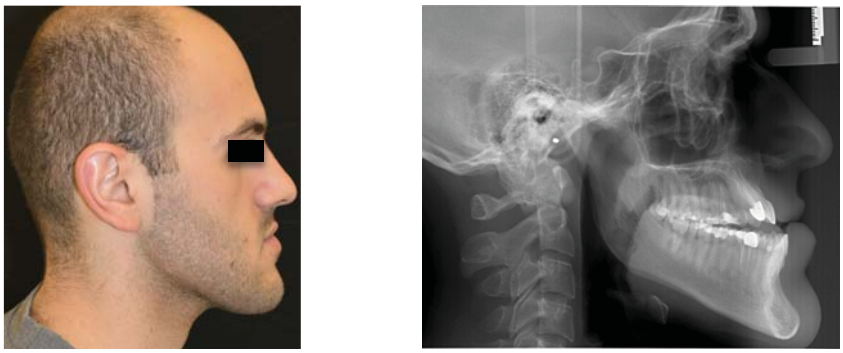


Figure I.1: Mandibular prognathism on a facial profile view (A) and a lateral cephalogram (B).

MP is usually characterized by one or more of the following factors:

1. Enlarged mandible relative to the maxilla.

2. Forward position of the mandible relative to the maxilla.

3. Forward rotation of the mandible, inducing a protrusive position of the chin and a reduced lower anterior facial height (Fischer-Brandies, Fischer-Brandies, & Acevedo, 1985).

The unpleasant facial appearance associated with MP may affect patients' self-confidence and lead to psychological and social problems. The discrepancy between upper and lower jaws can also lead to functional problems including low masticatory efficiency and deficiency in speech production/articulation (English, Buschang, & Throckmorton, 2002). The most common speech sound errors are consonants (zh, ch, sh, z) and the types of errors are distortion and substitution (Hu, Zhou, & Fu, 1997).

In most instances, MP is not evident in early childhood because the condition is developmental and often recognized after the eruption of teeth; hence, there is a late diagnosis of MP in some patients. Accordingly, a higher incidence of this condition is observed in the transitional and adult dentitions than in the primary dentition. In fact, with growth of the general skeleton, MP emerges progressively and accelerates in puberty due to excessive forward growth of the mandible, but does not reach its complete expression until the individual is fully mature (H. P. Chang, Tseng, & Chang, 2006; Shira & Neuner, 1976). This is the reason of delaying orthognathic surgery till the completion of growth in patients having a true mandibular macrognathism.

In growing patients, early facial growth modification (orthopedic treatment) is an effective method to correct or reduce the severity of the condition and possibly avoid later orthognathic surgery. This approach will facilitate a more favorable environment for normal growth and reduce potential psychosocial problems. In adults with mild to moderate affection status, the treatment of MP can consist of a dento-alveolar compensation of the skeletal problem by proclination of maxillary incisors and/or

retroclination of mandibular incisors. The combination of orthodontic treatment and orthognathic surgery involving the mandible with or without the maxilla (one jaw surgery or double jaws surgery) is needed for adult patients with severe MP to correct the skeletal discrepancy in order to provide a better facial appearance and function (H. P. Chang et al., 2006; Sperry, Speidel, Isaacson, & Worms, 1977).

Therefore, MP is possibly the most challenging orthodontic problem to diagnose and treat. One of the likely reasons for the difficulty is that the etiology of a jaw disproportion (thus its interception) for a specific individual is not fully known. Of course in frequent instances, MP has a familial history; the "large lower jaw" phenotype is observed in successive generations of the family with various severities (various expressivity) but unfortunately, relatively few genetic studies have been conducted on families with a high incidence of MP.

When establishing a treatment plan for a patient with MP, the most essential consideration is future growth. Treatment decisions should be based on the direction, amount, duration and pattern of craniofacial growth and specially its completion. Craniofacial growth, particularly mandibular growth, is highly variable and is reported to continue into the late developmental stages (young adulthood: 18 years on average) corresponding with the complete eruption of the 2nd and 3rd molars (Baccetti, Reyes, & McNamara, 2007). Thus, close observation and follow-up of midfacial and mandibular growth during adolescence, particularly during the second or third stage of orthodontic treatment, are essential to ensure stability of the effects of the applied treatment. Furthermore, the efficacy of using dentofacial orthopedics to alter or redirect facial growth in MP growing patients is still controversial and early determination of the borderline between patients who can be treated non-surgically (orthopedically) and

those in whom orthognathic surgery is a necessity is still poorly defined. In fact, patients may suffer from the long duration of treatment including one or more phases using facemask, notwithstanding the possibility of orthognathic surgery in adulthood, along with the associated frustration, loss of cooperation and potential side effects.

B. Significance

Identifying the candidate genes responsible of the development and familial transmission of MP in different ethnic populations is a major advancement in orthodontics and dentofacial orthopedics because it can help comprehend the molecular mechanisms of jaw development and how these mechanisms may influence the response to orthodontic and orthopedic treatment. Also, better understanding the specific genes contributing to variation in the risk for MP in the Mediterranean population can help estimate the genetic susceptibility to this condition in families with affected individuals. By extension, the research may help the identification of environmental factors that may be addressed in early treatment.

In addition, by early forecast of the condition following a blood test to assess the patient's genes, earlier treatment may be instituted (if needed) to try to intercept the development of MP, or at least minimize maxillary retrognathism at a time early enough to forego or even avoid a severe malocclusion. More importantly, present intervention may be foregone in favor of a later orthognathic surgery when true MP is genetically determined as a "certainty", in order to prevent a long treatment. In other words, if MP could be defined genetically in a precise manner in different ethnic populations, rather than phenotypically, we would approach to treatment of the condition in a more rational and effective approach. Such achievement can improve treatment modalities and

outcome and help in the prevention of moderate to severe cases, potentially reducing the frequency of maxillofacial surgery. Molecular genetic information can also be used to accurately predict long-term growth changes and may ultimately lead to the utilization of gene therapy. Recent gene mapping and linkage analysis give hope that the genetic determinants of facial development in general and MP in particular will be better understood in the near future.

An emphasis should be placed on studying the heritable patterns of each skeletal morphologic characteristic that may contribute to MP. However, only a few genome-wide family-based linkages have been performed in different ethnic populations to identify the gene(s) involved in the trait, and no previous study was conducted in the Mediterranean population, underscoring the fact that the genetic determinants of MP remain unclear.

In past attempts at deciphering the genetic components of MP, maxillary retrognathism may have been the dominant trait of the malocclusion. In this study, the genetic determination is based on the assumption that mandibular macrognathism is genetically determined, while maxillary retrognathism is mostly environmentally induced. Accordingly, the inclusion criteria were set in a stringent way that may lead to proper identification of a candidate gene(s).

C. Research objectives

The aims of this study are to:

1- Explore the inheritance pattern of MP in Mediterranean families.

2- Identify candidate loci and gene(s) responsible of the development and familial transmission of MP in the studied Mediterranean population.

3- Evaluate the skeletal and dento-alveolar cephalometric characteristics of individuals affected by MP.

(1) and (2) are the main aims of the study as we principally seek to clearly rule in or out the involvement of the estimated 30000 genes of the human genome in MP.

D. Hypothesis

Our main hypotheses are that:

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

2- Inheritance may be related more to mandibular macrognathism.

CHAPTER II
LITERATURE REVIEW

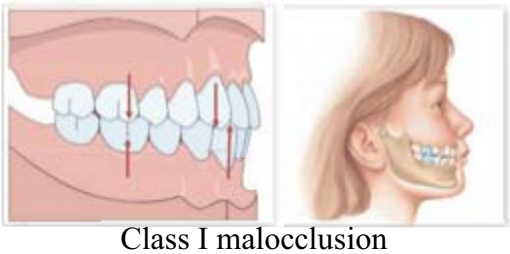
A. Definitions and concepts

1. Malocclusion

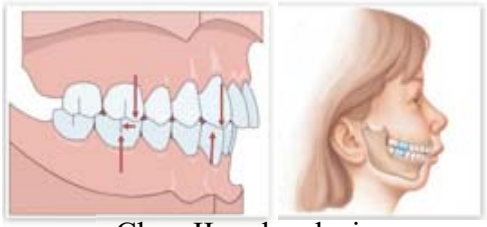
A malocclusion is a misalignment or incorrect relation between the teeth of the two dental arches when they approach each other after jaw closure. The term was invented by the "father of modern orthodontics" Edward H. Angle as a derivative of the term “occlusion”, which refers to the normal manner in which opposing teeth meet. Angle was the first to classify malocclusion in a paper he published in 1899, entitled “Classification of malocclusion”. His system is based on the relative position of the permanent first molars. Angle believed that the anteroposterior dental base relationship could be evaluated reliably from the permanent first molars, as their position does not change following their eruption. If the first molars are missing, the canine relationship is used.

Angle described three basic types of malocclusion: Class I, Class II and Class III. In Class I or molar neutrocclusion, present in approximately 50-55% of the U.S population, the mesio-buccal cup of the maxillary first molar occludes in the central groove of the mandibular first molar. In Class II or molar distocclusion, found in 15% of the U.S. population, the maxillary first molar is anterior to the buccal groove of the mandibular first molar; maxillary incisors may be proclined (increased overjet: division 1) or retroclined (increased overbite: division 2). In class III or molar mesiocclusion, which is the least prevalent (1-5% of the U.S. population), 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 (negative overjet) (Figure II.1) (Angle, 1899, 1907; Proffit, Fields, & Moray, 1998).



Class I malocclusion



Class II malocclusion

Class III malocclusion

Figure II.1: The three types of malocclusion and associated faces
(Adapted from source: https://dentodontics.com/2015/09/09/angles-classification-of-malocclusion. Accessed: March 30, 2017).

2. Genome, genotype and genes

a. Human genome

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

chromosome is made up of the double helical DNA that is wrapped around proteins called histones. Those proteins enable the DNA units to be tightly packed into the nucleus of the cells and also play an important role in regulating when and where the cells will use portions of the genetic information contained in the genome (Golbabapour, Abdulla, & Hajrezaei, 2011).

Each human being inherits a total of 46 chromosomes; 22 homologous pairs of chromosomes called autosomes and one pair of sex chromosomes that are homologous (X,X) in females and only partly homologous (X,Y) in males, which make the individual unique. Each pair is formed by one chromosome that is a copy of the original maternal chromosome and another chromosome that is a copy of the original paternal chromosome.

b. Genes

Each chromosome contains many genes, which represent the smallest physical and functional units of inheritance. Genes are specific sequences of bases that encode instructions for the synthesis of a specific polypeptide via a messenger RNA intermediate (mRNA) or the synthesis of a specific RNA molecule (e.g., transfer RNA, ribosomal RNA, and noncoding regulatory RNA molecules such as microRNA or long noncoding RNA). In fact, most codons (sequence of three nucleotides) in the mRNA lead to the addition of an amino acid to a growing polypeptide chain, which may ultimately become a protein (Figure II.2). Each person normally inherits two copies of every gene within the genome: one gene copy on the autosome or sex chromosome of maternal origin and the other gene copy on the autosome or sex chromosome of paternal origin (Hartsfield, 2011). Our genes only make up 2% of the estimated 3.2 billion base

pairs present in the human genome and the average gene is 3000 nucleotide base pairs in length. The remainder consists of non-coding regions (Human Genome Program, 2008).

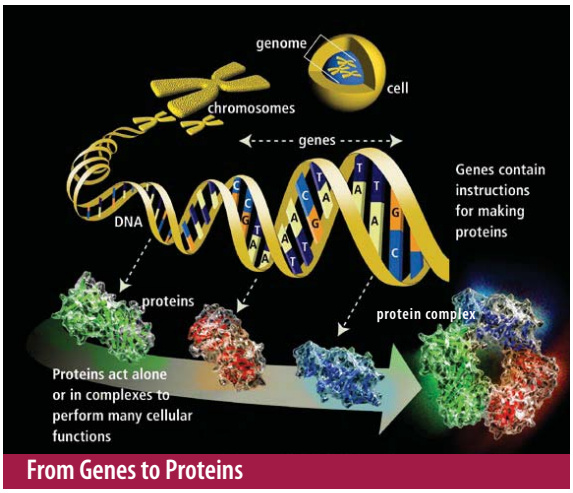


Figure II.2: Structural components of the genome (Human Genome Program, 2008).

Every gene resides in a specific location referred to as a locus. Genes at the same locus on a pair of homologous chromosomes are called alleles. One allele would be a copy of the maternal allele and the other a copy of the paternal allele. When the two alleles are identical, the individual is said to be homozygous for that locus. When the two alleles are different in the DNA sequence, the individual is said to be heterozygous for that locus (Hartsfield, 2011).

c. DNA variation

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

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

An example of a SNP is a frameshift mutation (also called framing error or reading frame shift) caused by INDELS (Insertions or Deletions) of a number of nucleotides in a DNA sequence that is not divisible by three. Due to the triplet nature of gene expression by codons, the insertion or deletion can change the reading frame (grouping of codons), resulting in a completely different translation from the original. A nonsense mutation is also an example of a SNP, defined as a point mutation in a DNA sequence that results in a premature stop codon (or termination codon) within the mRNA that signals a termination of translation into proteins. Those two types of mutations are called “disruptive mutations”, having a high putative impact on protein function and structure.

Another example of a SNP is a missense mutation defined as a point mutation in which a single nucleotide change results in a codon that codes for a different amino acid. This type of mutation has a moderate putative impact on protein function and structure (Human Genome Program, 2008).

d. Genotype

A genotype refers to the combination of alleles at a given locus within the genome that codes for a particular trait. Two organisms whose genes differ at even one

locus are said to have different genotypes. The transmission of genes from parents to descendants is controlled by precise molecular mechanisms the discovery of which began with Mendel (Hartsfield, 2011).

3. Phenotype

The phenotype can be thought of as a clinical expression of an individual's specific genotype. It is the observable properties, measurable features and physical characteristics of an individual (Baltimore, 2001). It is created by summation of the effects arising from an individual's genotype and the environment in which the individual develops over a period of time. MP is a trait, which is a particular aspect or characteristic of the phenotype that has a specific mode of inheritance. The genetic influences on traits are monogenic (predominantly single gene with the possibility of other smaller genetic and environmental factors) or complex (many genetic and environmental factors) (Hartsfield, 2011).

4. Modes of inheritance and penetrance

a. Modes of inheritance

The nature of family-based (familial) traits can be studied by constructing family trees called pedigrees in which males are denoted by squares and females by circles, with the affected individuals having their symbol filled. Pedigrees illustrate the family history in first-degree relatives of the patient (siblings and parents), second-degree relatives (half-siblings, aunts, uncles, grandparents) and third-degree relatives (first cousins) (Hartsfield, 2011).

i- Autosomal dominant

The mode of inheritance is autosomal dominant when a trait is present as the result of only one copy of a particular allele (example: “A”) in a heterozygous allele pair (example: “Aa”). The trait is also expressed in the presence of a homozygous allele pair (example: “AA”) (Figure II.3).

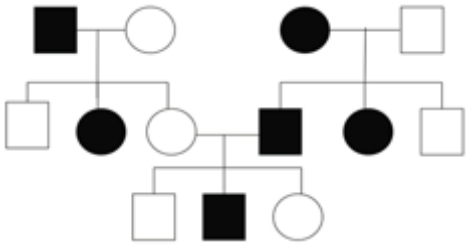


Figure II.3: Three-generation pedigree of a family with an autosomal dominant trait (Hartsfield, 2011).

ii- Autosomal recessive

The mode of inheritance is autosomal recessive when a trait is only present when both alleles at the locus are the same (example: “aa”); in other words, when the individual is homozygous for “a”. The symbols for presumed carriers (heterozygotes) of the autosomal recessive gene are filled in halfway (Figure II.4).

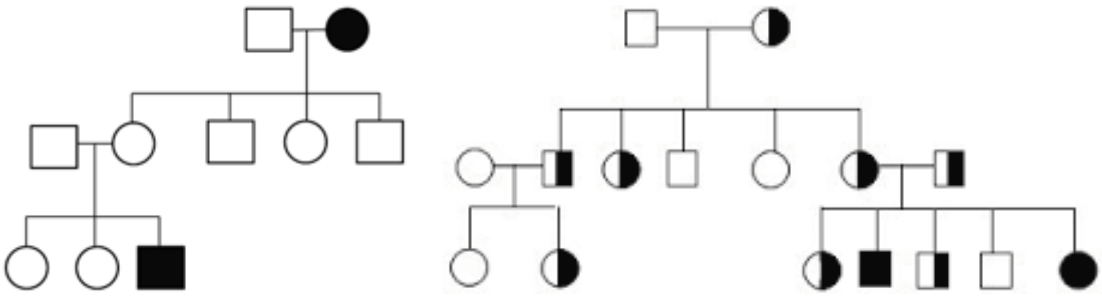


Figure II.4: Three-generation pedigree of a family with an autosomal recessive trait (Hartsfield, 2011).

iii- X-linked recessive

The mode of inheritance is X-linked recessive when a mutation in a gene on the X chromosome causes the phenotype to be expressed in males (who are necessarily hemizygous for the gene mutation because they have one X and one Y chromosome) and in females who are homozygous for the gene mutation. The symbols for presumed female carriers (heterozygotes) of the X-linked recessive gene have a dot in the middle of the circle (Figure II.5).

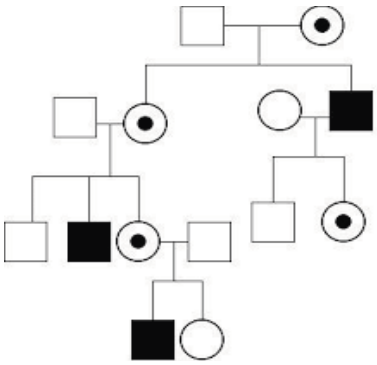


Figure II.5: Four-generation pedigree of a family with an X-linked recessive trait (Hartsfield, 2011).

iv- X-linked dominant

The mode of inheritance is X-linked dominant when a mutation in a gene on the X chromosome causes the phenotype to be expressed in males (who are necessarily hemizygous for the gene mutation because they have one X and one Y chromosome) and in females who are homozygous or heterozygous for the gene mutation (Figure II.6).

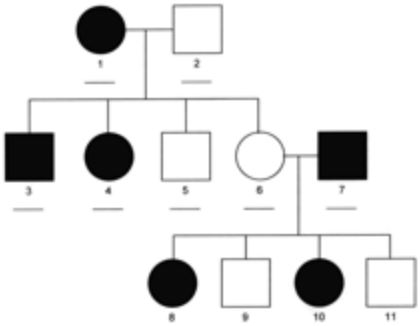


Figure II.6: Three-generation pedigree of a family with an X-linked dominant trait (Hartsfield, 2011).

Table II.1: Summary of the modes of inheritance (Hartsfield, 2011).

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

b. Modes of penetrance

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

- Complete penetrance: when all individuals who have the trait-causing mutation have clinical symptoms of the trait.

- Highly penetrant: when the trait is almost always apparent in an individual carrying the allele.

- Incomplete penetrance or reduced penetrance: when some individuals fail to express the trait, even though they carry the allele. Those individuals are able to have offsprings with the trait.

- Low penetrance: it will only sometimes produce the trait with which it has been associated at a detectable level (U.S. National Library Of Medicine, 2016).

c. Measures of heritability

Heritability is a static used in genetics to estimate how much variation in a phenotypic trait in a population is due to genetic variation among individuals in that population. Heritability increases when genetic factors are contributing more variation or when non-genetic (environmental) factors are contributing less variation. H^2 is a common measure of heritability, which reflects a specific mathematical formula that embeds all the genetic contributions to a population's phenotypic variance. The values of H^2 range between 0 and 1. A trait with a heritability estimate of 1 would be expressed with complete positive correlation to genotypic factors theoretically, as measured by comparing the concordance of the phenotype to the percentage of genes in common among twins or other siblings. By comparison, a trait with a heritability of 0.5 would have half its variability of concordance (from individual to individual) positively correlated with the percentage of genes in common. $H^2 \geq 0.5$ reflects a high heritability and $H^2 \leq 0.2$ suggests a low heritability (Hartsfield, 2011; Wray & Visscher, 2008).

5. Sanger and Next Generation sequencing techniques

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

a. Sanger sequencing

Developed by Frederick Sanger and his colleagues in 1977, the Sanger sequencing technique is based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. It requires a single-stranded DNA template (DNA to be sequenced), a DNA primer (starting point for DNA synthesis on the strand of DNA to be sequenced), a DNA polymerase, normal deoxynucleoside triphosphates (dNTPs), and modified dideoxynucleoside triphosphates (ddNTPs) that terminate DNA strand elongation (chain terminators). Four individual DNA synthesis reactions are performed. The four reactions include normal A, G, C, and T dNTPs and each contains a low level of one of four ddNTPs: ddATP, ddGTP, ddCTP, or ddTTP. The four reactions can be named A, G, C and T, according to which of the four ddNTPs was included. The DNA to be sequenced is added to the 4 reactions. Most of the time, DNA polymerase will add a proper dNTP to the growing strand it is synthesizing in vitro, but at random locations, it will instead add a ddNTP. When it does, that strand will be terminated at the ddNTP just added. If enough template DNAs are included in the reaction mix, each one will have the ddNTP inserted at a different random location, and there will be at least one DNA terminated at each different nucleotide along its length for as long as the in vitro reaction can take place. The ddNTPs that terminate the strands have specific fluorescent labels covalently attached to them. After the reaction is over, it is subject to capillary electrophoresis. All the newly

synthesized fragments, each terminated at a different nucleotide and so each a different length, are separated by size. Smaller fragments will migrate more. As each differently sized fragment exits the capillary column, a laser excites the fluorescent tag on its terminal nucleotide. From the color of the resulting fluorescence, a computer can keep track of which nucleotide was present as the terminating nucleotide. The computer also keeps track of the order in which the terminating nucleotides appeared, which is the sequence of the DNA used in the original reaction (Sanger & Coulson, 1975).

b. Next Generation Sequencing

In 2009, Next Generation Sequencing (NGS) or deep sequencing platform appeared on the market as a revolutionary technology that performs sequencing of millions of small DNA fragments in parallel "massive parallel sequencing". NGS can be used to sequence entire genomes (Whole Genome Sequencing: WGS) or constrained to specific areas of interest, including a whole exome (all 22 000 coding genes) (Whole Exome Sequencing: WES) or small numbers of individual genes. NGS has many advantages over the traditional sequencing technique known as Sanger sequencing: much faster, captures a broader spectrum of mutations, produces more data, genomes can be interrogated without bias, needs significantly less template DNA and is cost effective. However, the Sanger method remains in wide use for smaller-scale projects, validation of NGS results and for obtaining especially long contiguous DNA sequence reads (> 500 nucleotides) (Behjati & Tarpey, 2013; Pareek, Smoczynski, & Tretyn, 2011). The technique of NGS will be described in details in the material and methods section.

6. Terms related to the genetic analysis

a. Quality score

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

b. Filter status

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

c. Putative impact

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

d. Allele count and Minor Allele Frequency (MAF)

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

e. Approximate read depth

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

f. Inbreeding coefficient

The inbreeding coefficient (F) is the probability of autozygosity, i.e. the probability that a zygote obtains two copies of the same ancestral gene from both its parents because they are related (Human Genome Program, 2008).

g. Yield

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

h. GC-content

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

B. Incidence and prevalence

MP is found throughout the world. However, its prevalence varies among populations, indicating that ethnicity is a risk factor for MP. The highest incidence has been observed in Asian populations (2.1 to 19.9%), especially among Japanese and Chinese and the lowest incidence in Caucasian populations (0.48 to 4.3%) (Allwright & Bundred, 1964; Emrich, Brodie, & Blayney, 1965). No conclusive findings are documented concerning the gender similarities or differences related to the incidence of MP. Some found no gender differences (Joshia, Hamdanb, & Fakhouri, 2014; Solow & Helm, 1968), while some others found an increased incidence in males (El-Mangoury & Mostafa, 1990) and others found an increased incidence in females (Wood, 1971).

C. Craniofacial and dental morphological features

MP is clinically heterogeneous and can be associated with many combinations of skeletal and dental components that lead to the forward positioning of the mandible relative to the maxilla.

1. Craniofacial features

Craniofacial features that have been associated with the development of MP include:

- Overdeveloped mandible (long total mandibular length Co-Gn or Co-Pog) or anteriorly positioned mandible (increased SNB angle) with or without an underdeveloped (small Ptm-A) or backwardly positioned maxilla (small SNA angle),
- Steep mandibular plane angle,
- Obtuse gonial and fronto-nasal angles,
- Greater vertical lower face height,
- Small cranial base angle that may displace the glenoid fossa anteriorly to cause a forward positioning of the mandible (Battagel, 1993a; H. Chang, Hsieh, & Tseng, 2005; H. Chang, Lin, & Liu, 2006; Mitani, Sato, & Sugawara, 1993; Sato, 1994).

Studies have shown also that cranial base length can also affect the position of the jaws. Many Class III patients have a shorter anterior cranial base and longer posterior cranial base when compared with Class I controls. These features result in a more anteriorly positioned glenoid fossa, which then positions the mandible further anteriorly (Baccetti, Antonini, Franchi, Tonti, & Tollaro, 1997; Battagel, 1993b; H. Chang, Hsieh, et al., 2005; H. Chang et al., 2006; Dhopaktar, 2002).

Those skeletal characteristics are reflected in the facial features by smoothing of the labiomental fold, a prominent chin and a prognathic and concave profile.

2. Dental features

The dental characteristics of MP include an edge-to-edge incisal relationship or a negative overjet (anterior crossbite) and a Class III relationship between the permanent first molars. Anteroposterior dento-alveolar compensations include the tendencies toward proclination of maxillary incisors and retroclination of mandibular incisors (to a lesser extent), leading to a smaller interincisal angle. The skeletal position of mandible impacts on the inclination of incisors (H. P. Chang et al., 2006; Robinson, Speidel, Isaacson, & Worms, 1972; Sperry et al., 1977).

3. Sexual dimorphism

The main features concerning sexual dimorphism in Class III malocclusion at pubertal and postpubertal ages (13 years and older) translate into the following traits in female subjects when compared with male subjects: shorter anterior cranial base, shorter midfacial (Co-PtA) and mandibular (Co-Gn) lengths, shorter upper and lower anterior facial heights (ANS-Me), smaller maxillomandibular differential (ANB angle and Wits appraisal), less extruded upper molars and incisors and less extruded lower incisors (Baccetti, Reyes, & McNamara, 2005).

D. Etiology of Class III malocclusion

1. Maxillary retrognathism

Class III malocclusion associated with maxillary retrognathism may be of genetic origin but environmental factors have been inculpated as possibly predominant, as stipulated by the concept of developmental or “intragrowth orthopedics” (Ghafari, 2004). According to this concept, an early anterior cross bite sustained by mandibular forward positioning (caused by inherited macro and/or prognathic mandible, occlusal

interferences, habits, or to improve breathing), may induce forces that inhibit maxillary forward growth and produce maxillary retrognathism that otherwise would not exist. This process would occur particularly in instances of deep overbite (Ghafari, 2004; Ghafari & Haddad, 2005).

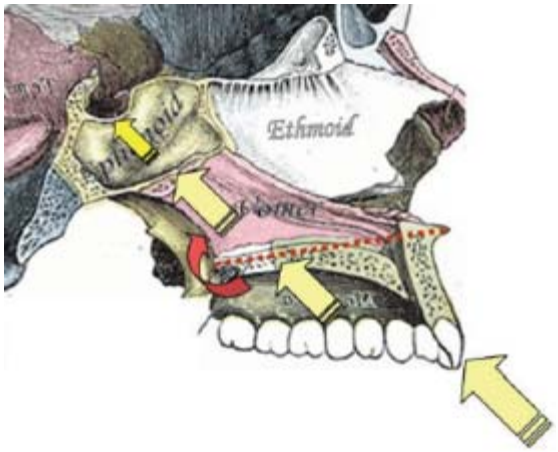


Figure II.7: Concept of developmental or "intragrowth orthopedics" (Anatomic illustration adapted from Gray's Anatomy, 2005).

2. Mandibular prognathism

The etiology of Class III malocclusion underlined with MP remains wide ranging and complex, involving both genetic and environmental factors (Kraus, Wise, & Frie, 1959; Litton, Ackerman, Isaacson, & Shapiro, 1970). The genetic component is predominant during embryonic craniofacial morphogenesis, particularly when the size of the mandible is significantly greater than the average (macrognathic mandible), but the environment plays a role postnatally, particularly during facial growth (Ghafari, Haddad, & Saadeh, 2011; Mossey, 1999). The environmental causes found to be involved in the development of MP include congenital anatomic defects (cleft lip/palate), imbalances in the endocrine system, hormonal disturbances, habitual

posture, trauma (instrumental deliveries) and nasal obstruction by enlarged tonsils (H. Chang, Chuang, & Yang, 2005; H. P. Chang et al., 2006; Macari & Ghafari, 2006; Pascoe, Hayward, & Costich, 1960; Schoenwetter, 1974). The latter was Angle's only explanation for the etiology of Class III malocclusion: "Deformities under this class begin at about the age of the eruption of the first permanent molars, or even much earlier, and are always associated at this age with enlarged tonsils and the habit of protruding the mandibule, the latter probably affording relief in breathing." Angle stipulated that as the mesio-buccal cusp of the maxillary first molar begins to engage the distal incline of the distobuccal cusp of the mandibular first molar, the mandible is mechanically thrust forward. The muscles would then exert abnormal force on the mandible and stimulate it to abnormal growth and malformation (Angle, 1899). While this concept has not born proof through research or the natural experiments of daily practice, the linkage between anterior mandibular positioning and breathing is in general plausible, regardless of the etiology of mouth breathing.

However, the genetic component plays a substantial role in the etiology of MP since it is the most commonly inherited malocclusion, followed by the long face syndrome (Proffit, Fields & Sarver, 2014). The strong genetic influence on MP is supported by the observation of familial aggregation (Litton et al., 1970). In fact, studies have shown a significantly high incidence of MP in the relatives of affected probands (Kraus et al., 1959). One of the most famous examples of the hereditary pattern of MP is the Habsburg jaw, which has been observed in 23 successive generations of the Spanish royal family. MP is believed to have been passed on and exaggerated over time through royal intermarriage, which caused acute inbreeding (Wolff, Wienker, & Sander, 1993) (Figure II.8).

Figure II.8: Members of the Habsburg royal family (Grabb, Hodge, Dingman & Oneal, 1968).

E. Inheritance pattern

The inheritance pattern of MP is polemic. Familial aggregation observed in this trait has been ascribed to a variety of genetic models including autosomal-recessive inheritance (Downs, 1928), autosomal-dominant inheritance (Kraus et al., 1959), dominant inheritance with incomplete penetrance (El-Gheriani et al., 2003; Wolff et al., 1993), or a polygenic threshold model (Litton et al., 1970).

These outcomes indicate that MP has a polygenetic or multifactorial model of inheritance in the vast majority of families which most likely results from mutations in numerous genes, with a threshold for expression (Yamaguchi, Park, Narita, Maki, & Inoue, 2005). Nevertheless, pedigree and linkage analyses showed that MP has definitive patterns of Mendelian inheritance and is segregated mainly in an autosomal-dominant manner with variable expressivity, incomplete penetrance and an estimated heritability ratio $H^2 = 0.316$ (Cruz et al., 2008; El-Gheriani et al., 2003; Frazier-Bowers, Rincon-Rodriguez, Zhou, Alexander, & Lange, 2009; Wolff, Wienker, & Sander, 1993). This value lies nearly in the middle zone between low ($H^2 \leq 0.2$) and high ($H^2 \geq 0.5$) heritability (see section A.4.c, page 16). It is lower than the heritability ratio of cleft lip/palate, which a higher heritable craniofacial condition at $H^2 = 0.76$ (Woolf, 1971).

F. Genetic studies

While research in humans holds great promise, animal models have led the way. In particular, multiple studies have been completed in transgenic mice that manifest MP (Machicek, 2007). Introduction of the U.S. Human Genome Project (HGP) in 1990 (completed in April 2003) was a breakthrough that aimed at determining the sequence of chemical base pairs that make up human DNA, identifying and mapping all the genes of the human genome from both a physical and a functional standpoint.

This increasingly detailed knowledge of the human genome at the DNA level forms the basis of understanding the genetic transmission and gene action with more precision. Therefore, it became possible to construct comprehensive genetic maps to locate and identify genes underlying susceptibility to disease (Sawicky, Samara, Hurwitz, & Passaro, 1993). However, genetics is still not well defined in non-syndromic conditions.

1. Mandibular prognathism

Recent progress in molecular genetics like the genome-wide linkage scan technology has allowed the investigation of susceptibility genes that underlie the development of MP. Genome Wide Association Studies (GWAS) are defined as studies comparing DNA markers across the genome in people with a disease or trait to people without the disease or trait. However, only few GWAS have been done, indicating that the genetic determinants of MP are still poorly understood. In addition, the highly variable sub-phenotype within and across populations is an obstacle to determining the genetic basis of this dentofacial phenotype (Li, Li, Zhang, & Chen, 2011). The few genetic mapping studies performed in different ethnic populations identified several chromosomal regions or loci that might harbor susceptibility genes for MP (Table II.2).

Yamaguchi et al. in 2005 were the first to map susceptibility loci to chromosomes 1p35-36, 5p13, 6q25 and 19p13.2 in affected sibling pairs from Korean and Japanese families. The loci 1p35.2 and 1p36.12 harbor positional candidate genes of interest, which include *MATN1*, *HSPG2* and *ALPL* respectively.

- *MATN1* gene encodes a non-collagenous protein secreted by chondrocytes and dominantly expressed in cartilage. It is involved in the formation of filamentous networks in the extracellular matrices of various tissues, mainly cartilage (Deák, Wagener, Kiss, & Paulsson, 1999) and has a definitive function in endochondral bone formation by influencing the growth plate of developing long bones (Hansson, Heinegård, Piette, Burkhardt, & Holmdahl, 2001).
- *HSPG2* gene provides instructions for making a protein called perlecan that is involved in cell signaling, adhesion of cells to one another, formation of new blood vessels (angiogenesis) and maintenance of basement membranes and cartilage throughout life. It also plays a critical role at the neuromuscular junction where signals are relayed to trigger muscle contraction (U.S. National Library Of Medicine, 2016).
- A*LPL* gene provides instructions for making an enzyme called alkaline phosphatase that plays an important role in growth and development of bones and teeth and is active in many other tissues, particularly the liver and kidneys (U.S. National Library Of Medicine, 2016).

On the other hand, a linkage study in Brazilian families showed that 1p36, 6q25 and 19p13.2 were not associated with the condition (Cruz et al., 2011).

Another GWAS carried out in four Hispanic families from a Colombian background, revealed five suggestive loci: 1p22.1, 3q26.2, 11q22, 12q13.13 and 12q23 (Frazier-Bowers et al., 2009). Follow-up studies on the 1p35.2 and 1p35.3 loci have

shown 2 plausible candidate genes that present susceptibility to MP: *MATN1* in Koreans (Jang et al., 2010), and *EPB41* in a Chinese population (Xue, Wong, & Rabie, 2010). *EPB41* gene encodes a major structural component of the membrane skeleton of erythrocytes that makes a crucial contribution to the structural integrity of the centrosome and mitotic spindle and plays a role in cell division (Conboy, 1993). In addition, the C-terminal domain (CTD) of the *EPB41* protein, a sequence domain that is unique to members of the 4.1 protein family, is associated with a growing number of protein–protein interactions, which have been shown to be important for the regulation of cell growth (Sun, Robb, & Gutmann, 2002).

In 2010, Li et al. performed a genome-wide linkage analysis with two MP pedigrees of Chinese Han people and detected a novel chromosomal region 4p16.1 potentially linked to MP, which harbors positional candidate genes: *EVC* and *EVC2*. *EVC2* encodes a protein that functions in bone formation and skeletal development. Mutations in this gene as well as in a neighboring gene (*EVC)*, which lies in a head-to-head configuration, cause an autosomal recessive skeletal dysplasia that is also known as chondroectodermal dysplasia or cause acrofacial dysostosis Weyers type, a disease that combines limb and facial abnormalities. As mutations in these genes always cause skeletal dysplasia, the authors suggest that some novel mutations in *EVC2* and *EVC* may be relevant to the form of MP (Li, Zhang, Li, & Chen, 2010).

In 2011, Li et al. identified one suggestive locus also in the Chinese Han population: 14q24.3-31.2. Two functional genes, *TGFβ3* and *LTBP2* are candidate genes within this locus and may be involved in the development of MP. *TGF-β* is one of the most important growth factors in the formation, differentiation and homeostasis of bone tissue and plays a crucial role in the regulation of cell proliferation, differentiation,

apoptosis and migration. Regarding the effects of *TGFB3* on craniofacial development, reports have shown a close relationship to the mineral maturation matrix (Reutter et al., 2008). *LTBP2* is known to play a structural role within elastic fibers and affects extracellular matrix homeostasis (Saharinen, Hyytiäinen, Taipale, & Keski-Oja, 1999). In a recent in vitro study, *LTBP2* was found to assist in the process of chondrogenic dedifferentiation (Goessler et al., 2005).

A study including white, African American, Hispanic and Asian subjects was done by Tassopoulou-Fishell et al. in 2012. They provided further evidence of a role of the 12q24.11 locus in the development of MP. They found an association between a marker in *MYO1H* (rs10850110) and the MP phenotype, while a 7p locus variation associated with MP was described in Brazilian and Colombian populations (Falcão-Alencar, Otero, & Cruz, 2010). In 2017, Cruz et al. confirmed also the association between the marker rs10850110 in *MYO1H* and MP. *MYO1H* is a Class I myosin that is necessary for cell motility, phagocytosis, and vesicle transport (Tassopoulou-Fishell, Deeley, Harvey, Sciote, & Vieira, 2012).

Whole-exome sequencing (WES) in an Estonian family showed that the missense variation on p.Ser182Phe in the *DUSP6* gene on chromosome 12q22-23 was linked to MP (Nikopensius et al., 2013). The protein encoded by this gene negatively regulates proteins that are associated with cellular proliferation and differentiation (U.S. National Library Of Medicine, 2016). Moreover, two loci (1q32.2 and 1p22.3) were found to be susceptibility regions of MP in the Japanese population, and *PLXNA2* and *SSX2IP* are considered to be candidate genes. *PLXNA2* encodes a protein involved in nervous system development, while *SSX2IP* is involved in cancer (Ikuno et al., 2014).

Furthermore, an association between polymorphism in the *COL2A* gene (located within the region 12q13.11) and MP was found by Xue et al. in 2014 in Chinese people. *COL2A1* is expressed in cartilage and is important for craniofacial growth. It provides instructions for making one component of the type II collagen (Garofalo et al., 1991).

Guan et al. (2015) suggested that 2 single-nucleotide polymorphisms (rs2738, rs229038) of *ADAMTS1* gene, which is located in the region 21q21.3, were significantly associated with MP in the Chinese population. *ADAMTS1* is a family of extracellular proteases involved in proteolytic modification of cell surface proteins and extracellular matrices (Apte, 2009; Rehn, Birch, Karlstrom, Wendel, & Lind, 2007).

A research study done in Italy revealed that the Gly1121Ser variant in the *ARHGAP21* gene (loci 10p12.1-12.3) that is involved in tubulin metabolism, was shared by all MP individuals included in the study, which shows that it may be associated with MP (Perillo et al., 2015).

In summary, the main candidate chromosomal regions associated with MP comprise the loci located on the following chromosomes:

- 1 (1p22.1, 1p22.2, 1p22.3, 1p35.2, 1p35.3, 1p36.12, 1q32.2),
- 3 (3q26.2),
- 4 (4p16.1),
- 5 (5p12, 5p13),
- 6 (6q25),
-10 (10p12.1, 10p12.3),
- 11 (11q22.2, 11q22.3),
- 12 (12q13.11, 12q13.13, 12q22, 12q23, 12q24.11),
- 14 (14q24.3, 14q31.2),
- 19 (19p13.2),
- 21 (21q21.3) (Tomaszewska, Kopczyński, & Flieger, 2013).

Table II.2: Summary of the susceptibility genes for MP found by the genetic mapping studies done in different ethnic populations.

GENE NAME	LOCUS	POPULATION	REFERENCE
MATN1, *HSPG2* and *ALPL*	1p35.2-36.12	Korean and Japanese	Yamaguchi et al., 2005
MATN1	1p35.2	Korean	Jang et al., 2010
EPB41	1p35.3	Chinese	Xue et al., 2010
EVC and *EVC2*	4p16.1	Chinese Han	Li et al., 2010
TGFβ3 and *LTBP2*	14q24.3-31.2	Chinese Han	Li et al., 2011
MYO1H (rs10850110)	12q24.11	White, African American, Hispanic, and Asian	Tassopoulou-Fishell et al., 2012 Cruz et al., 2017
DUSP6 (p.Ser182Phe variant)	12q22-23	Estonian	Nikopensius et al., 2013
PLXNA2 and *SSX2IP*	1q32.2, 1p22.3	Japanese	Ikuno et al., 2014
COL2A1	12q13.11	Chinese	Xue et al., 2014
ADAMTS1 (rs2738, rs229038)	21q21.3	Chinese	Guan et al., 2015
ARHGAP21 (Gly1121Ser variant)	10p12.1-12.3	Italian	Perillo et al., 2015

As already mentioned, it is suspected that mutations or polymorphisms occurring in the listed genes (Table II.2) may have a considerable effect on the incidence of MP. In addition, to-date, the several mutations and polymorphisms have been confirmed in the genes encoding specific growth factors and other signaling molecules involved in the bone and cartilage morphogenesis of the craniofacial area. Growth factors and cytokines affect the target cells through specific receptors, stimulating the cells to proliferate, differentiate and increase the gene expression as well as to further secrete biologically important substances. For example, excessive forward mandibular growth was related to the increased expression of IHH (Indian Hedgehog Homolog) and PTHrP (Parathyroid Hormone-Related Protein), promoting differentiation of mesenchymal stem cells into specialized bone and cartilage cells as well as their further proliferation; such proteins promote increased cartilage growth (Rabie, Tang, Xiong, & Hagg, 2003; Tang, Rabie, & Hagg, 2004). The growth factor *VEGF* and transcription factors *SOX9* and *RUNX2* hold an essential role in the differentiation of chondrocytes; therefore they are potential markers in searching for the genetic causes of MP (Pei, J, & Chen, 2008).

Another genetic factor taken into account when looking for the etiology of MP is the receptor of the growth hormone, which has a key role in the development of the bone structures and morphogenesis of the craniofacial area. Studies carried out on the Japanese population confirm a strong correlation between variant Pro561Thr in the Growth Hormone Receptor (GHR) and incidence of MP (Yamaguchi, Maki, & Shibasaki, 2001).

The varying results of these genome-wide linkage studies in different ethnic groups indicate that MP might be a complex condition and that the causative gene of MP may not be unique (polygenetic trait). Therefore, these studies strongly support locus heterogeneity in the development of MP and the genes concerned in the etiology of this feature may be correlated with the ethnicity of the population.

2. Other malocclusions

Many genetic studies were performed to explore the candidate genes that are responsible of the development of some malocclusions, mainly the Class II, the congenital absence of teeth and the primary failure of eruption. However, these studies are less frequent than those done to explore the inheritance pattern of MP since the latter is the most commonly inherited malocclusion (Proffit, Fields & Sarver, 2014).

There is evidence that Class II/2 has a genetic component based upon a twin study in which all 20 monozygotic twin pairs were concordant for Class II/2, while only 10.7% of 28 dizygotic twin pairs were concordant. The much lower concordance for dizygotic twins suggests that more than one genetic factor contributes to Class II/2 (Markovic, 1992). Further evidence for Class II/2 to have a polygenic complex etiology was found in a study of 68 Class II/2 patients, with a relative risk of first-degree relatives of the patients to have a Class II/2 of 3.3 to 7.3 (Morrison, Hartsfield, Foroud

& Roberts, 2008). Harris (1975) reported that the craniofacial skeletal patterns of individuals with Class II/1 malocclusions are familial. He suggested a polygenic inheritance and autosomal dominance models, with incomplete penetrance and variable expressivity for Class II subdivision 1 and 2, respectively A study on Japanese individuals revealed that those having a SNP at the Growth Hormone Receptor (GHR) gene locus had a significantly smaller mandibular ramus length (Yamaguchi, Maki, & Shibasaki, 2001). Gutierrez et al. (2010) detected an association between a SNP within the *NOGGIN* gene and mandibular hypoplasia, whereas Zebrick et al. (2014) demonstrated that R577X is associated with Class II and deep-bite skeletal malocclusions. More recently, Da Fontoura et al. (2015) related SNPs within *SNAI3* and *FGFR2* to an increased risk of a class II phenotype and an accentuated convex profile.

Class II/2 was reported to also include inherited dental developmental anomalies, especially dental agenesis, which is at least 3 times more common in this malocclusion than in the general population (Basdra, Kiokpasoglou & Stellzig, 2000; Hartsfield, 2011). Dental agenesis in general is most often familial in origin and usually observed as an "isolated" trait (i.e., nonsyndromic). However, it may also occur as part of a syndrome, especially in one of the many types of ectodermal dysplasias. Genetic factors are thought to play a major role in most of these conditions with autosomal dominant, autosomal recessive, X-linked, and multifactorial inheritance reported (Mostowska, Kobielak & Trzeciak, 2003). One of the most common patterns of hypodontia involves maxillary lateral incisors. Their agenesis can be an autosomal dominant trait with incomplete penetrance and variable expressivity as evidenced by the phenotype sometimes "skipping" generations, sometimes being a peg-shaped lateral instead of agenesis, and sometimes involving one or the other or both sides. However, a

polygenic mode of inheritance has also been proposed (Woolf, 1971). Numerous mutations in transcription factors and growth factor–related genes involved in dental development have been shown to have roles in human dental agenesis, including *PAX9, MSX1, EDARADD* and *AXIN2* (Bergendal, Klar, Stecksen-Blick, Norderyd & Dahl, 2011).

Genetic factors have also been involved in primary failure of eruption, including mainly the *PTHR1* gene (Decker et al., 2008).

G. Orthodontic studies on heritability of malocclusion

The classical twin research design, involving comparison of similarities in monozygotic or identical pairs and dizygotic or fraternal pairs, is one of the most effective methods to quantify the relative contributions of genetic (nature) and environmental (nurture) factors to variation in many variables in orthodontics and in other medical fields. Twins are invaluable for studying these important questions because they disentangle the sharing of genes and environments. In fact, dizygotic twins act as a control, as they share the same early environmental factors, but are not genetically alike. Researchers look for traits that show a greater similarity in identical twins than in nonidentical twins. This approach indicates a shared genetic basis for the trait and is expressed as a percentage known as the heritability (Garn et al., 1960; Moorrees, 1962; McNamara, 1973; Townsend, Richards, Hughes, Pinkerton & Schwerdt, 2003).

1. Growth and development

Garn et al. (1960) studied dental developmental stages for mandibular first and second molars in two sets of triplets and suggested that dental development is

mostly genetically determined. Picacintini (1962) applied the co-twin study method on six sets of same sex triplets in whom the zygosity had previously been determined to a high degree of probability. He concluded that the combined incremental growth of maxilla and mandible of monozygotic co-twin show significantly smaller intrapair differences compared to that of dizygotic co-twins. This result proves the role of heredity in governing growth of these bones.

2. Craniofacial complex

Kraus et al. (1959) found in a twin study almost perfect concordance in the craniofacial complex of monozygotic triples whereas only a low degree of concordance was noted in dizygotic triplets. They concluded that the morphology of all bones of the craniofacial complex is under rigid control of hereditary factors. Accordingly, there is a high heritability rate for craniometric variables compared to a low to moderate rate for dento-alveolar variables (Amini & Borzabadi-Farahani, 2009). Correlations between inheritance and craniometric variables increase from age 4 to age 20 and then decrease with age to the extent that few variables for subjects at age 20 have a correlation significantly different from zero (Harris & Johnson, 1991).

In a cephalometric study of craniofacial variation in 56 twin pairs, Horowitz et al. (1960) reported a highly significant genetic variation in the anterior cranial base and mandibular body lengths. They also stated that the upper face height was the more stable element in the facial profile, as it did not contribute greatly to the genetic variability of the face as a whole. Other authors indicated that vertical skeletal variables (in particular total and lower anterior facial heights) have more heritability and are seemingly under a stronger genetic control than horizontal ones (Amini & Borzabadi-Farahani, 2009). Heritability was apparently expressed more anteriorly than posteriorly.

Šidlauskas et al. (2016) studied the heritability of mandibular cephalometric variables in twins with completed craniofacial growth and concluded that the shape and sagittal position of the mandible was under stronger genetic control than its size and vertical relationship to cranial base.

3. Tooth size and occlusal variations

Detlefsen (1928) stated that tooth shape and size, as well as arch shape and size were largely determined by hereditary constitutional factors. Lundstrom (1948) studied tooth size and occlusion in twins and concluded that heredity was an important factor in malocclusion. Concordance was found in 87.3% of monozygotic twins and 84.6% of dizygotic twins with class I occlusion, and in 67.7% of monozygotic twins and 10% of dizygotic twins who had class II. Horowitz et al. (1958) carried out a study on 54 pairs of like sexed adult twins and found a strong genetic component of variability of the four maxillary and four mandibular incisors. The canine demonstrated relatively low hereditary component of variability. Corruccini and Potter (1980) studied occlusal variation in a sample of 32 monozygotic and 28 dizygotic twins pairs and reported that teeth displacement and crossbite were the most significantly heritable criteria of occlusion, whereas significant heritability of overjet, buccal segment relation, overbite, and tooth rotation/ displacement could not be documented. Townsend et al. (2009) related moderate to high heritability proportions (>60%) for many dental features such as dental spacing, arch dimensions and Bolton type tooth size discrepancies. Conversely, overbite (53%) and overjet (28%) presented lower heritability. These results demonstrate a considerably increased environmental component of variance in occlusion.

CHAPTER III

MATERIAL AND METHODS

A. Target population

1. General characteristics

This is a prospective case-control study including a sample that consisted of 8 Mediterranean families known to include growing and/or adult subjects diagnosed with MP (because of previous or ongoing treatment of some of them at the Division of Orthodontics and Dentofacial Orthopedics of the American University of Beirut Medical Center, AUBMC). 51 Mediterranean families were first approached and their pedigrees drawn. 14 families including 81 subjects underwent the data and biospecimen collection procedure, but we selected 8 families to undergo the genetic analysis because they have the largest number of affected individuals over many generations, the most severe conditions and depending on the mode of transmission of the phenotype and the budget. IRB approval was granted before initiation of the study for both levels of investigation: first to evaluate the existing radiographs under specified regulations, then for the family inheritance part. Privacy of the subjects was only accessed by the research group members; therefore there was no potential risk of breach of confidentiality. The research was funded by the Medical Practice Plan (MPP) and the University Research Board (URB).

2. Inclusion criteria

To be included in the study, the families should be part of the Mediterranean population and comprise several affected individuals over many generations. Patients

having an ANB angle ≤ 0 degrees (Cruz et al., 2008) and/or a negative Wits appraisal ≥ −2.0 mm (Alexander, McNamara, Franchi, & T, 2009) along with an increased mandibular length (Co-Gn) above one standard deviation of the norm for their age, a normal/long maxillary length (ANS-PNS) and a dental Class III malocclusion (with an anterior edge to edge, cross-bite or OJ because of severely proclined maxillary incisors as a compensation) were diagnosed as affected (Table III.1). Not only the affected individuals were included, but also some of the non-affected relatives as controls.

Table III.1: Criteria to diagnose an individual as affected.

Criteria to diagnose the individual as affected	
ANB ≤ 0 degrees	Negative Wits appraisal ≥ -2.0 mm
Normal/long maxillary length	Anterior edge to edge, cross-bite or OJ because of severely proclined maxillary incisors as a compensation
Increased mandibular length above one standard deviation of the norm for the specific age (mandibular macrognathism)	

3. Exclusion criteria

-Subjects having undergrowth of the maxilla (short maxillary length ANS-PNS).

-Subjects having congenital disorders such as cleft lip/palate or a general physical disease.

B. Families selection and recruitment process

51 subjects from the Mediterranean population diagnosed with MP (because of previous or ongoing treatment at the Division of Orthodontics and Dentofacial Orthopedics, AUBMC) were approached and pedigrees of their corresponding families were drawn in order to elucidate the families' structure (number of affected and non-affected males and females, mode of inheritance, consanguinity). Then, the families

whose pedigrees show only 1 or 2 affected subjects were ruled out (n=20) and the following recruitment process was adopted on the remaining families:

a- The selected subjects who are followed in our division were first approached by the study coordinator and asked if they are willing to participate in the research project.

b- The ones who agreed were detailed more about the study, handed a written consent form and given enough time to sign it. There are specific consent forms depending on the age of the participant (child between 7-12 years, adolescent between 13-17 years, adult and parental consents). The consent form contains information about the aims of the study, the procedure, the risks and benefits and a confidentiality section.

c- Then, they were asked about their family histories, including the affection status of other individuals in their family and 3 specific questions: do you have a congenital disorder? Did you undergo a previous genetic test? Is there consanguinity in your family?

d- They were given flyers (invitation to participate in a study) to distribute them to their affected and non-affected relatives. The flyers include general information about the study, its purpose, benefits, location… If the relatives are interested in the study, they can contact the research team (at (01) 350 000 ext. 5702) for further information and/or participation process.

e- The relatives who decided to enroll were 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- Then, the subjects underwent the data and biospecimen collection procedure that consisted of a clinical examination, a lateral cephalometric radiograph (done only on subjects on whom features of a Class III malocclusion were noted clinically) and 5cc of

blood collection (from some affected and non affected subjects). 14 Mediterranean families (12 Lebanese, 2 Lebanese/Syrian) including 81 subjects (42 males, 39 females; 40 affected, 41 non-affected) accepted to participate in the study and underwent the data and biospecimen collection procedure. Therefore, in total, 5cc of blood was collected from 81 subjects and a lateral cephalometric x-ray was taken on 36 affected subjects (18 males, 18 females). However, the genetic analysis was only performed on 8 families (7 Lebanese, 1 Lebanese/Syrian) because the budget is limited (Table III.2,3). Those 8 families were selected because they have the largest number of affected individuals over many generations, the most severe conditions and depending on the mode of transmission of the phenotype that was analyzed by visual inspection. The 8 selected families include 49 subjects who had 5cc of blood collected: 24 males and 25 females; 27 affected and 22 non-affected. Out of the 49 selected subjects, a lateral cephalometric x-ray was taken on 22 affected subjects (13 males, 9 females) (Table III.4). Each one of the 49 selected subjects was assigned a specific code that includes the family code (A-H) followed by an Arabic numeral (1-13) (Figures III.2-III.8).

Table III.2: Distribution of the ethnicities included in this study.

Families who were first approached and their pedigree drawn	**Families who had data and biospecimens collected**	**Families selected for the genetic analysis**
51 (44 Lebanese, 3 Lebanese/Syrian, 1 Syrian, 1 Syrian/Iraqi, 1 Palestinian/Lebanese, 1 Lebanese/Algerian)	**14** (12 Lebanese and 2 Lebanese/Syrian)	**8** (7 Lebanese and 1 Lebanese/Syrian)

Table III.3: Detailed summary of the number of subjects who had data and biospecimens collected.

	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**
14 families who had data and biospecimens collected	**42**	**39**	**40**	**41**	**18**	**18**
Total	**81**		**81**		**36**	

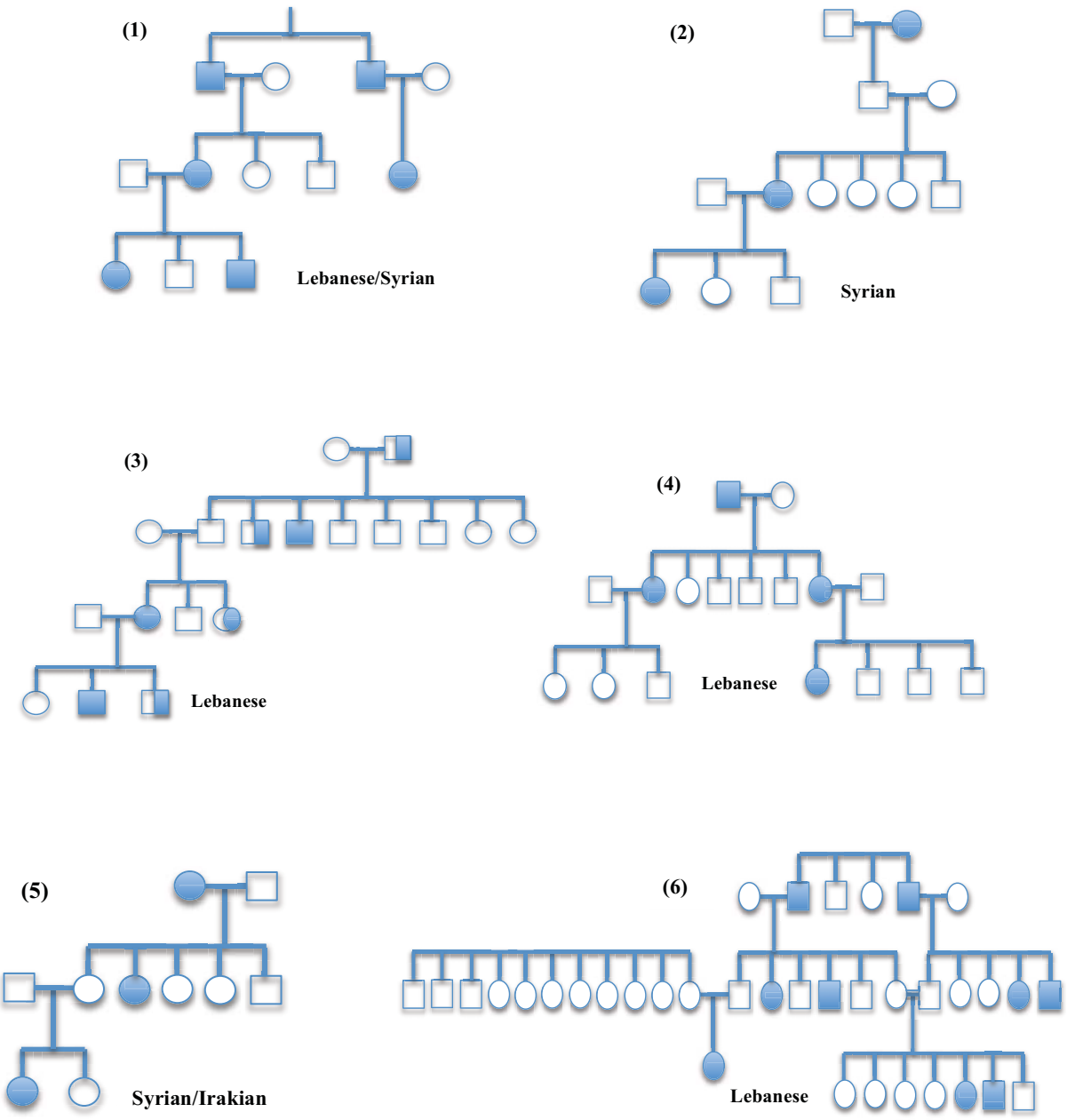
Table III.4: Summary of the demographic characteristics of the 8 selected families.

	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	3	5	5	3	1	3
Family (B)	Lebanese/Syrian	2	1	2	1	2	0
Family (C)	Lebanese	4	4	5	3	2	1
Family (D)	Lebanese	3	4	4	3	1	2
Family (E)	Lebanese	1	3	2	2	1	1
Family (F)	Lebanese	8	5	5	8	3	1
Family (G)	Lebanese	2	3	3	2	2	1
Family (H)	Lebanese	1	-	1	-	1	-
Total		**24**	**25**	**27**	**22**	**13**	**9**
TOTAL		**49**		**49**		**22**	

C. Families' structure (pedigrees)

1. Pedigrees of the 51 approached families

Pedigrees of the 51 Mediterranean families that were approached are illustrated below (Figure III.1):



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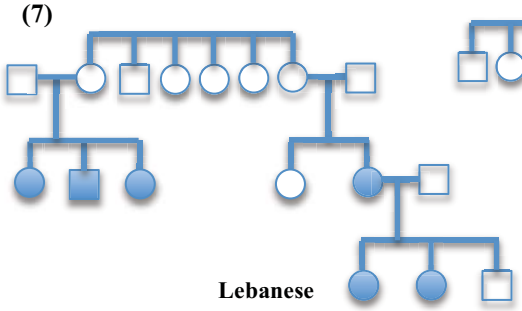
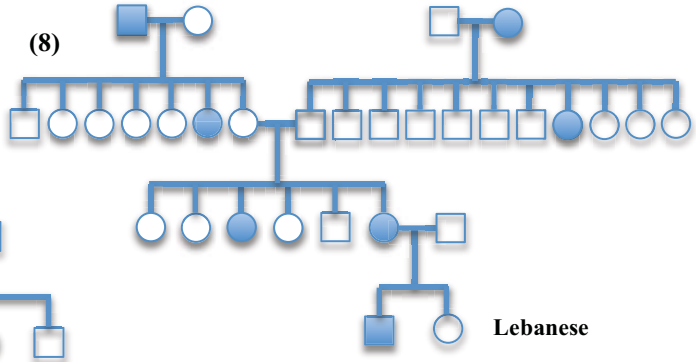


Lebanese

(8)



Lebanese

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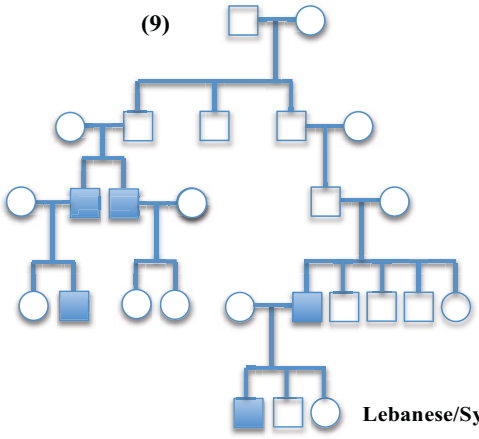

Lebanese/Syrian

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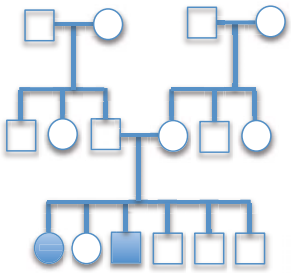
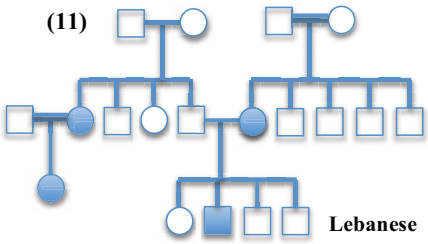


Palestinian/Lebanese

(11)



Lebanese

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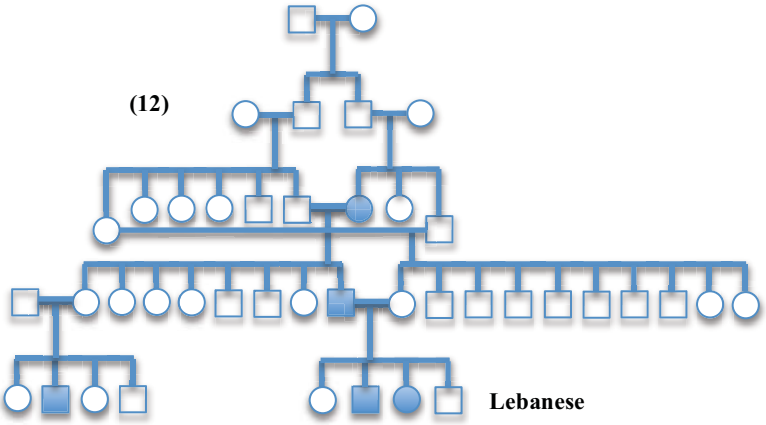

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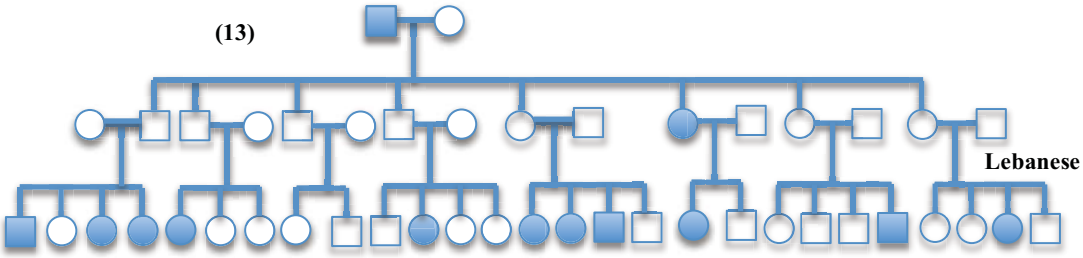

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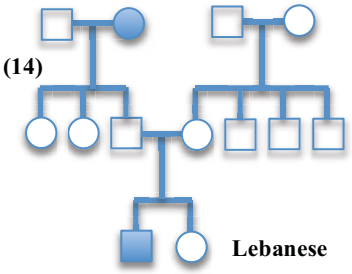

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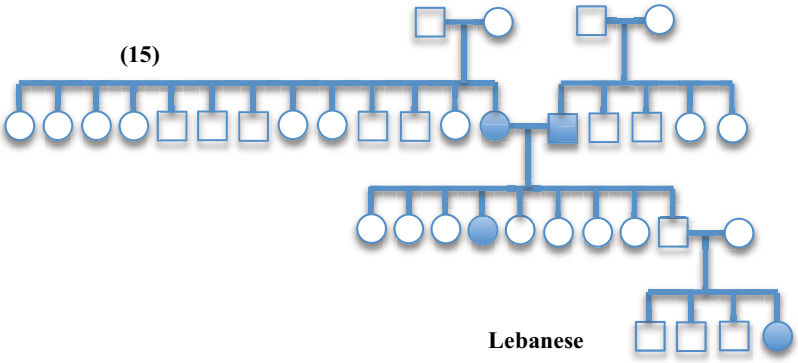

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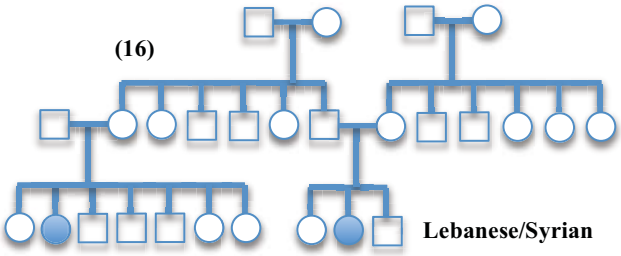

Lebanese/Syrian

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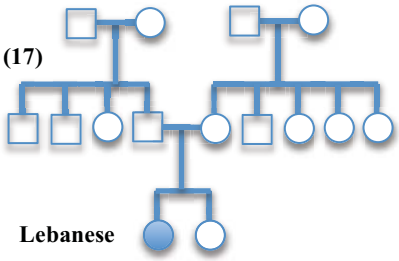

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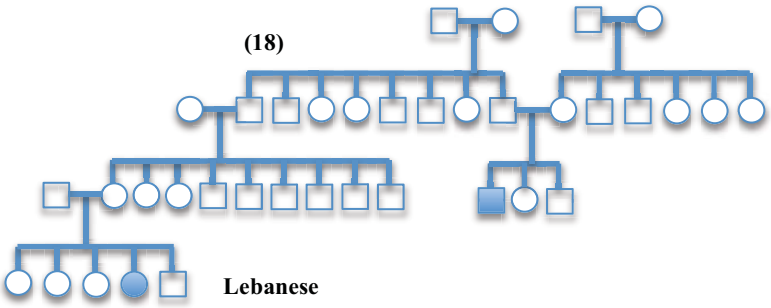

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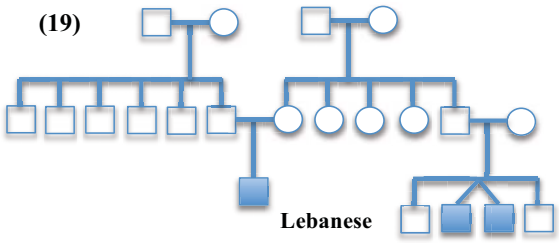

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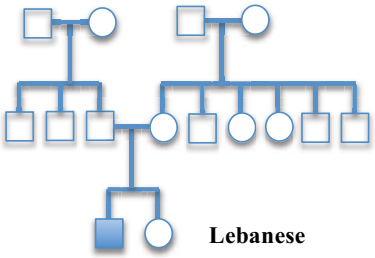

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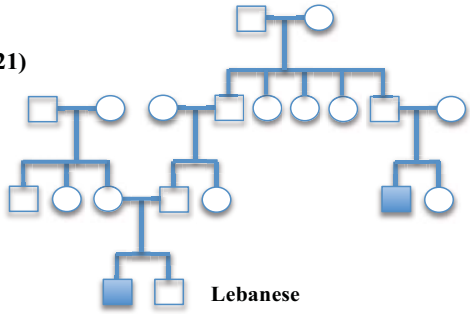

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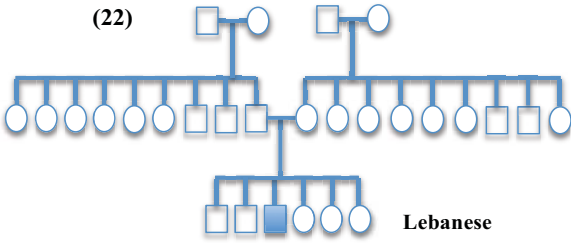

Lebanese

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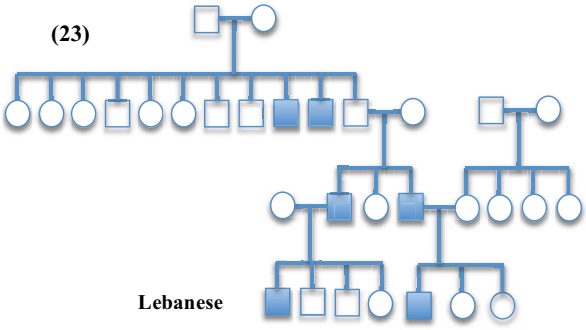

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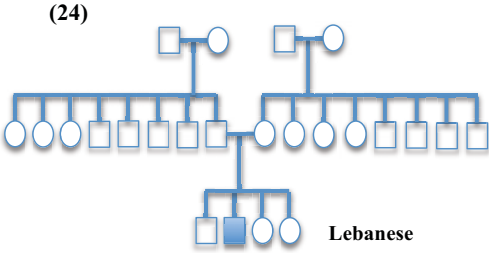

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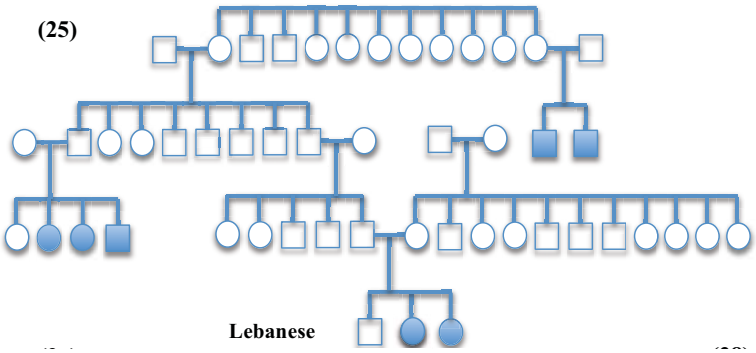

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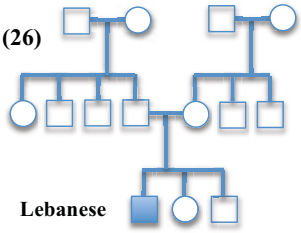

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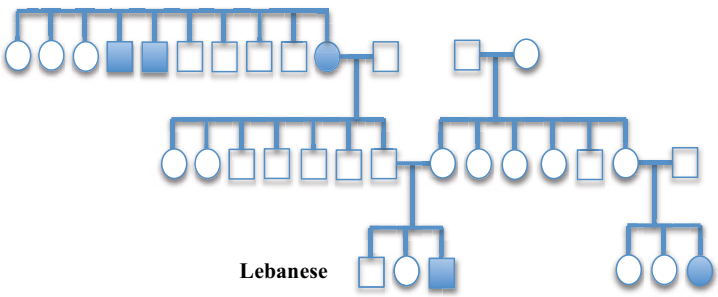

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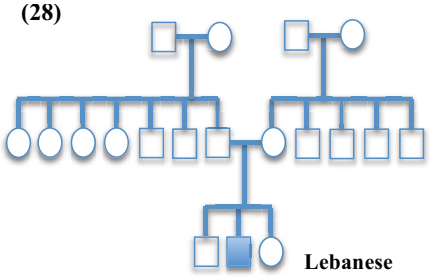

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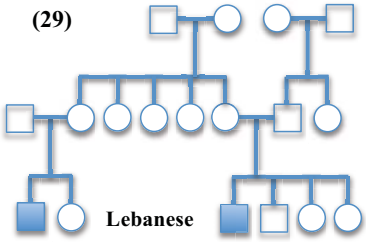

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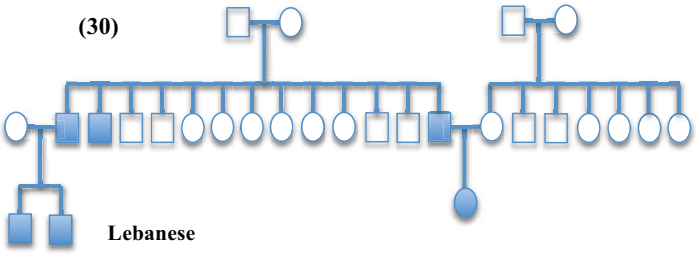

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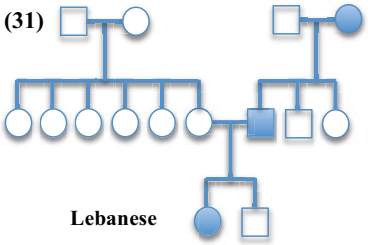

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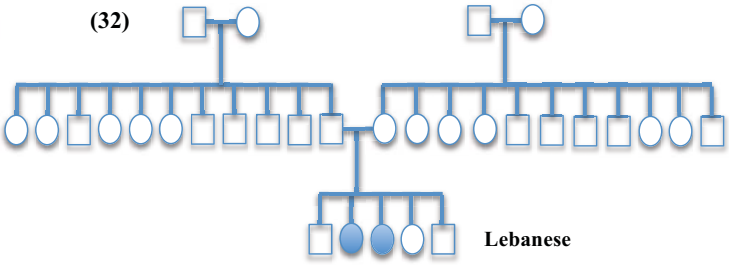

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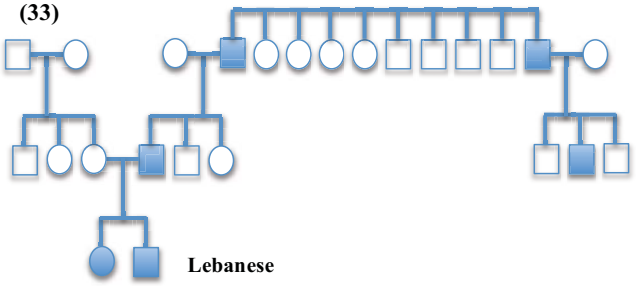

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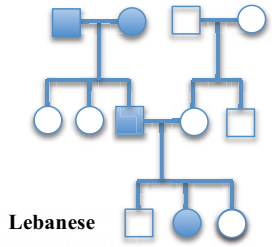

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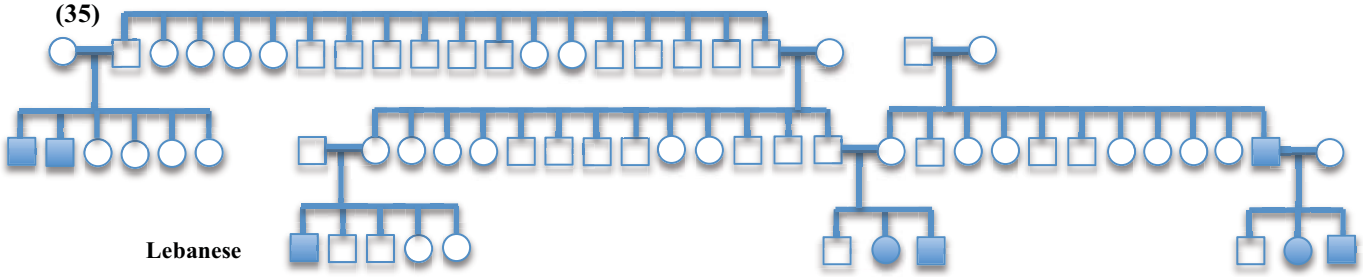

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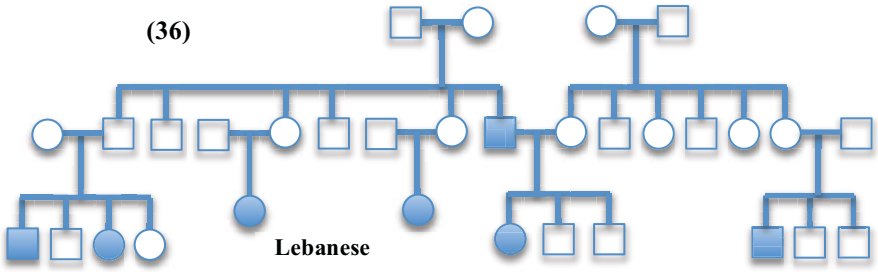

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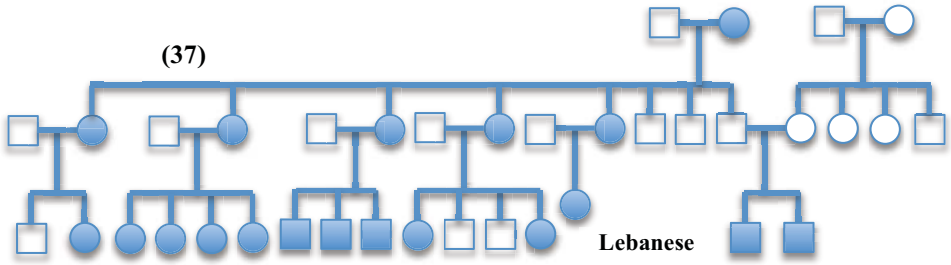

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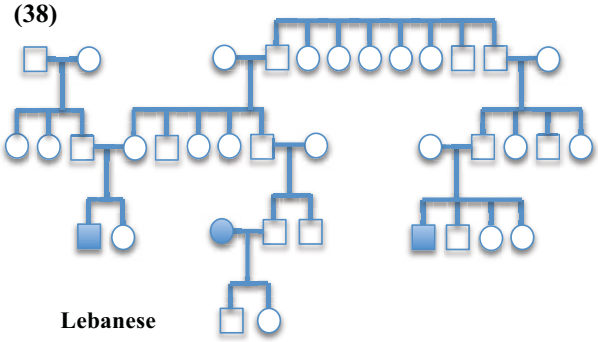

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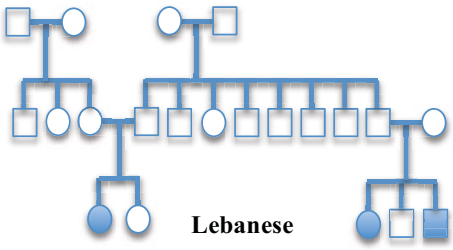

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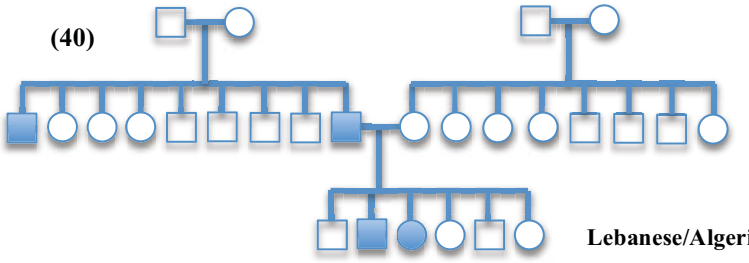

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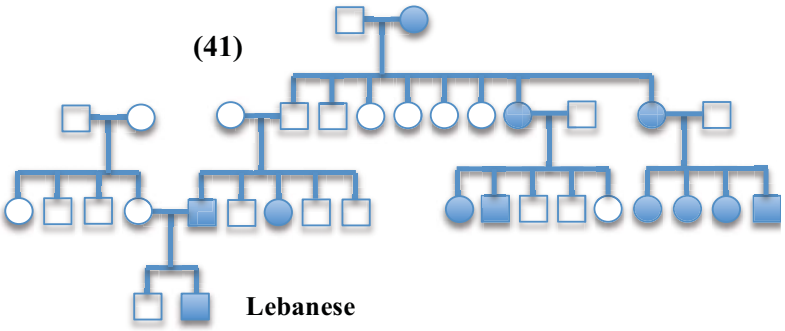

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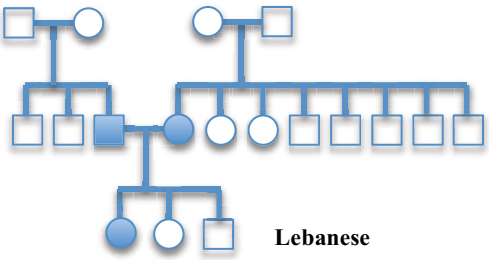

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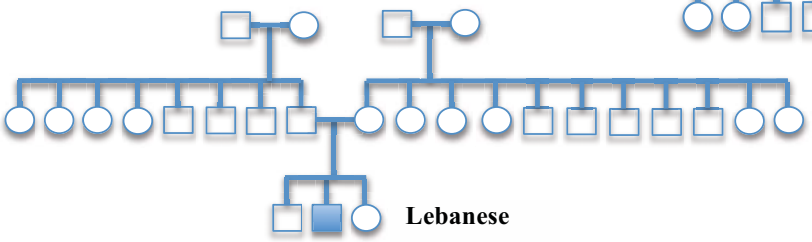

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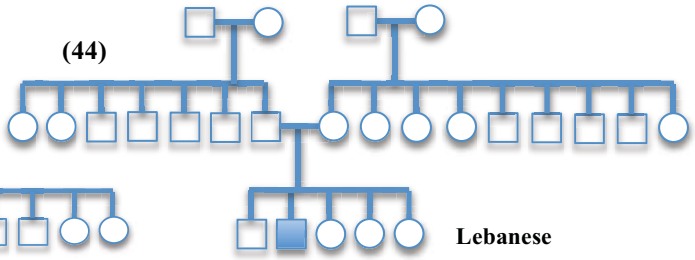

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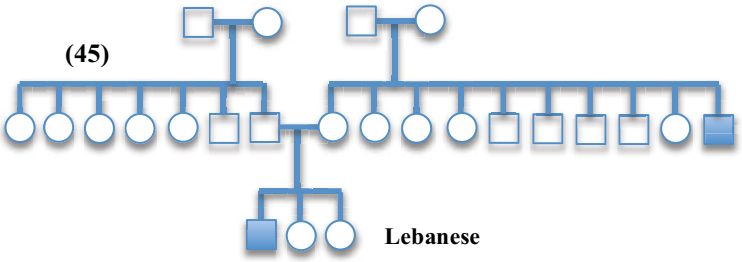

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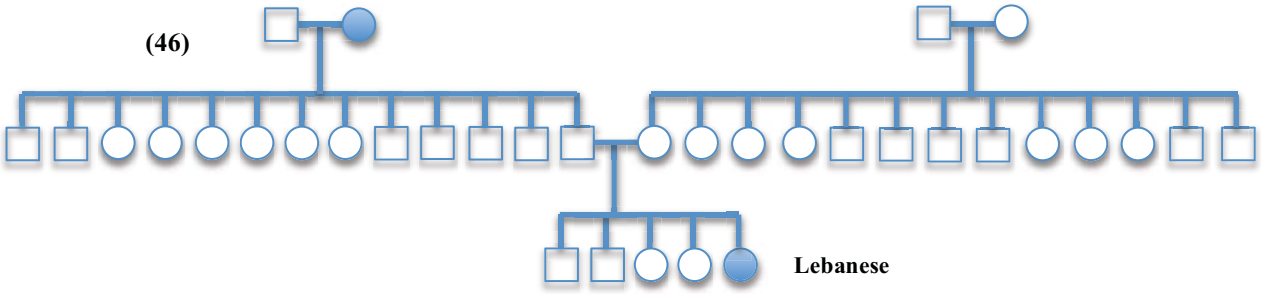

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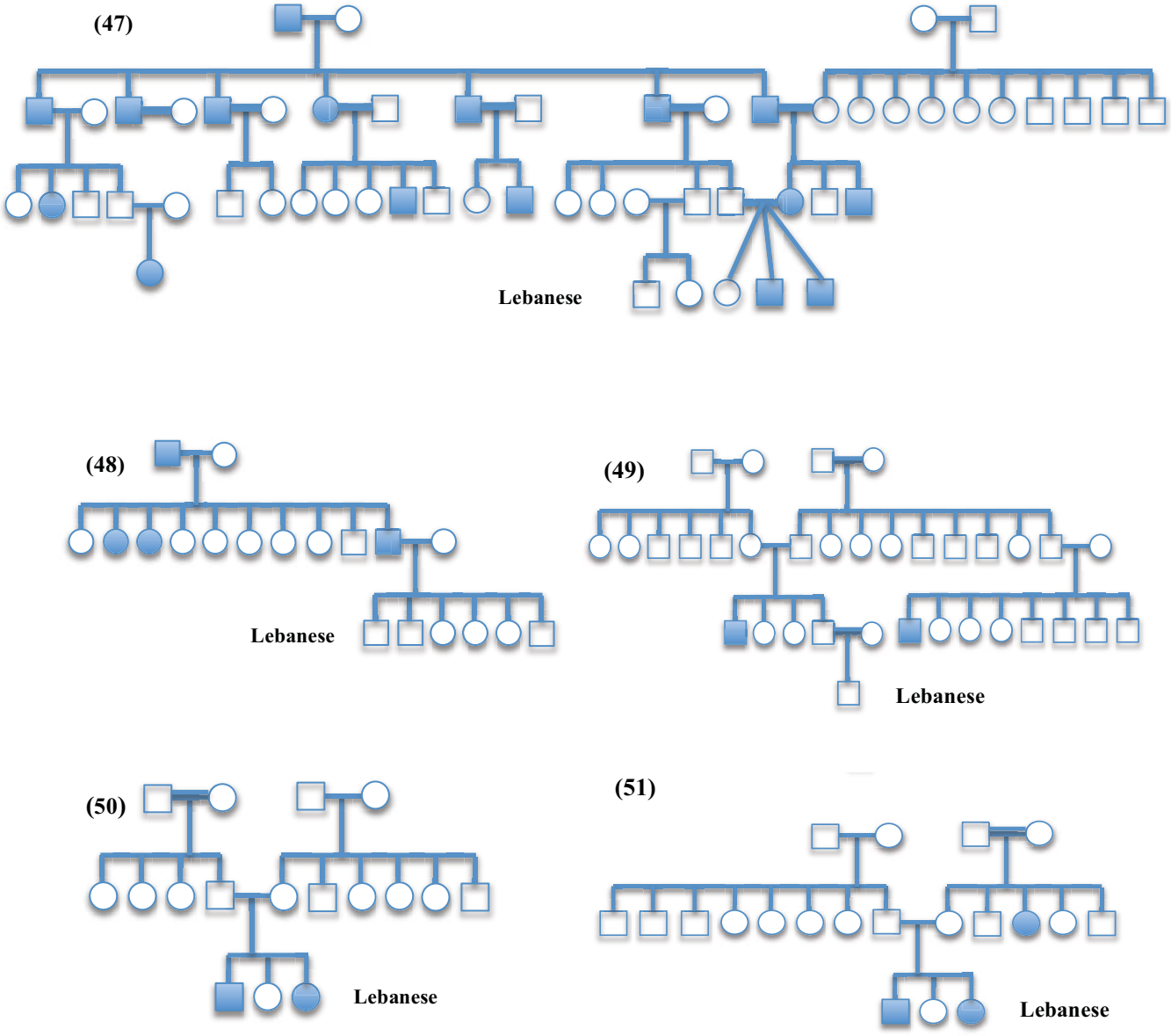

Figure III.1: Pedigrees of the 51 Mediterranean families.

The 14 families that underwent the data and biospecimen collection procedure correspond to the following pedigrees: 1, 3, 7, 8, 9, 11, 12, 13, 34, 35, 44, 47, 49, 51 => 12 Lebanese and 2 Lebanese/Syrian.

2. Pedigrees of the 8 selected families

The 8 families that were selected for the genetic analysis are illustrated below (Figures III.2-III.9), with the subjects from whom blood was collected underlined in red and on whom a lateral cephalometric x-ray was taken underlined in green. The selected subjects are numbered using Arabic numerals. A double horizontal line indicates consanguinity. 7 families are Lebanese and 1 is Lebanese/Syrian.

1-

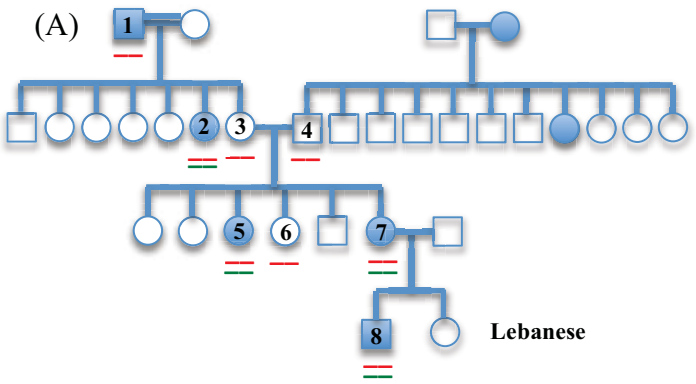

Figure III.2: Pedigree of the selected family (A).

Family's structure: pedigree comprised of 4 generations with a total of 31 individuals including 7 affected and 24 non-affected. More females (n=3) than males (n=2) exhibit the phenotype. Consanguinity is noted in the family. The mode of inheritance is autosomal dominant. Blood was collected from 8 subjects (5 affected, 3 non-affected) and a lateral cephalometric x-ray was taken on 4 affected subjects.

2-

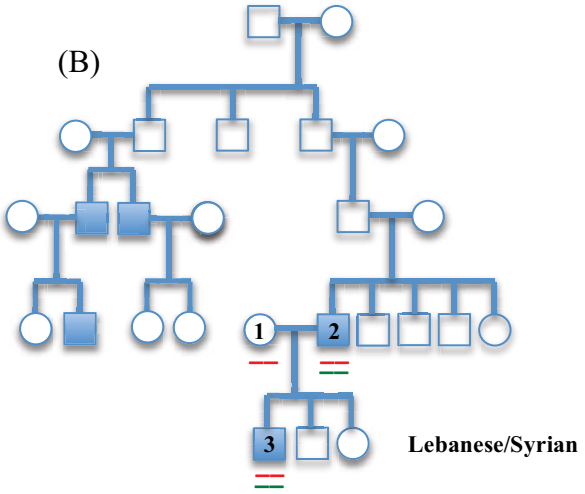

Figure III.3: Pedigree of the selected family (B).

Family's structure: pedigree comprised of 5 generations with a total of 26 individuals including 5 affected and 21 non-affected. More males (n=5) than females (n=0) exhibit the phenotype. Consanguinity is not noted in the family. The mode of inheritance is autosomal dominant. Blood was collected from 3 subjects (2 affected, 1 non-affected) and a lateral cephalometric x-ray was taken on 2 affected subjects.

3-

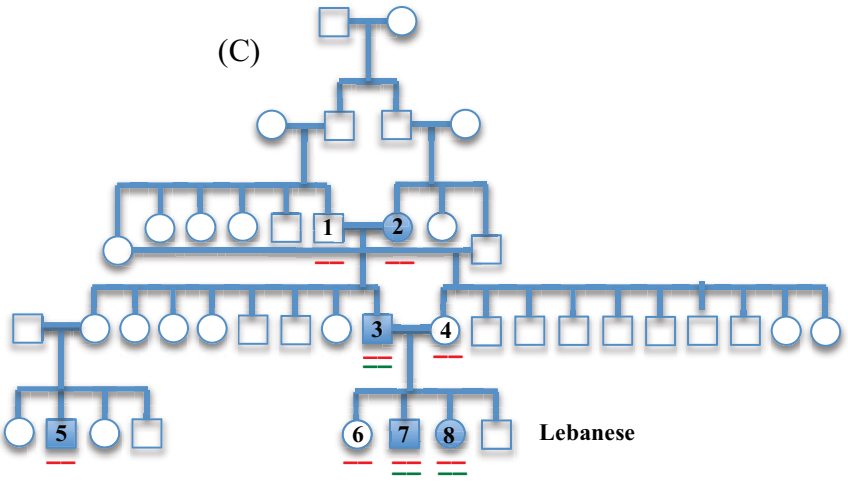

Figure III.4: Pedigree of the selected family (C).

Family's structure: pedigree comprised of 5 generations with a total of 42 individuals including 5 affected and 37 non-affected. More males (n=3) than females (n=2) exhibit the phenotype. Consanguinity is noted in the family. The mode of inheritance is autosomal dominant. Blood was collected from 8 subjects (5 affected, 3 non-affected) and a lateral cephalometric x-ray was taken on 3 affected subjects.

4-

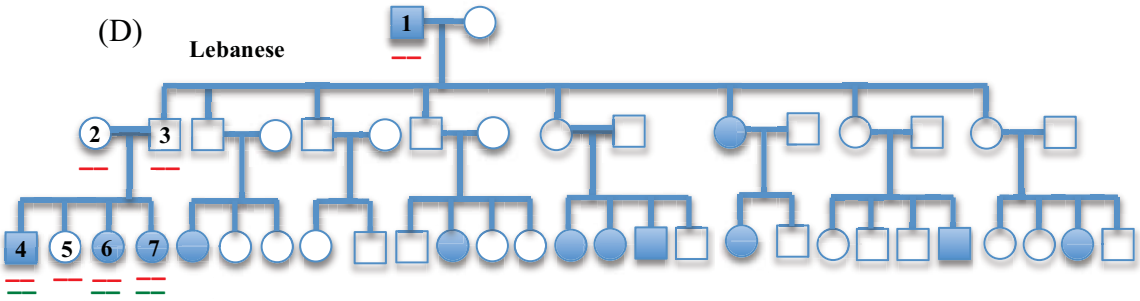

Figure III.5: Pedigree of the selected family (D).

Family's structure: pedigree comprised of 3 generations with a total of 45 individuals including 13 affected and 32 non-affected. More females (n=9) than males (n=4) exhibit the phenotype. Consanguinity is noted in the family. The mode of inheritance is autosomal dominant. Blood was collected from 7 subjects (4 affected, 3 non-affected) and a lateral cephalometric x-ray was taken on 3 affected subjects. However, DNA sequencing and analysis was not performed on subject #4 because of poor blood quality.

5-

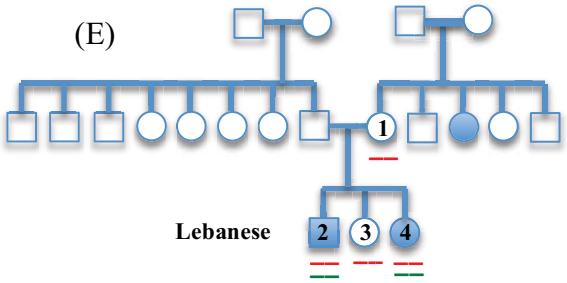

Figure III.6: Pedigree of the selected family (E).

Family's structure: pedigree comprised of 3 generations with a total of 20 individuals including 3 affected and 17 non-affected. More females (n=2) than males (n=1) exhibit the phenotype. Consanguinity is noted in the family. The mode of inheritance is recessive. Blood was collected from 4 subjects (2 affected, 2 non-affected) and a lateral cephalometric x-ray was taken on 2 affected subjects.

6-

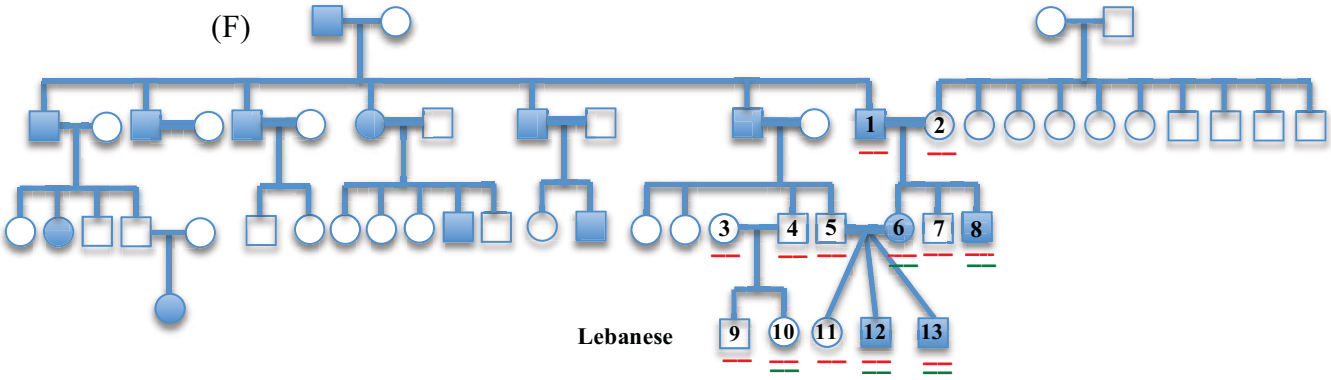

Figure III.7: Pedigree of the selected family (F).

Family's structure: pedigree comprised of 4 generations with a total of 55 individuals including 16 affected and 38 non-affected. More males (n=12) than females (n=4) exhibit the phenotype. Consanguinity is noted in the family. The mode of

inheritance is autosomal dominant. Blood was collected from 13 subjects (5 affected, 8 non-affected) and a lateral cephalometric x-ray was taken on 4 affected subjects. DNA sequencing and analysis was performed on all subjects except F9 because of poor blood quality.

7-

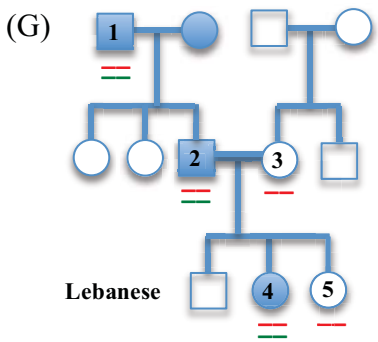

Figure III.8: Pedigree of the selected family (G).

Family's structure: pedigree comprised of 3 generations with a total of 12 individuals including 4 affected and 8 non-affected. Equal number of males and females exhibit the phenotype (n=2). Consanguinity is noted in the family. The mode of inheritance is autosomal dominant. Blood was collected from 5 subjects (3 affected, 2 non-affected) and a lateral cephalometric x-ray was taken on 3 affected subjects.

8-

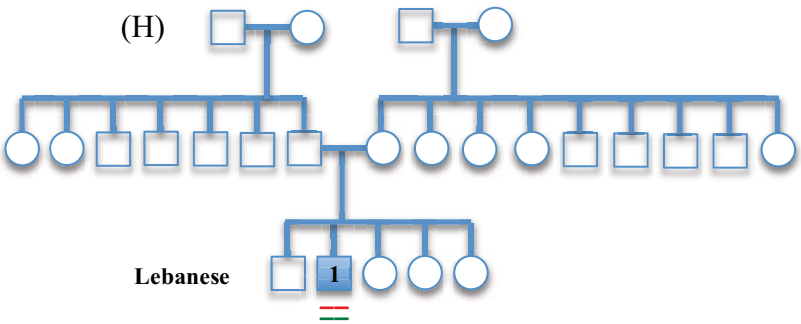

Figure III.9: Pedigree of the selected family (H).

Family's structure: pedigree comprised of 3 generations with a total of 25 individuals including 1 affected and 24 non-affected. The phenotype is expressed only in 1 male individual. Consanguinity is not noted in the family. The mode of inheritance is recessive. Blood and lateral cephalometric x-ray were only performed on the 1 affected subject. This individual was selected to undergo the genetic analysis because of the severe phenotype that he expresses, despite that fact that he is the only affected individual in his family.

In summary, 5cc of blood was withdrawn from 49 subjects (27 affected, 22 non-affected) and a lateral cephalometric x-ray was taken on 22 affected subjects. Out of the 49 samples collected, the genetic analysis was performed on 47 samples (26 affected, 21 non-affected) because of poor blood quality of 2 samples. It should be noted that consanguinity and an autosomal mode of inheritance are present in 6 out of the 8 families and a recessive inheritance pattern is present in 2 out of the 8 families.

D. Clinical examination and cephalometric analysis

1. Description of the means of diagnosis

The selected subjects who are part of the 14 families were diagnosed by means of a clinical examination and a lateral cephalometric radiograph (done only on subjects on whom features of a Class III malocclusion were noted clinically). Extra oral pictures were also taken on the selected subjects: face with and without a smile, right and left profiles with and without a smile. Pre-treatment pictures of patients who did or are under orthodontic treatment in our division, were gathered from the departmental database where pictures of all patients are stored. The lateral cephalometric radiograph 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. Subjects already under treatment would have had the series of records taken before initiation of therapy. The pre-treatment lateral cephalogram that is housed in the departmental radiologic software (CLINIVIEW) was used in this study.

The lateral cephalograms were digitized and analyzed by one investigator (PG) 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.

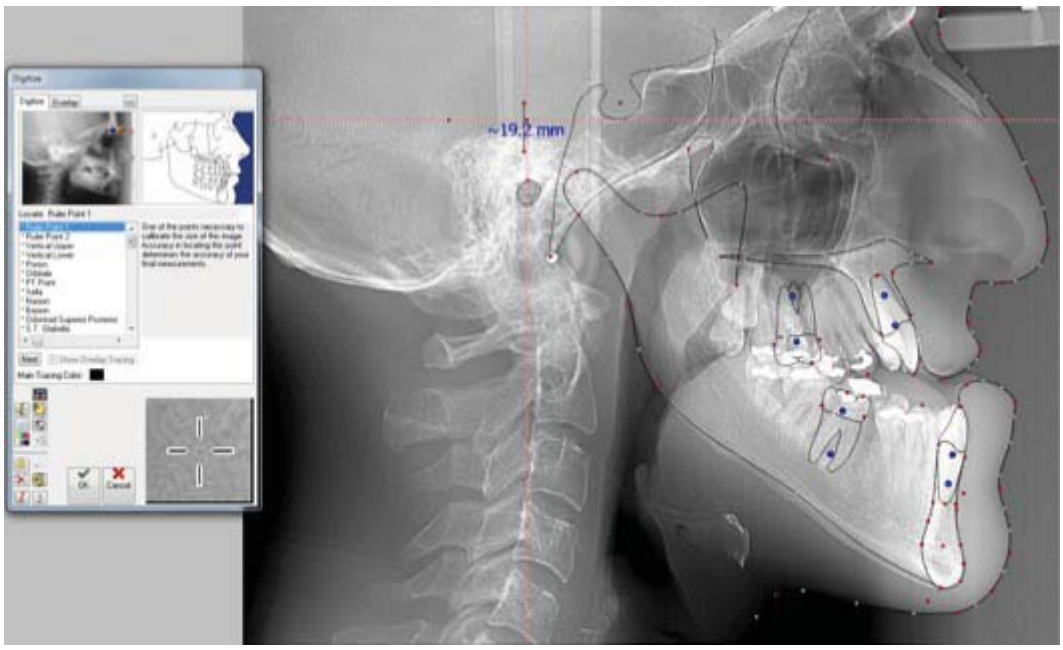

Figure III.10. Digitized lateral cephalogram with soft and hard tissue landmarks.

2. Cephalometric landmarks

All landmarks used for the digitization are shown in Figure.III.11, and the corresponding definitions are displayed in Tables III.5, 6 and 7.

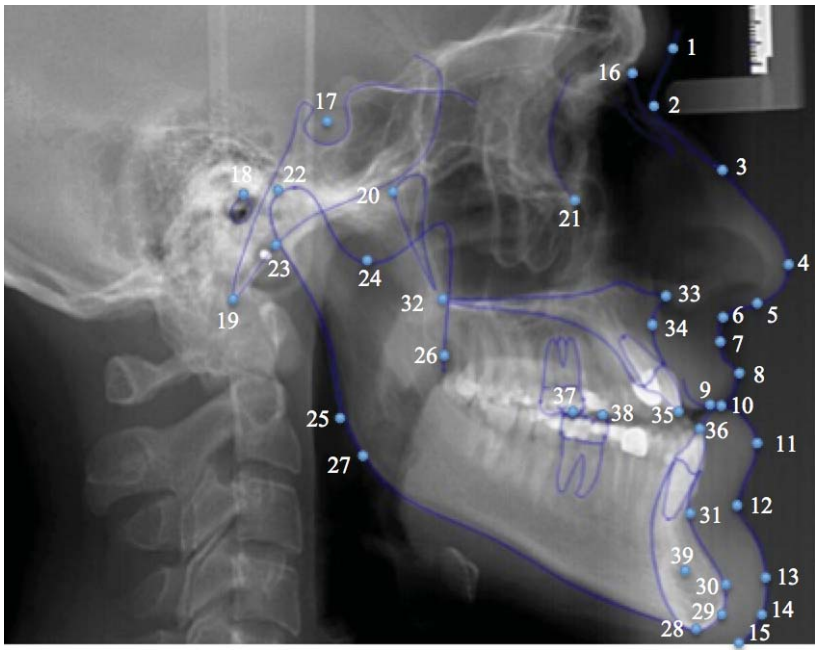

Figure III.11: Lateral cephalometric tracing showing the used anatomic landmarks.

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

Angular and linear measurements were done in order to: 1. Gauge the characteristics of the cranial base and each jaw, as well as the relationships of the jaws

to the cranial base and to each other and 2. Select/confirm the patients affected by MP.

The cephalometric measurements included the following:

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.12)

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	

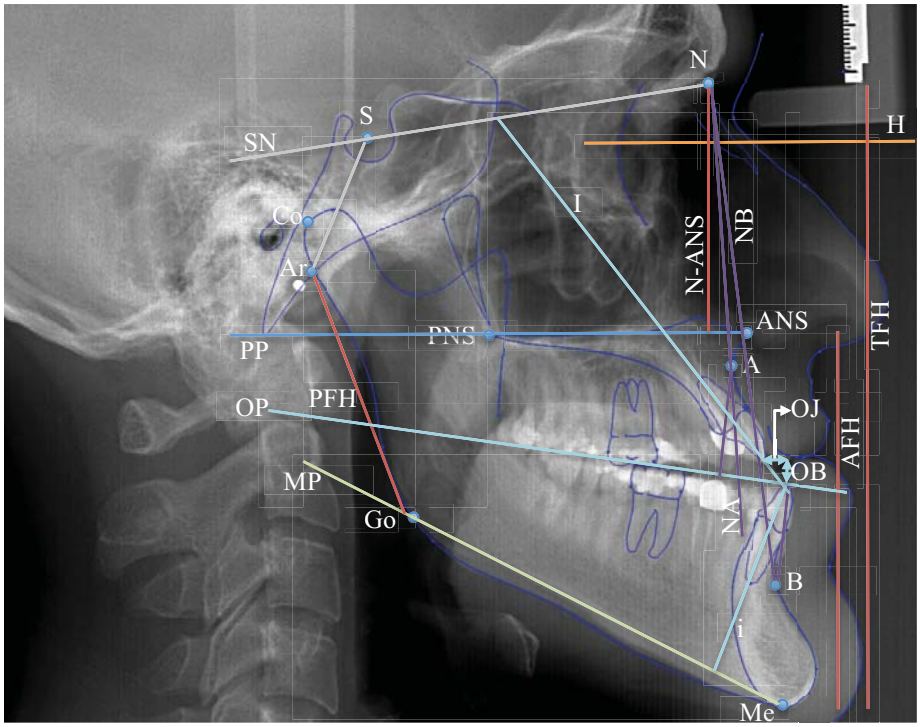

Figure III.12: Lateral cephalometric tracing with landmarks and angles describing the cranial base, maxilla, facial heights, vertical and sagittal relationships between the jaws and dento-alveolar relationships.

b) Measurements at the level of the mandible (Table III.9, Fig. III.13)

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

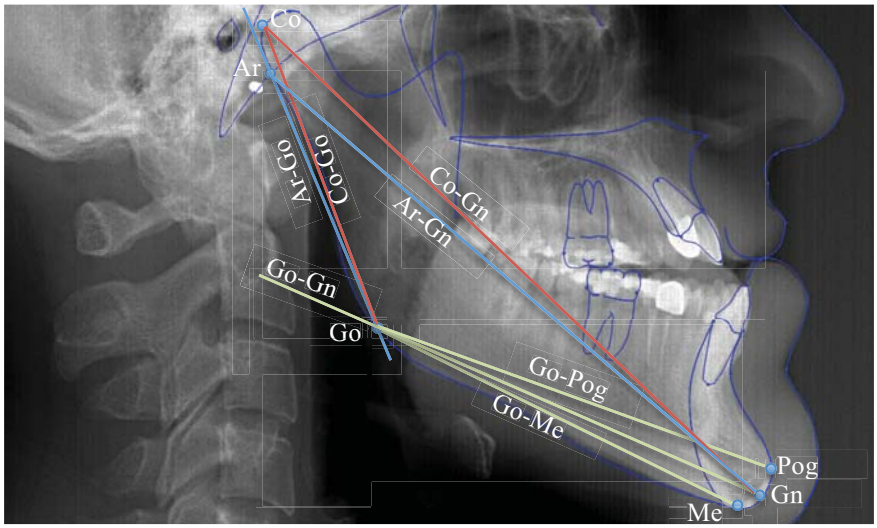

Figure III.13: Lateral cephalometric tracing with mandibular landmarks and angles.

c) Measurements at the level of the symphysis (Table III.10,11; Fig. III.14,15)

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

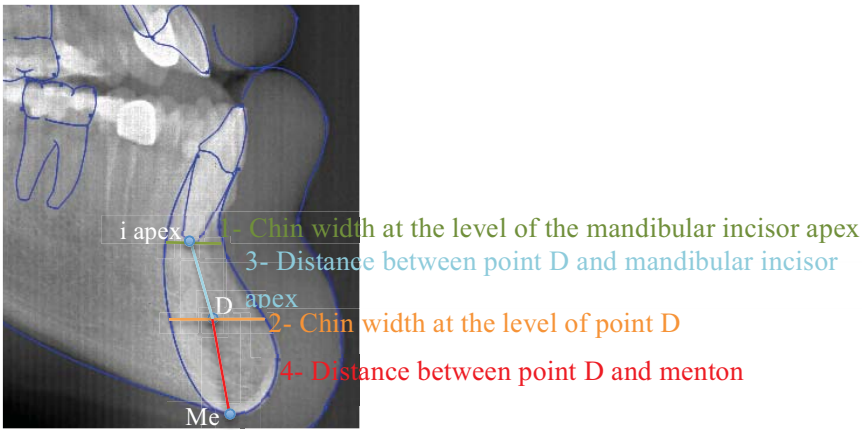

Figure III.14: Cephalometric tracing describing the relationship between point D, mandibular incisor and menton.

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

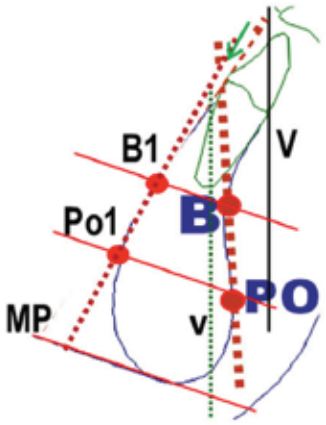

Figure III.15: Chin drawing from cephalometric radiograph indicating the component analysis of the symphysis (Ghafari & Macari, 2014).

As mentioned in the inclusion criteria, all patients having an ANB angle of ≤ 0 degrees (Cruz et al., 2008) and a negative Wits appraisal ≥ −2.0 mm (Alexander et al., 2009) along with an increased mandibular length (Co-Gn) above one standard deviation of the norm for their age, a normal maxillary length (ANS-PNS) and an anterior cross-bite were diagnosed as affected. Subsequent to the analysis of these measurements, the pedigrees drawn to elucidate the families' structure were adjusted (if needed) and the Z score was calculated for each individual, depending on the value of his 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.

The potential risks associated with the lateral cephalometric x-ray include radiation dose. However, the effective dose of a single cephalogram is 1.7 mrem, an exposure considered minor. For the patient, this radiograph is part of the diagnostic battery of records normally taken and justified because it is needed for proper diagnosis. For other subjects, the radiograph may represent an opportunity for evaluation of present or past problems in the relations between the jaws. They would be asked if they had had the same x-ray in the past year, in which case, and pending good quality of the radiograph, no additional exposure would be needed. Accordingly, the listed potential risks are considered minimal and therefore, the benefits of this study outweigh its potential risks.

4. Repeated measurements

Intra-examiner reliability of the measurements was assessed by choosing randomly, re-digitizing and analyzing 7 lateral cephalograms (20% of the total sample) 5 months after initial digitization. Spearman correlation test was performed for intra-class examiner and gave an average correlation coefficient of 0.960.

5. Statistical analysis

First, a two sample independent T test was made to assess differences at the level of the cephalometric measurements between group 1 (involving the 22 affected subjects included in the genetic analysis) and group 2 (involving the 45 affected subjects that are part of the approached families but not included in the genetic analysis). 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 8 selected families to evaluate differences between them.

E. Genetic procedure

1. Blood collection

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

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

1- Positioning of the patient in a chair.

2- Selection of a suitable site for venipuncture, by placing a tourniquet 3 to 4 inches above the selected puncture site on the patient.

3- Palpation for a vein.

4- When a vein is selected, cleansing of the area using alcoholic pad, in a circular motion, beginning at the site and working outward.

5- Allowing the area to air dry.

6- Asking the patient to make a fist, then grabbing the patient's arm using the thumb and swiftly inserting the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface.

7- Removal of the tourniquet when the last tube is filling.

8- Removal of the needle from the patient's arm using a swift backward motion.

9- Placement of gauze immediately on the puncture site, applying and holding adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, taping a fresh piece of gauze or Band-Aid to the puncture site (Figure III.16).

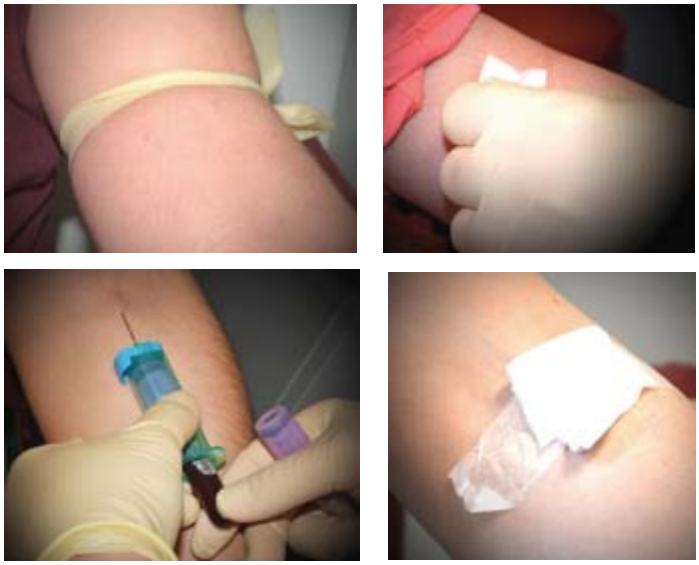

Figure III.16: Different steps of blood withdrawal.

Then, the blood samples were preserved in the refrigerator at a temperature of 4°C, awaiting DNA extraction (done 0 to 10 days maximum after blood withdrawal).

The potential risks associated with blood withdrawal include bruising, pain, hematoma, and slight possibility of infection or fainting. However, blood withdrawal

was done at the hospital (AUBMC), by a specialized nurse or physician, using a clean needle. The AUBMC Laboratory Medicine rules and regulations were followed, including any information provided by this department on a routine basis. Therefore, these potential risks are considered minimal and the benefits of this study outweigh its potential risks. In fact, these potential risks didn't occur to anyone of the participants in this study.

2. Saliva collection

In the case of one family, saliva was collected instead of blood on three individuals (children aged 5, 7 and 10 years) due to the preference of their family not to have blood withdrawn from them because of their age. In the case of another family, saliva was also collected instead of blood on one 40 years old individual because he is out of town most of the time. However, those 2 families were not part of the 8 families that were selected to undergo the Next Generation Sequencing (NGS) step. Saliva collection provides smaller amounts of DNA than blood and the DNA yield is 2-fold lower from saliva than from blood, that's why the ideal is to extract DNA from blood but saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping because of the ease of collection, the convenient storage of saliva samples, the high response rate and the high DNA quality. Therefore, saliva samples are a good alternative to blood samples in epidemiologic studies (Abraham et al., 2012; Hansen, Simonsen, Nielsen, & Hundrup, 2007; Nunes et al., 2012; Rylander-Rudqvist, Hakansson, Tybring, & Wolk, 2006).

Saliva collection was performed using the Oragene.Discover self-collection kit. It is an effective method for obtaining high-quantity and high-quality DNA. The kit

contains a cap (including a stabilizing liquid) and an empty container in which the individuals have to spit saliva (Figure III.17).

Figure III.17: Oragene.Discover self-collection kit.

The procedure of saliva collection involves the following steps:

1- Start spitting your saliva into the empty container.

2- Spit until the amount of liquid saliva (not bubbles) reaches the level shown in figure III.18.

3- Put the container on a flat surface and screw the cap onto the container. Then, make sure the cap is closed tightly. If you have difficulty closing the cap completely, turn the cap slightly counter-clockwise and then try again.

4- Tighten the cap firmly and shake gently for 10 seconds. Then, recycle outer packaging.

Most people take between 2 and 5 minutes to deliver a saliva sample following steps 1 to 4 (Figure III.18).

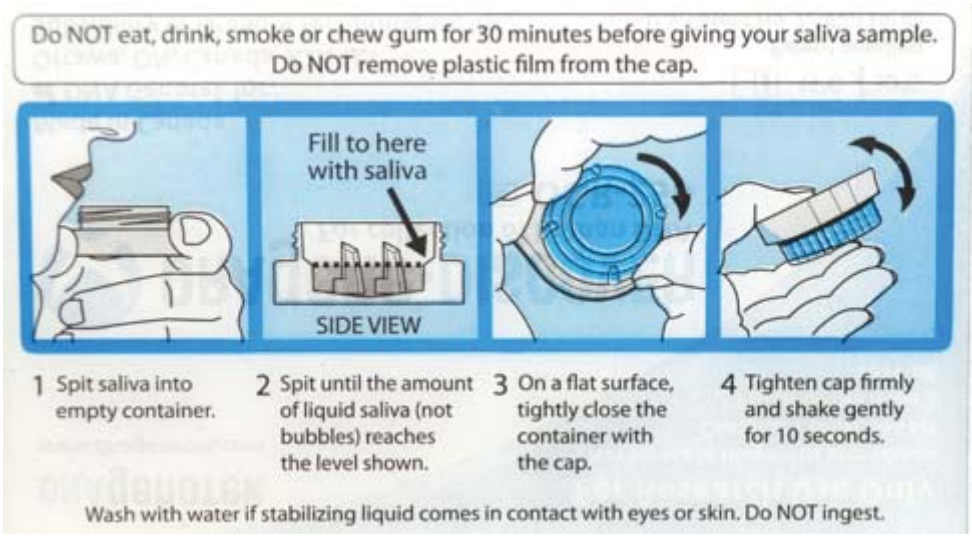

Figure III.18: Technique of saliva collection using Oragene.Discover self-collection kit.

Saliva samples were labeled according to the same coding system used for the blood samples. Then, they were stored at a temperature between 15 and 30°C, awaiting DNA extraction.

Collection precautions include the following:

- Do not eat, drink, smoke or chew gum 30 minutes before giving your saliva sample.
- Do not remove the plastic film from the cap.
- Wash with water if the stabilizing liquid comes in contact with your eyes or skin.
- Do not ingest.

3. DNA extraction

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

It includes the following steps:

1- In a 15ml falcon tube (conical centrifugation tube), put:

2ml blood,

+ 200 µl protease (5.5ml H_2O to the powder) (to denature the proteins and keep the DNA intact).

+ 2.4ml lysis buffer (breaks open cells and nuclear membranes but also exposes the DNA to proteins).

2- Mix by inversion 15 times, vortex for 1 min to mix the liquid and then incubate at 70°C for 10 min (boil the water on the heater).

3- Add 2ml of ethanol 100% to precipitate the DNA from the lysed cells.

4- Mix by inversion 10 times and vortex for 30 sec.

5- Remove half of the volume (≈ 3ml) and pour into the purifying falcon tube (with membrane).

6- Centrifuge at 3600 for 10 min at 15°C to separate the DNA from the reagents and proteins during the cell lysis step.

7- Decant the supernatant and add the remaining volume of the lysed blood + 3.6ml in the purifying tube.

8- Centrifuge at 3600 for 10 min at 15°C.

9- Add 2ml of washing buffer 1 to clean the reagent then centrifuge at 3600 for 10 min.

10- Add 2ml of washing buffer 2 also to clean the reagent, then centrifuge at 3600 for 10 min. At this stage, the DNA moved to the bottom of the tube.

11- Air-dry for 7 min to evaporate the ethanol.

12- Transfer to a new falcon tube, add 150µl of dilution buffer and incubate for 5 min at room temperature.

13- Centrifuge for 10 min at 3600. Repeat the elution step if the quantity is small.

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

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

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

The process of DNA extraction from saliva samples is identical to the one used for blood samples, except that saliva is introduced in the falcon tube instead of blood.

4. Genetic analysis

DNA was first quantified and assessed for quality using the Nanodrop at the American University of Beirut Molecular Core Facility. Then, the experimental genetic procedure was done in four main steps: captured library preparation, cluster generation, sequencing and data analysis (Figure III.19). For this purpose, the samples were sent to

Macrogen Laboratory in South Korea (dna.macrogen.com) since we don't have yet at AUB a high throughput sequencer (HiSeq).

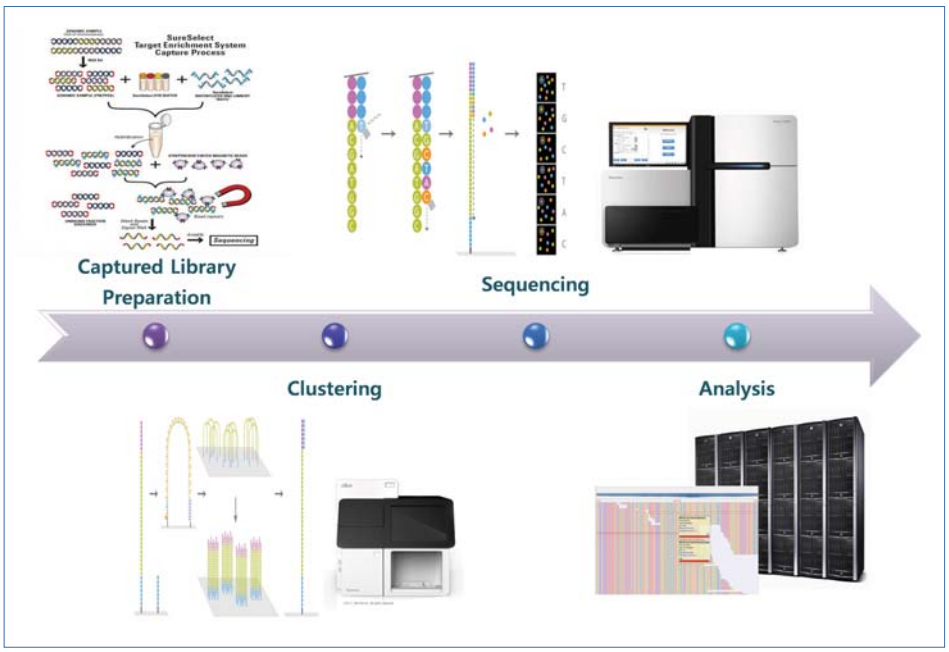

Figure III.19: Four main steps of the genetic experimental procedure following DNA extraction *(Adapted from source: http://dna.macrogen.com. Accessed: March 30, 2017).*

a. Captured library preparation:

After DNA extraction, a random fragmentation of the DNA was performed, followed by a library hybridization during which the DNA fragments of a few hundred base pairs were added to the target enrichment capture kit Agilent SureSelect V6-Post. The SureSelect Target Enrichment workflow is a solution-based system utilizing ultra-long -120 mer biotinylated cRNA baits- to capture regions of interest, enriching them out of a NGS genomic fragment library. The kit fragment includes primers at both ends (formed of 20 intron nucleotides) that are the same for all fragments, in addition to a unique primer (bar code) for each individual. The DNA fragments that include only

exons had their both ends ligated to the kit fragment and the other DNA fragments were eliminated (Figure III.20). Then, the libraries were amplified by emulsion PCR during which DNA is amplified on micro beads. After PCR amplification, for each one of the annealed fragments, the number of copies read (read depth) was indicated. To be reliable, the read depth should be >40-50 for the preliminary analysis and then >20 if a result was not found during the preliminary analysis.

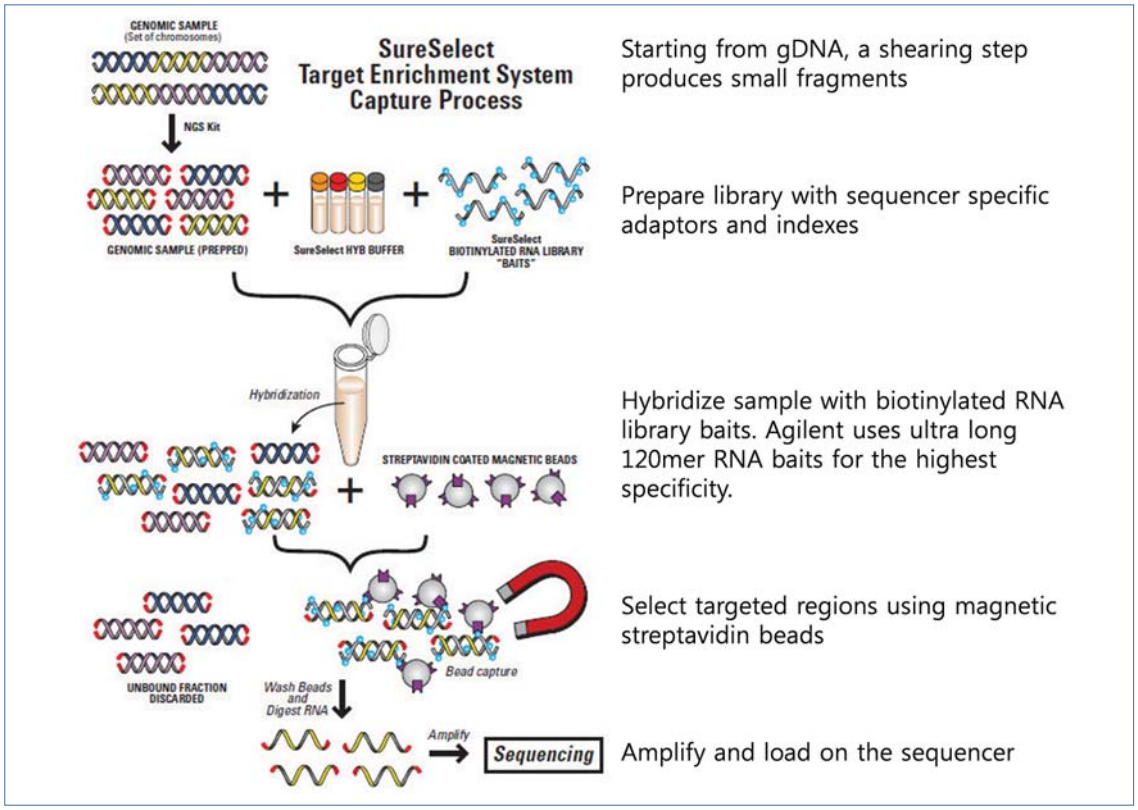

Figure III.20: Detailed procedure of the captured library preparation step
(Adapted from source: http://dna.macrogen.com. Accessed: March 30, 2017).

b. Cluster generation:

The goal is to detect groups of genes that exhibit similar expression patterns by subdividing the genes in such a way that similar ones fall into the same sub-class

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

c. Next Generation sequencing (NGS):

The libraries underwent Whole Exome Sequencing (WES) on a HiSeq2000/2500 Illumina platform to determine the exact sequence of nucleotides (adenine, guanine, cytosine and thymine). A flow cell containing millions of unique clusters is loaded into the HiSeq 2000/2500 for automated cycles of extension and imaging (Figure III.21). Sequencing-by-Synthesis is through the use of polymerase-catalyzed addition of four proprietary fluorescently labeled nucleotides with “reversible terminators”. Each sequencing cycle occurs in the presence of all four nucleotides leading to higher accuracy than methods where only one nucleotide is present in the reaction mix at a time. This cycle is repeated, one base at a time, generating a series of images each representing a single base extension at a specific cluster. Hundreds of thousands to hundreds of millions of sequencing reactions occur simultaneously, which refers to the term “massive parallel sequencing”.

Figure III.21: Hiseq 2000/2500 Illumina sequencer *(Adapted from source: http://dna.macrogen.com. Accessed: March 30, 2017).*

d. Data analysis:

Several softwares (GWA, Picard, GATK and Snpeff) were used to map and analyze the sequencing data with a primary focus on variant discovery and genotyping as well as strong emphasis on data quality assurance, while using the normal databases as a reference. 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. Single nucleotides and deletion/insertion variants were generated using the GATK software, and during the 1st stringent filtering, only the passed variants having a high putative impact (disruptive mutation: frameshift or stop codon) and a coverage read >40-50 were analyzed; if no result was found, an analysis was performed on the variants with a read depth >20. In family E, which has a recessive inheritance pattern, the selected variants were also homozygous. In addition, the synonymous mutations that don't affect protein function and the intronic regions that annealed to the primers of the kit were removed. Therefore, the number of the

remaining variants was reduced from 100000 to 550-600 on average in all families and 300 on average in family E.

During the 2nd filtering, a comparison was done between individuals of the same family in an attempt to find common gene(s) between the affected ones that is(are) not noted in the genetic material of the non-affected ones. In other words, we aim to find a mutation that segregates with the phenotype.

Then, the analysis was repeated by selecting only the passed variants having a moderate putative impact and a MAF ≤ 0.001 in the normal population and a comparison was also done between individuals of the same family in order to find common gene(s) between the affected ones.

In families B and G where a large number of common genes was noted, 2 subfilterings were performed to reduce the number of genes. The 1st subfiltering consisted of keeping only the genes that have a MAF equal to 0. The 2nd subfiltering is a complimentary biochemical interface done to prove that the results obtained by sequencing affect protein function. Assessment of the effects of the filtered variants on protein function was done in silico using both the Polyphen2 and SIFT softwares. During this 2nd subfiltering, which is applicable only on the variants having a moderate putative impact, the genes having the Polyphen and SIFT predictions as "Damaging" were kept and the others were filtered out.

In family H, which includes 1 affected individual, only the passed variants having a high putative impact and a Minor Allele Frequency (MAF) ≤ 0.01 were kept. Then, the genes having several mutations were filtered out and the potential genes were highlighted.

After the several steps of filtering, we ended up with a reduced number of possible candidate genes, so depending on their function, some of them were filtered out and the other(s) were considered as candidate gene(s) for MP. 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 MP.

Then, a search was done to check if the potential candidate gene(s) is(are) present in individuals that are part of the 7 other selected families and the candidate gene(s) found in this study were compared to those discovered by previous studies published in the literature to assess if common genes are present.

It should be noted also that this is a straightforward genetic approach to identify gene(s) implicated in the condition. No statistical power analysis is needed because the analysis is not a linkage analysis, which requires SNP genotyping across the genome, but rather a genotype-phenotype linkage based on the results of the WES. The latter takes into account all the variables (e.g. level of 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

Most of the pedigrees drawn to represent the structure of the 51 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 reported affected males and females (n=2).
- More families with male predominance and with at least 3 affected males.
- More families with no affected females.
- Equally split affected individuals in the 1st generation: 10 males and 10 females.
- 20 families having affected siblings in the youngest generation; 4 of them were selected for the genetic analysis and consanguinity is noted in those 4 families.

Table IV.1: Number and average of generations, affected males and females in the 51 Middle Eastern families.

	Generations	**Affected females**	**Affected males**
Total number in 51 families	165	110	119
Average	3.59	2.39	2.59

Table IV.2: Pedigree analysis of the 51 Middle Eastern families.

	N	**%**		**N**	**%**
Families with more affected females	19	39.2	**Families with more affected males**	29	56.9
Families with at least 3 affected females	12	63.2	**Families with at least 3 affected males**	19	65.5
Families with no affected females	15	29.4	**Families with no affected males**	6	11.8
Families with affected males in the 1st generation	10	19.6	**Families with affected females in the 1st generation**	10	19.6
Families with affected siblings in the youngest generation	20	39.2	**Selected families with affected siblings in the youngest generation**	4	20

B. Cephalometric analysis

1. Measurements related to the 51 approached families

When statistical analysis was performed on measurements related to the 69 individuals that are part of the 51 approached families, the following results were noted. Most importantly, mandibular length is represented by the average Co-Gn, which is 115.95 and the average Co-Pog that is equal to 113.06. Maxillary length is denoted by the average ANS-PNS that is equal to 49.90. Facial divergence is represented by the average MP/SN, which is equal to 37.05, 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). Measurements related to the sagittal relationship of the jaws show an average SNA angle of 80.56, SNB angle of 82.11, ANB angle of -1.44 and AO-BO of -8.29 denoting, on average, an orthognathic maxilla, a tendency toward a prognathic mandible and a skeletal Cl III. Dento-alveolar measurements show proclined maxillary incisors and retroclined mandibular incisors as a dento-alveolar compensation of the Class III malocclusion. Finally, the average OJ is equal to -1.29, indicating an anterior cross bite (Table IV.3).

After excluding individuals that are part of the 8 selected families, a statistical analysis was done on measurements related to the 45 individuals that are part of the remaining approached families. Average mandibular length measurements Co-Gn and Co-Pog are 113.60 and 110.89, respectively. Average maxillary length measurement ANS-PNS and facial divergence measurement MP/SN are equal to 49.21 and 36.81, respectively. Measurements related to the sagittal maxillomandibular relation show an average SNA angle of 80.27, SNB angle of 81.64, ANB angle of -1.18 and AO-BO of -7.87. Dento-alveolar measurements denote also proclined maxillary incisors and

retroclined mandibular incisors representing a dento-alveolar compensation of the Class III malocclusion. Lastly, the average OJ is equal to -0.65 (Table IV.4). All those measurements are smaller than those displayed in Table IV.3.

Table IV.3: Measurements related to individuals that are part of the 51 approached families (n=69).

Measurement	Average	Standard deviation	Maximum	Minimum
SN	65.71	4.86	77.8	54.7
S-Ar	36.20	5.09	48.5	25.3
SN/H	11.48	4.92	21.7	-4.7
N-S-Ar	123.08	6.58	156.6	107.5
Ar-Go-Gn	129.30	7.01	146.9	109.9
Ar-Go-Me	132.29	6.95	150.2	112.6
Co-Go-Me	127.36	7.01	145.7	109
Ar-Gn	114.45	13.72	152.3	79.3
Co-Gn	115.95	13.81	152.1	81.5
Co-Pog	113.06	13.19	147.8	79.6
Co-Go	54.09	9.34	77.2	35.1
Ar-Go	42.67	7.79	61.8	27.6
Go-Me	76.15	10.77	98.3	55.1
Go-Gn	81.52	9.82	105.3	54.8
Go-Pog	76.31	8.71	95.5	52.9
Chin width at apex of i	7.77	2.02	12	2.9
Chin width at level of D	12.58	2.04	17.4	7.4
D- i apex	9.34	2.41	14.5	4.6
D-Me	10.72	2.06	15.6	6.8
Ant slope of the chin/ V	10.15	8.82	31.2	-9.5
Post slope of the chin/ V	-19.77	8.79	2.1	-38.3
Angle Ant/ Post slopes	31.88	9.52	58.1	9.8
ANS-PNS	49.90	4.94	59.4	37.6
N-ANS	50.76	6.07	64.4	33.9
ANS-Me (AFH)	65.45	8.44	83.1	44.5
PFH	45.26	7.82	63.5	29.3
LFH/TFH	56.16	2.09	61.9	51.8
AFH/PFH	143.47	24.31	188.4	1.43
MP/SN	37.05	7.43	54.5	16.4
MP/H	30.05	7.43	47.5	9.4
PP/MP	28.47	6.18	42.7	12.1
PP/H	-2.88	4.48	11.6	-11.6
SNA	80.56	4.12	91.2	70.4
SNB	82.11	4.55	94.5	71.8
ANB	-1.44	2.93	3.9	-9.5
AOBO	-8.29	6.01	1.9	-32.8
I/NA	27.70	8.00	46.8	5.3
I-NA	4.57	2.89	10.5	-3.5
I/SN	108.47	9.22	128.4	87.9
I/PP	117.45	8.49	139.3	99.2
i/NB	22.36	6.65	36	6.1
i-NB	4.029	2.13	9.2	-0.9
i/MP	82.98	8.33	98.4	59.5
I/i	130.95	11.91	158.6	109.9
OJ	-1.29	3.34	4.9	-12.3
OB	0.13	2.93	5.5	-10

Table IV.4: Measurements related to individuals that are part of all the approached families, except the 8 selected families (n=45).

Measurement	Average	Standard deviation	Maximum	Minimum
SN	65.48	4.95	74.5	54.7
S-Ar	35.92	5.41	48.5	25.3
SN/H	11.43	4.79	21.7	-4.7
N-S-Ar	122.78	5.27	136.4	107.5
Ar-Go-Gn	129.44	7.290	146.6	109.9
Ar-Go-Me	132.26	7.28	150.2	112.6
Co-Go-Me	127.61	7.05	143.4	109
Ar-Gn	111.75	13.12	142.5	79.3
Co-Gn	113.60	13.51	142.8	81.5
Co-Pog	110.89	12.83	140.9	79.6
Co-Go	52.18	8.95	74.4	35.1
Ar-Go	41.13	7.56	61.3	27.6
Go-Me	75.20	11.20	95.4	55.1
Go-Gn	80.16	9.87	99.8	54.8
Go-Pog	75.34	8.94	92.5	52.9
Chin width at apex of i	7.83	1.87	12	4.6
Chin width at level of D	12.43	2.07	17.4	7.4
D- i apex	8.82	2.26	13.2	4.6
D-Me	10.27	1.90	14.4	6.8
Ant slope of the chin/ V	9.31	9.63	31.2	-9.5
Post slope of the chin/ V	-20.05	8.50	2.1	-38.3
Angle Ant/ Post slopes	30.99	9.95	58.1	9.8
ANS-PNS	49.21	5.06	58.7	37.6
N-ANS	49.29	5.76	57.7	33.9
ANS-Me (AFH)	64.44	7.67	79.7	44.5
PFH	43.99	7.38	62.5	29.3
LFH/TFH	56.53	1.81	61.9	52.8
AFH/PFH	147.51	16.50	188.4	115.8
MP/SN	36.81	7.32	54.5	16.4
MP/H	29.81	7.32	47.5	9.4
PP/MP	28.99	5.92	42.7	16.3
PP/H	-3.62	4.14	7.4	-11.6
SNA	80.27	4.17	91.2	71.7
SNB	81.64	4.68	94.5	71.8
ANB	-1.18	2.93	3.9	-9.2
AOBO	-7.87	5.61	1.9	-31.6
I/NA	27.31	7.51	43	9.1
I-NA	4.82	2.65	10.5	0.1
I/SN	107.86	8.81	128	90.3
I/PP	116.12	7.60	133.3	100.4
i/NB	21.30	6.86	36	6.1
i-NB	3.94	2.29	9.2	-0.9
i/MP	82.94	8.60	98.4	59.5
I/i	132.00	11.87	158.6	112.7
OJ	-0.65	3.09	4.9	-8.1
OB	0.38	2.82	5.5	-10

2. Measurements related to the 8 selected families

Mandibular length (Co-Gn) value of the initial affected patient of each selected family confirms the presence of a mandibular macrognathism. The difference between the patients' values and the norm ranges between 6.9 and 31.7, indicating an increased mandibular length that is more than 1SD above the norm. The average Co-Gn value of the 8 initial affected patients is 127.01, which is greater than the norms by more than 1SD (Table IV.5).

Table IV.5: Mandibular length analysis of the initial affected patient of each selected family.

Initial affected patient	Age	Co-Gn value	Average of 8 initial affected patients	Difference: Co-Gn value - average affected	Co-Gn Norm (per age)	Difference: Co-Gn value-norm
Family A	19	121.2	127.01	-5.81	109.9	11.3
Family B	24.4	152.1		25.09	120.4	31.7
Family C	17.5	130.7		3.69	117.6	13.1
Family D	29.1	116.8		-10.21	109.9	6.9
Family E	26	119.3		-7.71	109.9	9.4
Family F	21.7	135.5		8.49	120.4	15.1
Family G	7.7	103.2		-23.81	92.4	10.8
Family H	22.6	137.3		10.29	120.4	16.9

Statistical analysis performed on measurements related to the 22 individuals that are part of the 8 selected families confirms the affection status and presence of characteristics of MP in those subjects. Specifically, an average Co-Gn of 120.65 and Co-Pog of 117.41 indicate a long mandibular length (mandibular macrognathism). An average ANS-PNS of 51.28 designates a normal maxillary length and an average MP/SN of 37.54 denotes a tendency to a hyperdivergent facial pattern with an upward inclination of SN relative to the horizontal (N is higher than S). An average SNA angle of 81.10, SNB angle of 83.05, ANB angle of -1.96 and AO-BO of -9.13 refer to a skeletal Class III malocclusion underlined by an orthognathic maxilla and prognathic mandible. Dento-alveolar measurements indicate also proclined maxillary incisors and

retroclined mandibular incisors as a dento-alveolar compensation of the Class III malocclusion. Finally, an average OJ of -2.64 suggests an anterior crossbite (Table IV.6). All those measurements are greater than those displayed in Tables IV.3 and IV.4.

Table IV.6: Measurements related to individuals that are part of the 8 selected families (n=22).

Measurement	Average	Standard deviation	Maximum	Minimum
SN	66.16	4.81	77.8	59.6
S-Ar	36.73	4.49	45.1	28.9
SN/H	11.59	5.39	18.6	-0.7
N-S-Ar	123.68	8.80	156.6	113.4
Ar-Go-Gn	129.03	6.74	146.9	118.9
Ar-Go-Me	132.34	6.56	149.7	121.2
Co-Go-Me	126.85	7.25	145.7	111.6
Ar-Gn	119.83	13.86	152.3	94.1
Co-Gn	120.65	13.80	152.1	93.3
Co-Pog	117.41	13.38	147.8	90.8
Co-Go	57.92	9.26	77.2	37.9
Ar-Go	45.75	7.61	61.8	28.8
Go-Me	78.04	10.06	98.3	61.5
Go-Gn	84.25	9.56	105.3	68.2
Go-Pog	78.25	8.28	95.5	64.5
Chin width at apex of i	7.64	2.38	11.7	2.9
Chin width at level of D	12.89	2.03	17.3	9.5
D- i apex	10.39	2.47	14.5	5.4
D-Me	11.62	2.08	15.6	7.3
Ant slope of the chin/ V	11.83	6.97	30.5	-1.1
Post slope of the chin/ V	-19.21	9.37	1.9	-34.7
Angle Ant/ Post slopes	33.66	8.66	47.9	10.6
ANS-PNS	51.28	4.59	59.4	42.4
N-ANS	53.68	5.77	64.4	43.3
ANS-Me (AFH)	67.46	9.91	83.1	48.3
PFH	47.57	8.44	63.5	30.8
LFH/TFH	55.44	2.43	60.4	51.8
AFH/PFH	135.38	35.02	170.7	1.43
MP/SN	37.54	7.92	54.1	20.5
MP/H	30.54	7.92	47.1	13.5
PP/MP	27.42	6.69	41.4	12.1
PP/H	-1.42	4.93	11.6	-7.6
SNA	81.10	4.07	86.8	70.4
SNB	83.05	4.18	89	72.3
ANB	-1.96	2.98	3	-9.5
AOBO	-9.13	6.96	-0.2	-32.8
I/NA	28.52	9.36	46.8	5.3
I-NA	4.03	3.38	10.1	-3.5
I/SN	109.70	10.29	128.4	87.9
I/PP	120.16	9.82	139.3	99.2
i/NB	24.42	6.00	34.9	13.7
i-NB	4.22	1.81	7.3	1.2
i/MP	83.07	8.15	97.6	64.1
I/i	128.71	12.31	153.8	109.9
OJ	-2.64	3.59	2.5	-12.3
OB	-0.37	3.23	4.4	-6.6

3. Comparison between individuals and families

A two sample independent T test and an ANOVA test compared means of the cephalometric measurements of group 1 (involving the 22 affected subjects included in the genetic analysis) and group 2 (involving the 45 affected subjects that are part of the approached families but not included in the genetic analysis). The objective was to assess differences between the 2 groups. The results of the statistical test are displayed in Table IV.7. Greater values are noted in group 1 except for the following measurements: Ar-Go-Gn, Co-Go-Me, chin width at apex of i, posterior slope of the chin/V, LFH/TFH, AFH/PFH, PP/MP, PP/H, I-NA, i/MP, I/I and OB; however the difference is not statistically significant except for PP/H. This denotes the presence of more severe skeletal and dento-alveolar MP features in group 1 that was selected for the genetic analysis. The measurements that present a statistically significant p-value ($\leq$ 0.05) are:

- Ar-Gn
- Co-Pog
- Ar-Go
- D-Me
- PP/H
- Co-Gn
- Co-Go
- D-i apex
- N-ANS
- OJ

This indicates that mainly, mandibular length in group 1 is significantly greater than in group 2 and the anterior cross bite is significantly more severe in group 1.

Table IV.7: Comparison between measurements of the subjects included and excluded from the genetic analysis.

Measurement	Mean (group 1)	Mean (group 2)	P value
SN	65.95	65.48	0.706
S-Ar	36.94	35.88	0.405
SN/H	11.59	11.43	0.903
N-S-Ar	124.06	122.78	0.449
Ar-Go-Gn	129.21	129.44	0.897
Ar-Go-Me	132.60	132.26	0.848
Co-Go-Me	127.25	127.61	0.843
Ar-Gn	119.95	111.75	0.016
Co-Gn	120.90	113.60	0.035
Co-Pog	117.41	110.89	0.048
Co-Go	57.95	52.18	0.013
Ar-Go	45.72	41.13	0.018
Go-Me	78.25	75.20	0.263
Go-Gn	84.30	80.16	0.092
Go-Pog	78.18	75.34	0.194
Chin width at apex of i	7.44	7.83	0.459
Chin width at level of D	12.83	12.43	0.427
D- i apex	10.83	8.82	0.0025
D-Me	11.86	10.27	0.0026
Ant slope of the chin/ V	11.58	9.31	0.318
Post slope of the chin/ V	-19.58	-20.05	0.840
Angle Ant/ Post slopes	34.40	30.99	0.167
ANS-PNS	51.40	49.21	0.078
N-ANS	53.5	49.29	0.0052
ANS-Me (AFH)	68.45	64.44	0.069
PFH	47.39	43.37	0.042
LFH/TFH	55.86	56.53	0.232
AFH/PFH	138.50	147.51	0.153
MP/SN	38.16	36.81	0.499
MP/H	31.16	29.81	0.499
PP/MP	27.99	28.99	0.534
PP/H	-1.367	-3.62	0.044
SNA	80.95	80.46	0.642
SNB	82.53	81.64	0.462
ANB	-1.61	-1.18	0.571
AOBO	-8.50	-7.87	0.683
I/NA	28.54	27.31	0.560
I-NA	4.08	4.82	0.324
I/SN	109.44	107.77	0.487
I/PP	119.93	115.76	0.064
i/NB	24.37	21.81	0.147
i-NB	4.38	3.94	0.474
i/MP	82.90	82.94	0.983
I/i	128.55	132.00	0.276
OJ	-2.45	-0.69	0.039
OB	-0.35	0.35	0.350

Group 1: 22 affected subjects included in the genetic analysis.
Group 2: 45 affected subjects (part of the approached families) but not included in the genetic analysis.
P value ≤ 0.05: statistically significant.

Descriptive statistics, including means, standard deviations and p-values, was performed on cephalometric measurements related to each selected family in order to evaluate differences between the 8 families. The results are displayed in Tables IV.8.A and IV.8.B. The measurements that present a statistically significant p-value (≤ 0.05) across the 8 selected families are:

- Ar-Go-Gn
- Co-Go-Me
- D-i apex
- Angle Ant/Post slopes
- MP/H
- SNA
- AOBO
- i/MP
- Ar-Go-Me
- Chin width at apex of i
- Posterior slope of the chin/V
- MP/SN
- PP/MP
- ANB
- i/NB
- OJ

Table IV.8.A: Descriptive statistics of cephalometric measurements of the selected families A-D.

Measurement	Family A (n= 4)		Family B (n= 2)		Family C (n= 3)		Family D (n= 3)	
	μ	σ	μ	σ	μ	σ	μ	σ
SN	64.98	6.36	74.05	5.30	64.50	2.33	64.47	1.99
S-Ar	37.54	4.75	37.45	.07	41.33	4.27	31.37	3.37
SN/H	7.92	6.36	7.60	4.38	11.47	5.20	15.93	1.86
N-S-Ar	130.54	14.77	117.1	.99	119.63	1.89	128.67	5.06
Ar-Go-Gn	124.8	3.38	132.9	3.96	125.37	3.33	134.33	3.26
Ar-Go-Me	128.24	3.83	136.1	4.10	128.8	2.62	137.5	4.42
Co-Go-Me	121.28	5.84	129.6	2.55	124.3	3.24	134.57	4.48
Ar-Gn	114.3	13.26	139.75	17.75	122.63	8.36	116.33	6.65
Co-Gn	112.26	12.54	138.75	18.88	123.07	7.97	120	6.07
Co-Pog	108.68	12.06	134.85	18.31	120.17	7.15	116.3	6.07
Co-Go	53.18	7.14	66.85	7.28	56.70	4.72	54.53	3.18
Ar-Go	42.96	4.93	54.4	2.55	43.87	3.00	41.70	2.42
Go-Me	78.18	14.20	88.05	14.50	84.23	10.36	76.97	5.76
Go-Gn	82.64	10.57	95.30	14.14	90.27	9.29	82.10	5.78
Go-Pog	77.7	9.069	87.35	11.53	83.63	7.75	75	4.92
Chin width at apex of i	7.86	2.22	7.20	0	6.47	1.30	3.3	.53
Chin width at level of D	13.6	1.84	13.15	2.05	12.33	.95	11.13	1.56
D- i apex	9.78	1.64	13.2	1.84	11.67	2.06	15.27	3.43
D-Me	11.76	2.92	14.2	1.98	11.03	1.20	12.23	1.99
Ant slope of the chin/ V	12.02	6.28	22.75	10.96	12.57	7.71	3.77	5.93
Post slope of the chin/ V	-23.62	10.61	-10.9	5.52	-25.80	9.92	-34.57	5.99
Angle Ant/ Post slopes	35.04	6.14	27.9	5.09	31.03	4.54	48.30	3.52
ANS-PNS	49.78	3.28	55.75	3.75	50.70	4.97	49.73	4.07
N-ANS	47.16	4.14	55.20	5.52	56.4	7.18	55.97	1.27
ANS-Me (AFH)	60.76	9.51	75.35	10.96	71.53	6.99	74.73	9.21
PFH	43.14	6.06	56	2.26	45.97	6.07	42.70	1.68
LFH/TFH	55.92	3.17	57.45	1.06	55.83	3.99	56.97	2.55
AFH/PFH	112.87	66.75	134.2	14.00	155.53	17.10	170.7	21.63
MP/SN	29.64 (29.72)	5.31	34.25 (34.65)	5.59	38.13 (34.66)	3.60	54.20 (46.27)	3.95
MP/H	22.64	5.31	27.25	5.59	31.13	3.60	47.20	3.95
PP/MP	20.76	5.61	25.90	2.57	28.8	3.75	40.07	2.94
PP/H	1.00	7.40	.75	1.34	-2.13	4.01	-1.83	2.15
SNA	82.66 (82.58)	2.10	85.55 (85.15)	1.77	82.57 (86.04)	1.55	73.63 (81.56)	1.07
SNB	85.56 (85.48)	1.64	88.85 (88.45)	1.63	84.87 (88.34)	3.91	75.53 (83.46)	2.26
ANB	-2.92	3.04	-2.35	.21	-2.3	2.41	1.1	1.66
AOBO	-7.06	3.78	-11.8	5.94	-12.77	2.25	-4.83	2.25
I/NA	29.55	14.99	36.40	1.41	27.27	9.11	32.47	1.92
I-NA	4.55	4.80	6.65	1.77	3.07	3.33	4.80	.61
I/SN	112.93 (112.85)	13.95	121.95 (121.55)	3.18	109.83 (113.3)	10.46	106.1 (114.03)	2.95
I/PP	121.93	15.40	130.25	.07	119.13	8.64	120.23	3.842
i/NB	21.2	3.46	33.00	2.69	20.17	6.88	25.43	5.22
i-NB	2.05	.84	6.90	.57	3.267	1.86	6.43	4.06
i/MP	83.90	3.30	90.90	6.65	77.20	7.12	78.70	3.52
I/i	131.23	12.53	112.9	4.24	134.83	9.41	121	7.03
OJ	1.86e-08	1.86	-4.1	2.40	-3.60	.85	.033	1.36
OB	.97	.78	-2.15	2.90	.40	3.52	-.1	26

( ) Corrected values relative to SN/H

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

Table IV.8.B: Descriptive statistics of cephalometric measurements of the selected families E-H.

Measurement	Family E (n= 2)		Family F (n= 4)		Family G (n= 3)		Family H (n= 1)		P value
	μ	σ	μ	σ	μ	σ	μ	σ	
SN	66.05	.92	64.12	1.94	65.47	5.14	73.70	0	0.143
S-Ar	39.80	.28	36.50	2.83	37.20	6.84	32.20	0	0.204
SN/H	9.25	7.00	13.8	4.11	12.4	4.89	16.4	0	0.35
N-S-Ar	127.55	4.88	121.96	6.52	118.23	2.22	126.10	0	0.372
Ar-Go-Gn	120.75	2.62	131.06	4.85	129.47	4.90	146.90	0	0.001
Ar-Go-Me	124.05	4.03	134.56	4.33	133.2	3.67	149.7	0	0.001
Co-Go-Me	118.95	2.76	128.68	3.70	128.27	4.60	145.7	0	0.001
Ar-Gn	118.75	2.05	117.94	14.43	116.1	15.86	135.40	0	0.378
Co-Gn	121.1	2.55	119.8	16.13	118.33	12.51	137.3	0	0.363
Co-Pog	117.5	2.26	115.88	15.57	115.37	11.41	134.90	0	0.303
Co-Go	62.25	4.60	58.92	14.38	60.53	10.14	56.90	0	0.730
Ar-Go	49.20	2.55	46.36	12.28	46.60	9.10	46.90	0	0.672
Go-Me	79.65	4.31	72.6	6.439	74.5	5.05	81.5	0	0.616
Go-Gn	84	2.26	80.74	6.69	79.30	9.74	92.80	0	0.428
Go-Pog	79.60	.85	74.34	5.65	73.43	7.83	86.10	0	0.316
Chin width at apex of i	8.80	.71	9.82	1.77	7.73	.301	5.70	0	0.0021
Chin width at level of D	12.15	1.20	14.42	1.96	12.47	1.65	9.5	0	0.147
D- i apex	11.3	.42	8.42	1.74	9.60	3.68	10.2	0	0.0032
D-Me	12.1	1.27	11.76	1.92	11.47	3.67	10.2	0	0.877
Ant slope of the chin/ V	13	3.82	9.42	7.08	14.23	4.15	7.5	0	0.206
Post slope of the chin/ V	-4.95	9.69	-17.54	3.12	-11.97	4.37	-15.3	0	0.0089
Angle Ant/ Post slopes	24.3	19.37	33.9	5.43	37.30	7.93	26.5	0	0.052
ANS-PNS	52.70	1.56	52.90	6.79	50.20	3.48	51.40	0	0.807
N-ANS	50.95	2.33	56.36	5.86	52.77	5.68	58.70	0	0.147
ANS-Me (AFH)	67.30	1.56	68.34	12.01	64.43	11.15	79.80	0	0.442
PFH	50.85	.78	49.3	12.20	46.4	9.89	56.20	0	0.486
LFH/TFH	56.9	1.70	54.46	3.15	54.67	2.81	57.60	0	0.861
AFH/PFH	131.35	4.17	138.6	16.01	138.33	8.629	141.8	0	0.586
MP/SN	30.60 (29.35)	1.27	41.02 (35.22)	1.49	35.1 (30.7)	2.59	50.60 (44.20)	0	0
MP/H	23.60	1.27	34.02	1.49	28.1	2.59	43.60	0	0
PP/MP	22.55	6.43	30.68	3.07	25.8	4.45	33.60	0	0.0009
PP/H	-1.2	1.70	-3.44	5.42	-3.10	4.19	1.5	0	0.871
SNA	79.55 (80.80)	.21	81.80 (87.60)	2.14	83.53 (87.93)	1.19	71.80 (80.10)	0	0
SNB	82.40 (83.65)	2.12	82.33 (88.13)	2.52	83.17 (87.57)	2.98	79.80 (88.20)	0	0.899
ANB	-2.90	1.84	-.53	3.09	-.63	1.79	-8.10	0	0.048
AOBO	-4.20	0	-6.86	5.52	-5.63	4.73	-32.80	0	0.001
I/NA	29.3	3.82	27.7	1.38	20.95	17.89	24.6	0	0.665
I-NA	4.40	2.55	3.12	2.01	2.60	7.21	7	0	0.803
I/SN	108.85 (110.1)	3.61	109.62 (115.42)	1.52	101.7 (106.1)	19.52	95 (103.4)	0	0.376
I/PP	116.9	8.91	119.98	4.34	113.25	19.87	112	0	0.778
i/NB	22.6	7.92	28.62	3.55	22.85	3.32	14.6	0	0.048
i-NB	3.5	2.83	5.58	.87	3.45	1.48	3.40	0	0.094
i/MP	89.60	11.31	85.98	4.935	82.70	3.11	64.10	0	0.021
I/i	130.95	13.65	123.38	5.29	140.35	19.02	150.3	0	0.078
OJ	-2.9	2.26	-1.88	3.62	-3.75	3.04	-12.3	0	0.022
OB	1.85	2.33	.34	3.29	-3.5	4.38	-6.5	0	0.201

( ) Corrected values relative to SN/H

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

A summary of the families having the highest values of cephalometric measurements related to MP is presented in Table IV.9. The most severe skeletal and dento-alveolar features of MP are noted in families B and H (to a lesser extent).

Table IV.9: Families with the highest cephalometric measurements related to MP (most severe).

	Family with highest value	Average value in family B	Average value in family H
SN	B	74.05	73.70
Co-Gn	B	138.75	137.3
Co-Pog	H	134.85	134.90
Go-Gn	B	95.30	92.80
Go-Pog	B	87.35	86.10
ANS-PNS	B	55.75	51.40
MP/SN	D	34.25	50.60
SNA	B	85.55	71.70 (80.10)
SNB	B	87.85	79.80 (88.20)
ANB	H	-2.35	-8.1
AOBO	H	-11.8	-32.80
I/NA	B	36.40	24.6
I-NA	H	6.65	7
I/SN	B	121.95	95
I/PP	B	130.25	112
i/NB	B	33.00	14.6
i-NB	B	6.90	3.40
i/MP	B	90.90	64.10
OJ	H	-4.1	-12.3

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

The average main features of each selected family are presented in Tables IV.10.A-B. Each family expresses MP features with mainly an orthognathic/prognathic maxilla, a prognathic mandible and a dento-alveolar compensation. However, the starting point is the initial affected patient who shares mostly the same characteristics.

Table IV.10.A: Cephalometric characteristics of the selected families A-D.

		Family A (n=4)	Family B (n=2)	Family C (n=3)	Family D (n=3)
Inclination of SN/H	**P**	Normal	Normal	Normal	Upward (=2SD)
	F	Normal	Normal	Upward (=1SD)	Upward (>2SD)
Facial pattern	**P**	Normodivergent	Hyperdivergent (<1SD)	Normodivergent	Hyperdivergent (>1SD)
	F	Normodivergent	Normodivergent	Normodivergent	Hyperdivergent (>1SD)
Position of maxilla	**P**	Orthognathic	Prognathic (=1SD)	Orthognathic	Orthognathic
	F	Orthognathic	Prognathic (=1SD)	Prognathic (=1SD)	Orthognathic
Position of mandible	**P**	Prognathic (=2SD)	Prognathic (>3SD)	Prognathic (>2SD)	Prognathic (=1SD)
	F	Prognathic (>1SD)	Prognathic (=3SD)	Prognathic (>3SD)	Prognathic (=1SD)
Inclination of maxillary incisors	**P**	Proclined (>11SD)	Proclined (>5SD)	Proclined (>5SD)	Proclined (=3SD)
	F	Proclined (>2SD)	Proclined (>7SD)	Proclined (>1SD)	Proclined (=4SD)
Inclination of mandibular incisors	**P**	Retroclined (>1SD)	Retroclined (=1SD)	Retroclined (=4SD)	Retroclined (=5SD)
	F	Retroclined (>1SD)	Retroclined (=2SD)	Retroclined (>3SD)	Retroclined (=5SD)
Overjet	**P**	Positive	Negative	Negative	Edge to edge
	F	Positive	Negative (crossbite)	Negative (crossbite)	Edge to edge

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

Table IV.10.B: Cephalometric characteristics of the selected families E-H.

		Family E (n=2)	Family F (n=4)	Family G (n=3)	Family H (n=1)
Inclination of SN/H	**P**	Normal	Upward (=1SD)	Upward (>1SD)	Upward (=3SD)
	F	Normal	Upward (>1SD)	Upward (>1SD)	-
Facial pattern	**P**	Normodivergent	Normodivergent	Normodivergent	Hyperdivergent (=1SD)
	F	Normodivergent	Normodivergent	Normodivergent	-
Position of maxilla	**P**	Orthognathic	Orthognathic	Orthognathic	Orthognathic
	F	Orthognathic	Prognathic (>1SD)	Prognathic (>1SD)	-
Position of mandible	**P**	Prognathic (=1SD)	Prognathic (>3SD)	Prognathic (>1SD)	Prognathic (=2SD)
	F	Prognathic (=1SD)	Prognathic (=3SD)	Prognathic (>2SD)	-
Inclination of maxillary incisors	**P**	Proclined (>1SD)	Proclined (=2SD)	Well inclined	Proclined (<1SD)
	F	Proclined (>1SD)	Proclined (>1SD)	Well inclined	-
Inclination of mandibular incisors	**P**	Retroclined (=3SD)	Retroclined (=3SD)	Retroclined (=3SD)	Retroclined (>7SD)
	F	Well inclined	Retroclined (=1SD)	Retroclined (=3SD)	-
Overjet	**P**	Negative (crossbite)	Negative (crossbite)	Negative (crossbite)	Negative (crossbite)
	F	Negative (crossbite)	Negative (crossbite)	Negative (crossbite)	-

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

We can state that out of the 8 selected families:

- 6 have an average normodivergent facial pattern and 2 have an average hyperdivergent facial pattern with an upward inclination of SN to horizontal,
- 4 have an average orthognathic maxilla and prognathic mandible,
- 4 have an average prognathic maxilla and mandible (more than the maxilla),
- 8 show a dento-alveolar compensation involving either one arch (2 families) or both arches (6 families), along with an anterior cross bite in 6 families, an edge-to-edge relationship in 1 family and an overjet in 1 family.

4. Mandibular length analysis: Z score.

Mandibular length measurements (Co-Gn) and Z scores of each family and its individuals are presented in Tables IV.11-18. Subjects are numbered by ascending order of generations, which means by descending order of age.

In family (A):

- Co-Gn value ranges between 96.2 and 122.6.
- Z score value ranges between 1.16 and 3.34.
- The average Z score is equal to 2.49 (Table IV.11).

Table IV.11: Co-Gn values (mm) and Z scores of individuals that are part of the selected family (A).

Family (A)						
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**	**Average Z score**
1	51.3	113.8	109.9	3.8	1.03	**2.13**
2	30.9	122.6	109.9	3.8	3.34	
3	19	121.2	109.9	3.8	2.97	
4	5.7	96.2	91.9	3.7	1.16	

Initial affected patient

In family (B):

- Co-Gn value ranges between 125.4 and 152.1.
- Z score value ranges between 1.02 and 6.47.
- The average Z score is equal to 3.744897959 (Table IV.12).

Table IV.12: Co-Gn values (mm) and Z scores of individuals that are part of the selected family (B).

Family (B)						
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**	**Average Z score**
1	54.10	125.4	120.4	4.9	1.02	**3.74**
2	24.4	152.1	120.4	4.9	6.47	

Initial affected patient

In family (C):

- Co-Gn value ranges between 114.8 and 130.7.
- Z score value ranges between 0.67 and 2.85.
- The average Z score is equal to 1.88 (Table IV.13).

Table IV.13: Co-Gn values (mm) and Z scores of individuals that are part of the selected family (C).

Family (C)						
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**	**Average Z score**
1	52.2	123.7	120.4	4.9	0.67	**1.88**
2	17.5	130.7	117.6	4.6	2.85	
3	13.3	114.8	105.9	4.2	2.12	

Initial affected patient

In family (D):

- Co-Gn value ranges between 116.2 and 127.
- Z score value ranges between 1.35 and 1.82.
- The average Z score is equal to 1.61 (Table IV.14).

Table IV.14: Co-Gn values (mm) and Z scores of individuals that are part of the selected family (D).

Family (D)						
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**	**Average Z score**
1	34.9	116.2	109.9	3.8	1.66	
2	33.3	127	120.4	4.9	1.35	**1.61**
3	29.1	116.8	109.9	3.8	1.82	

Initial affected patient

In family (E):

- Co-Gn value ranges between 119.3 and 122.6.
- Z score value ranges between 0.45 and 2.47.
- The average Z score is equal to 1.46 (Table IV.15).

Table IV.15: Co-Gn values (mm) and Z scores of individuals that are part of the selected 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**
1	26	119.3	109.9	3.8	2.47	
2	22.9	122.6	120.4	4.9	0.45	**1.46**

Initial affected patient

In family (F):

- Co-Gn value ranges between 117.7 and 135.5.
- Z score value ranges between 2.05 and 3.63.
- The average Z score is equal to 2.88 (Table IV.16).

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

Family (F)						
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**	**Average Z score**
1	25	117.7	109.9	3.8	2.05	**2.88**
2	21.7	135.5	120.4	4.9	3.08	
3	14.5	124.7	110.7	5.1	2.75	
4	14.5	129.2	110.7	5.1	3.63	

Initial affected patient

In family (G):

- Co-Gn value ranges between 103.2 and 126.
- Z score value ranges between -0.94 and 3.38.
- The average Z score is equal to 1.82 (Table IV.17).

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

Family (G)						
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**	**Average Z score**
1	81.11	126	120.4	4.9	1.14	**1.82**
2	41.2	125	120.4	4.9	0.94	
3	7.7	103.2	92.4	3.2	3.38	

Initial affected patient

In family (H):

- Co-Gn value is 137.3.
- Z score value is 3.45 (Table IV.18).

Table IV.18: Co-Gn value (mm) and Z score of the individual that is part of the selected family (H).

Family (G)					
Subject number	**Age at x-ray time**	**Co-Gn value**	**Co-Gn mean**	**Co-Gn standard deviation**	**Z score**
1	22.6	137.3	120.4	4.9	3.45

Initial affected patient

To sum up, mandibular length Co-Gn ranges between 96.2 (family A) and 152.1 (family B), Z score value ranges between 1.16 (family A) and 6.47 (family B) and average Z score corresponding to the 8 selected families is equal to 2.42, with the highest value corresponding to family B and the lowest value to family A. The average Z score, being more than 2 standard deviations above the norm, indicates a mandibular macrognathism.

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). However, the Z score reaches maximum 3SD above the norm in the 2nd generation and 6.47 SD in the 3rd generation. The most severe scores correspond to the initial patient of family B, followed by families H and F. The variation in severity of the Z score across individuals confirms the variable expressivity of MP. In most of the families, the highest values correspond to the initial affected patient (Figures IV.1,2).

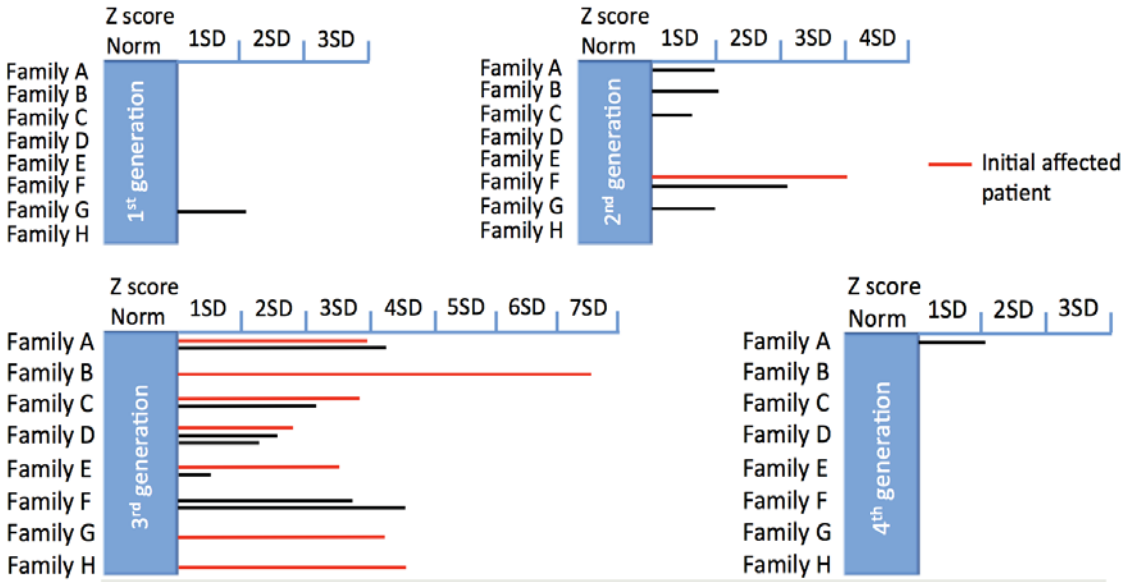

Figure IV.1: Distribution of the Z score by family across the different generations.

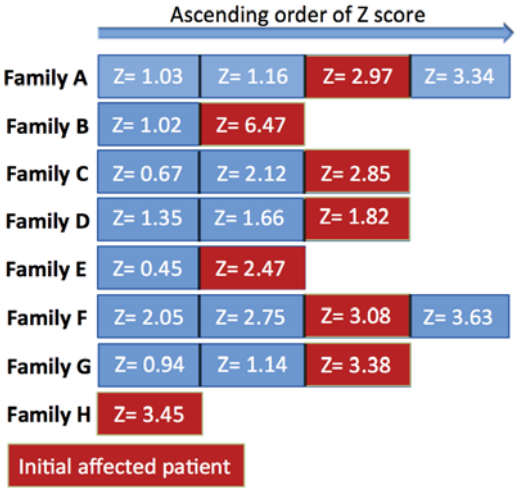

Figure IV.2: Order of severity of the Z score in each selected family.

C. Genetic analysis

Whole Exome Sequencing (WES) identified the genetic variants in exonic regions of the 47 subjects that are part of the 8 selected families, including the affected ones as well as the non-affected. For each individual, the results were first displayed on

a file named “fast q 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. Sample and families characteristics

a. Sample characteristics

The total number of read bases ranges between 5,569,143,232 and 10,281,893,728 with an average of 7,292,945,738 and a standard deviation of 962039749.6. The lowest value corresponds to individual A6 and the highest value to individual A5 (Table IV.19).

The total number of reads ranges between 55,140,032 and 101,800,928 with an average of 72,199,958 and a standard deviation of 9530786.1. The lowest value corresponds to individual A6 and the highest value to individual A5 (Table IV.19).

The GC-content ranges between 47.6% with an average of 49.66% and a standard deviation of 0.94%. The lowest value corresponds to individual G3 and the highest value to individual F5 (Table IV.19).

Q20 ranges between 90.6% and 98.2% with an average of 95.53% and a standard deviation of 3.06%. The lowest value corresponds to individual A2 and the highest value to individual D7 (Table IV.19).

Q30 ranges between 83.2% and 95.5% with an average of 91.09% and a standard deviation of 4.96%. The lowest value corresponds to individual A2 and the highest value to individual E3 (Table IV.19).

The average read length with forward and reverse read is stable across all individuals, with a value of 101.0 (Table IV.19).

The total yield, which is calculated by the formula total number of reads * average read length, ranges between 5,569 and 10,281 with an average of 7,292 and a standard deviation of 962.6. The lowest value corresponds to individual A6 and the highest value to individual A5 (Table IV.19).

The number of SNP ranges between 85,501 and 92,310 with an average of 89,412 and a standard deviation of 14556.67. The lowest value corresponds to individual D7 and the highest value to individual E2 (Table IV.19).

The number of INDEL (insertion or deletion) ranges between 8,966 and 11,678 with an average of 10,175 and a standard deviation of 641.39. The lowest value corresponds to individual D3 and the highest value to individual D5 (Table IV.19).

The total number of variants ranges between 94,467 and 103,319 with an average of 99,587 and a standard deviation of 2041.87. The lowest value corresponds to individual D3 and the highest value to individual E2 (Table IV.19).

Table IV.19: Summary of the genetic characteristics of the whole sample (n=47).

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	7,292,945,738	962039749.6	5,569,143,232	10,281,893,728
Total number of reads	72,199,958	9530786.1	55,140,032	101,800,928
GC-content (%)	49.655	0.94	47.6	51.4
Q20 (%)	95.53	3.06	90.6	98.2
Q30 (%)	91.0925	4.96	83.2	95.5
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	7,292	962.6	5,569	10,281
Number of SNP	89,412	14556.67	85,501	92,310
Number of INDEL	10,175	641.39	8,966	11,678
Total number of variants	99,587	2041.87	94,467	103,319

The numbers above indicate that the results of this study are consistent across the sample and have a quality comparable to the results of other laboratories and platforms.

b. Families characteristics

Below are displayed tables that summarize in details the average characteristics of each selected family in terms of number of reads, quality scores, read length, yield and number of variants (Tables IV.20→26).

-Family A:

Family A has an average total read bases of 7,123,387,132, 49.81% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 96.38% and 92.54% respectively, indicating that 96.38% of the fragments have 20 copies read and more and 92.54% of the fragments have 30 copies read and more. The average total yield, equal to 7,119 Mbp, is obtained by multiplying the total number of reads (70,491,457) by the average read length with forward and reverse read (101.0 bp). The addition of the number of SNP (90,452) and the number of insertion or deletion "INDEL" (10,385) resulted in a total number of variants equal to 100,836 (Table IV.20).

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

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	7,123,387,132	1383546107	5,569,143,232	10,281,893,728
Total number of reads	70,491,457	13715076.34	55,140,032	101,800,928
GC-content (%)	49.8125	0.57	48.7	50.5
Q20 (%)	96.3875	3.12	90.9	98.2
Q30 (%)	92.5375	5.07	83.6	95.5
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	7,119	1385.11	5,569	10,281
Number of SNP	90,452	1095.55	88,371	91,676
Number of INDEL	10,385	381.32	9,863	10,945
Total number of variants	100,836	1421.82	98,392	102,554

-Family B:

Family B has an average total read bases of 8,125,421,720, 48.25% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 95.5% and 91% respectively, indicating that 95.5% of the fragments have 20 copies read and more and 91% of the fragments have 30 copies read and more. The average total yield, equal to 8,125 Mbp, is obtained by multiplying the total number of reads (80,449,720) by the average read length with forward and reverse read (101.0 bp). The addition of the number of SNP (90,277) and the number of insertion or deletion “INDEL” (10,078) resulted in a total number of variants equal to 100,355 (Table IV.21).

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

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	8,125,421,720	201320736.5	7,983,066,462	8,267,776,978
Total number of reads	80,449,720	1993274.62	79,040,262	81,859,178
GC-content (%)	48.25	0.35	48	48.5
Q20 (%)	95.5	0.14	95.4	95.6
Q30 (%)	91	0.28	90.8	91.2
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	8,125	200.82	7,983	8,267
Number of SNP	90,277	361.33	90,021	90,532
Number of INDEL	10,078	193.75	9,941	10,215
Total number of variants	100,355	555.08	99,962	100,747

-Family C:

Family C has an average total read bases of 6,969,720,422, 50.64% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 95.43% and 90.98% respectively, indicating that 95.43% of the fragments have 20 copies read and more and 90.98% of the fragments have 30 copies read and more. The average total yield, equal to 6,978 Mbp, is obtained by multiplying the total number of reads (69,093,487) by the average read length with forward and reverse read (101.0 bp). The addition of the number of SNP (88,616) and the number of insertion or deletion "INDEL" (10,026) resulted in a total number of variants equal to 98,642 (Table IV.22).

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

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	6,969,720,422	543988305.1	6,077,759,032	7,960,466,702
Total number of reads	69,093,487	5751236.32	60,175,832	78,816,502
GC-content (%)	50.6375	0.45	50.0	51.4
Q20 (%)	95.425	3.57	90.6	98.2
Q30 (%)	90.975	5.78	83.2	95.5
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	6,978	580.94	6,077	7,960
Number of SNP	88,616	1852.21	86,677	91,908
Number of INDEL	10,026	639.25	9,241	11,040
Total number of variants	98,642	2135.59	96,720	102,948

-Family D:

Family D has an average total read bases of 6,711,134,072, 49.72% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 97.11% and 93.67% respectively, indicating that 97.11% of the fragments have 20 copies read and more and 93.67% of the fragments have 30 copies read and more. The average total yield, equal to 6,711 Mbp, is obtained by multiplying the total number of reads (66,446,872) by the average read length with forward and reverse read (101.0 bp).

The addition of the number of SNP (88,249) and the number of insertion or deletion "INDEL" (10,544) resulted in a total number of variants equal to 98,793 (Table IV.23).

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

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	6,711,134,072	387138577.4	6,081,680,458	7,084,126,062
Total number of reads	66,446,872	3833055.22	60,214,658	70,139,862
GC-content (%)	49.71666667	0.63	48.9	50.4
Q20 (%)	97.11666667	2.31	92.4	98.1
Q30 (%)	93.66666667	3.76	86	95.3
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	6,711	387.21	6,081	7,084
Number of SNP	88,249	2156.13	85,501	91,097
Number of INDEL	10,544	887.07	8,966	11,678
Total number of variants	98,793	2716.69	94,467	101,883

-Family E:

Family E has an average total read bases of 7,733,136,003, 49.45% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 96.65% and 92.93% respectively, indicating that 96.65% of the fragments have 20 copies read and more and 92.93% of the fragments have 30 copies read and more. The average total yield, equal to 7,733 Mbp, is obtained by multiplying the total number of reads (76,565,703) by the average read length with forward and reverse read (101.0 bp). The addition of the number of SNP (90,396) and the number of insertion or deletion "INDEL" (10,637) resulted in a total number of variants equal to 101,033 (Table IV.24).

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

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	7,733,136,003	924463701.7	7,095,716,216	9,105,062,534
Total number of reads	76,565,703	9153105.96	70,254,616	90,149,134
GC-content (%)	49.45	0.97	48.0	50.0
Q20 (%)	96.65	2.77	92.5	98.2
Q30 (%)	92.925	4.56	86.1	95.5
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	7,733	924.71	7,095	9,105
Number of SNP	90,396	1556.28	89,067	92,310
Number of INDEL	10,637	701.95	9,792	11,374
Total number of variants	101,033	2151.37	98,983	103,329

-Family F:

Family F has an average total read bases of 7,180,782,658, 49.1% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 94.59% and 89.43% respectively, indicating that 94.59% of the fragments have 20 copies read and more and 89.43% of the fragments have 30 copies read and more. The average total yield, equal to 7,180 Mbp, is obtained by multiplying the total number of reads (71,096,858) by the average read length with forward and reverse read (101.0 bp). The addition of the number of SNP (88,530) and the number of insertion or deletion "INDEL" (9,767) resulted in a total number of variants equal to 100,836 (Table IV.25).

Table IV.25: Summary of the average characteristics of family F.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	7,180,782,658	801775767.1	6,053,311,780	8,221,434,340
Total number of reads	71,096,858	7938373.93	59,933,780	81,400,340
GC-content (%)	49.1	0.88	48.0	50.2
Q20 (%)	94.58571429	2.97	91.5	98.0
Q30 (%)	89.42857143	4.72	84.6	95.1
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	7,180	801.83	6,053	8,221
Number of SNP	88,530	1599.25	85,811	89,986
Number of INDEL	9,767	609.89	9,030	10,596
Total number of variants	98,296	1989.11	94,841	100,334

-Family G:

Family G has an average total read bases of 8,237,504,975, 49.26% of which are guanine or cytosine nucleotides. The average quality scores Q20 and Q30 are 92.86% and 86.78% respectively, indicating that 92.86% of the fragments have 20 copies read and more and 86.78% of the fragments have 30 copies read and more. The average total yield, equal to 8,237 Mbp, is obtained by multiplying the total number of reads (81,559,455) by the average read length with forward and reverse read (101.0 bp). The addition of the number of SNP (90,518) and the number of insertion or deletion "INDEL" (9,877) resulted in a total number of variants equal to 100,394 (Table IV.26).

Table IV.26: Summary of the average characteristics of family G.

	Average	Standard deviation	Minimum	Maximum
Total read bases (bp)	8,237,504,975	916604665.6	7,366,248,150	9,657,690,498
Total number of reads	81,559,455	9075293.72	72,933,150	95,620,698
GC-content (%)	49.26	1.26	47.6	51.1
Q20 (%)	92.86	3.02	90.8	98.2
Q30 (%)	86.78	4.88	83.4	95.4
Average read length (bp)	101.0	0	101.0	101.0
Total yield (Mbp)	8,237	916.55	7,366	9,657
Number of SNP	90,518	1690.03	87,629	92,101
Number of INDEL	9,877	404.13	9,376	10,341
Total number of variants	100,394	2032.64	97,005	102,442

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: *HSPG2, ALPL, MATN1, EPB41, EVC, EVC2, TGFβ3, LTBP2, MYO1H* (rs10850110), *DUSP6* (p.Ser182Phe variant), *PLXNA2, SSX2IP, COL2A1, ADAMTS1* (rs2738, rs229038) and *ARHGAP21* (Gly1121Ser variant). None of the candidate genes,

loci or variants found to be associated with MP in previous studies was noted in the genotype of the individuals who are part of the 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 >40-50, a homozygosity status (in family E), and a high putative impact on protein structure and function. The number of variants was reduced from an average of 100,000 to an average of 550-600 in all families and 300 in family E.

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 except in families A and D where no shared gene was noted. In family B, 406 genes were found to be common between the 2 affected individuals when the non-affected subject was not filtered out. However, when we selected only the variants having a Minor Allele Frequency (MAF) ≤ 0.001, the number of shared genes was reduced to 132. When the non-affected subject was filtered out, 93 common genes were noted between the 2 affected individuals (Table IV.27).

Table IV.27: Common variants in 93 genes between affected individuals of family B following the 1st and 2nd filterings (high putative impact).

A2M	*AGL*	*AKAP3*	*AQP7*	*ARSD*	*ATP8B3*	*BAGE3*
BAGE4	*C14orf159*	*C17orf77*	*C2orf15*	*C2orf83*	*C5orf20*	*C9orf129*
CASP7	*CD177*	*CLLU1*	*CNOT1*	*CNTN5*	*CWH43*	*CYP4B1*
EFCAB6	*FAM111B*	*FAM83H*	*FXYD4*	*GAB4*	*GCSAML*	*GLT6D1*
GNA12	*IFNA10*	*IFNA17*	*IL32*	*IQGAP2*	*KIAA0040*	*KIAA1586*
KIR2DL3	*KRT24*	*KRT38*	*KRT83*	*KRTAP10-1*	*KRTAP10-4*	*KRTAP1-1*
KRTAP1-5	*KRTAP29-1*	*LCN10*	*LOC100130451*	*LOC643339*	*LOC643355*	*LRRIQ1*
MFSD6L	*MOB3C*	*MOK*	*MRGPRX3*	*MST1*	*NAT8B*	*NBPF9*
NME4	*NPHP4*	*OBSCN*	*OR10AD1*	*OR10J1*	*OR13C2*	*OR13C5*
OR1J2	*OR2B11*	*OR4C16*	*OR4X1*	*OR52N4*	*OR5K3*	*OR6C76*
OR7G3	*PATE4*	*PLCL2*	*PLK5*	*PPIG*	*RAD52*	*RAI1*
RPP1L1	*RYK*	*SETBP1*	*SLC5A9*	*STPG2*	*TCEB3CL*	*TGM4*
TMPRSS11A	*U2AF2*	*UBE2D3*	*WDR89*	*ZFYVE19*	*ZNF283*	*ZNF438*
ZNF727P						

In the remaining families, the number of common variants in specific genes between the affected individuals is:

- 1 in family C: *NFU1*.
- 232 in family E, when the non-affected subjects were not filtered out, which explains that some of these genes may be present also in the 2 non-affected individuals. However, when the non-affected subjects were filtered out, the number was reduced to 14: *OR5K4, OR5K3, GUCA1C, ATG3, HLA-A, CHN2, FZD6, ZFP41, CLECL1, GPATCH2L, NME4, PRR25, FLJ44313* and *KRTAP19-6.*
- 4 in family F: *C1orf167, OR13C2, ZNF883* and *NCR3LG*.
- 14 in family G: *UPP2, OR52J3, OAS1, OAS2, COPZ2, PEBP4, ANKRD30A, OR51F1, P2RX5, GCAT, APOBEC3B, SLC25A5, SEL1L* and *LMF2.* It should be noted that the gene *SLC25A5* is present also in the non-affected individual G3, on the same chromosome (X) and position, having a high putative impact and a heterozygozity but the filter is MG_INDEL (not PASS) (Tables IV.28-32).

Table IV.28: Summary of the common variants in specific genes between affected individuals of each family following the 1st and 2nd filterings (high putative impact).

	Family A	Family B	Family C	Family D	Family E	Family F	Family G
Gene 1	-	*93*	*NFU1*	-	*OR5K4*	*C1orf167*	*UPP2*
Gene 2	-		-	-	*OR5K3*	*OR13C2*	*OR52J3*
Gene 3	-		-	-	*GUCA1C*	*ZNF883*	*OAS1*
Gene 4	-		-	-	*ATG3*	*NCR3LG*	*OAS2*
Gene 5	-		-	-	*HLA-A*	-	*COPZ2*
Gene 6	-		-	-	*CHN2*	-	*PEBP4*
Gene 7	-		-	-	*FZD6*	-	*ANKRD30A*
Gene 8	-		-	-	*ZFP41*	-	*OR51F1*
Gene 9	-		-	-	*CLECL1*	-	*P2RX5*
Gene 10	-		-	-	*GPATCH2L*	-	*GCAT*
Gene 11	-		-	-	*NME4*	-	*APOBEC3B*
Gene 12	-		-	-	*PRR25*	-	*LMF2*
Gene 13	-		-	-	*FLJ44313*	-	*SLC25A5*
Gene 14	-		-	-	*KRTAP19-6*	-	*SEL1L*

Table IV.29: Summary of the characteristics of the shared variant in a specific gene between affected individuals of family C following the 1st and 2nd filterings (high putative impact).

Gene Name	Chromosome	Position	Zygosity	Effect	MAF
NFU1	2	69,659,126	HET or HOM	start_lost	.

Table IV.30: Summary of the characteristics of the shared variants in specific genes between affected individuals of family E following the 1st and 2nd filterings (high putative impact).

Gene Name	Chromosome	Position	Zygosity	Effect	Range of MAF
OR5K4	3	98,073,591	HOM	frameshift_variant	0.1319 - 0.5875
OR5K3	3	98,110,406	HOM	frameshift_variant	0.127 - 0.5964
GUCA1C	3	108,634,973	HOM	splice_donor_variant &intron_variant	0.1806 - 0.3084
ATG3	3	112,253,058	HOM	frameshift_variant	.
HLA-A	6	29,911,240	HOM	stop_gained	0.025 - 0.4732
CHN2	7	29,186,576	HOM	splice_donor_variant &intron_variant	0.7118 - 0.9881
FZD6	8	104,312,432	HOM	start_lost	0.0129 - 0.2704
ZFP41	8	144,332,012	HOM	start_lost	0.83 - 0.9254
CLECL1	12	9,885,707	HOM	frameshift_variant	0.38 - 0.7564
GPATCH2L	14	76,644,266	HOM	stop_gained	0 - 0.0099
NME4	16	450,140	HOM	frameshift_variant	0.126 - 0.4571
PRR25	16	863,355	HOM	frameshift_variant	0.0675 - 0.6876
FLJ44313	18	74,208,485	HOM	splice_acceptor_variant &splice_donor_variant &intron_variant	0.5952 - 0.9508
KRTAP19-6	21	31,913,981	HOM	frameshift_variant	0.7034 - 0.9266

Table IV.31: Summary of the characteristics of the common variants in specific genes between affected individuals of family F following the 1st and 2nd filterings (high putative impact).

Gene Name	Chromosome	Position	Zygosity	Effect	Range of MAF
C1orf167	1	11,844,520	HET	stop_gained	.
OR13C2	9	107,367,392 or 107,367,664	HET	frameshift_variant	0.2216 - 0.2326
ZNF883	9	115,759,519	HET	stop_gained	0.2236 or 0.2348
NCR3LG1	1	17,394,037	HET	frameshift_variant	0.3171 or 0.3183

Table IV.32: Summary of the characteristics of the shared variants in specific genes between affected individuals of family G following the 1st and 2nd filterings (high putative impact).

Gene Name	Chromosome	Position	Zygosity	Effect	Range of MAF
UPP2	2	158,958,551	HET	frameshift_variant &splice_region_variant	.
OR52J3	11	5,068,662	HET	stop_gained	0.2163 - 0.3385
OAS1	12	113,357,193	HET or HOM	splice_acceptor_variant &intron_variant	0.6042 - 0.6452
OAS2	12	113,448,288	HET or HOM	stop_lost	0.6054 - 0.6484
COPZ2	17	46,115,072 or 46,115,084	HOM	frameshift_variant	0.9996 - 0.9998
PEBP4	8	22,570,907	HET	frameshift_variant	0.0623 - 0.1436
ANKRD30A	10	37,419,292	HET	stop_gained	0.1869 - 0.2755
OR51F1	11	4,790,873	HET or HOM	frameshift_variant	0.2159 - 0.3171
P2RX5	17	3,594,276	HET	frameshift_variant	0.8904 - 0.9567
GCAT	22	38,204,035	HET	stop_gained	1
APOBEC3B	22	39,387,395	HET	frameshift_variant	1
LMF2	22	50,943,289	HET	frameshift_variant	1
SLC25A5	X	118,603,706	HET	frameshift_variant	1
SEL1L	14	81,965,790	HET	stop_lost	0.6925 - 0.7790

Then, as previously mentioned, the analysis was repeated by selecting only the passed variants having a moderate putative impact and a Minor Allele Frequency (MAF) ≤ 0.001 in the normal population and running a comparison between the individuals of the same family in order to find common gene(s) between the affected ones. Therefore, the number of variants was reduced to 10000-11000 on average in all

families (except family E) and the following results were found. Common gene(s) were noted in all families except family A. In the remaining families, the number of shared variants in specific genes between the affected individuals is:

- 730 in family B, when the non-affected subject was not filtered out. The number was reduced to 132 after filtering him out.
- 3 in family C: *PCYOX1, ERAP1* and *ZNF638*.
- 1 in family D: *MAP3K9*.
- 4 in family F: *SKI, WRAP73, SLFNL1* and *MPL*.
- 12 in family G: *TTC4, DYNC2LI1, FBXO42, SPATA6, DNTTIP2, PTPN7, FAM228A, ABCG5, FSHR, ELMOD3, IMMT* and *FABP1* (Table IV.33-37).

Table IV.33: Summary of the shared variants in specific genes between affected individuals of each family following the 2nd filtering (moderate putative impact).

	Family A	**Family B**	**Family C**	**Family D**	**Family F**	**Family G**
Gene 1	-	132	*PCYOX1*	*MAP3K9*	*SKI*	*TTC4*
Gene 2	-		*ERAP1*	-	*WRAP73*	*DYNC2LI1*
Gene 3	-		*ZNF638*	-	*SLFNL1*	*FBXO4*
Gene 4	-		-	-	*MPL*	*SPATA6*
Gene 5	-		-	-	-	*DNTTIP2*
Gene 6	-		-	-	-	*PTPN7*
Gene 7	-		-	-	-	*FAM228A*
Gene 8	-		-	-	-	*ABCG5*
Gene 9	-		-	-	-	*FSHR*
Gene 10	-		-	-	-	*ELMOD3*
Gene 11	-		-	-	-	*IMMT*
Gene 12	-		-	-	-	*FABP1*

Table IV.34: Summary of the characteristics of the shared variants in specific genes between affected individuals of family C following the 2nd filtering (moderate putative impact).

Gene Name	**Chromosome**	**Positions**	**Zygosity**	**Effect**	**Range of MAF**
PCYOX1	2	70,488470	HET	missense_variant	0.0006 - 0.0012
ERAP1	5	96,118,852 96,124,330 96,139,250	HET	missense_variant	0.0012 - 0.1512
ZNF638	2	71,654,175	HET	missense_variant	0.0006 - 0.0012

Table IV.35: Summary of the characteristics of the shared variant in a specific gene between affected individuals of family D following the 2nd filtering (moderate putative impact).

Gene Name	Chromosome	Position	Zygosity	Effect	Range of MAF
MAK3K9	14	71,275,773	HET	disruptive_inframe_deletion	0.6207 - 0.6310

Table IV.36: Summary of the characteristics of the shared variants in specific genes between affected individuals of family F following the 2nd filtering (moderate putative impact).

Gene Name	Chromosome	Position	Zygosity	Effect	MAF
SKI	1	2,160,390	HET	missense_variant	.
WRAP73	1	3,548,832	HET	missense_variant	.
SLFNL1	1	41,485,902	HET	missense_variant	.
MPL	1	43,804,340	HET	missense_variant	.

Table IV.37: Summary of the characteristics of the shared variants in specific genes between affected individuals of family G following the 2nd filtering (moderate putative impact).

Gene Name	Chromosome	Positions	Zygosity	Effect	Range of MAF
TTC4	1	55,182,300	HET	missense_variant	.
DYNC2LI1	2	44,004,010 44,021,826	HET	missense_variant	0.0008
FBXO4	1	16,577,908	HET	missense_variant	.
SPATA6	1	48,764,419	HET	missense_variant	.
DNTTIP2	1	94,342,564	HET	missense_variant	.
PTPN7	1	202,129,826	HET	missense_variant	.
FAM228A	2	24,413,298	HET	missense_variant	0.0002 - 0.0004
ABCG5	2	44,040401	HET	missense_variant	0.0008
FSHR	2	49,189921 49,191,041	HET	missense_variant	0.0010
ELMOD3	2	85,590,286	HET	missense_variant	0.0020 - 0.0024
IMMT	2	86,400,824	HET	missense_variant	0.0020 - 0.0028
FABP1	2	88,424,066	HET	missense_variant	0.0020 - 0.0028

Following the 1st subfiltering done on families B and G for the variants having a high putative impact, the number of common variants in specific genes was reduced to 26 in family B and 1 (*UPP2*) in family G (Table IV.38).

Table IV.38: Common variants in the 26 conserved genes in family B following the 1st subfiltering (high putative impact).

NPHP4	*MOB3C*	*CYP4B1*	*SLC5A9*	*AGL*	*LOC643355*	*NBPF9*
OR10J1	*KIAA0040*	*OBSCN*	*OR2B11*	*GCSAML*	*NAT8B*	*PPIG*
C2orf83	TGM4	*OR5K3*	*CWH43*	*TMPRSS11A*	*STPG2*	*UBE2D3*
C5orf20	*KIAA1586*	OR10X1	*IQGAP2*	*LOC100130451*		

Following the 1st subfiltering done on families B and G for the variants having a moderate putative impact, the number of common variants in specific genes was reduced to 104 in family B and 8 in family G (*TTC4, FBXO42, SPATA6, MCOLN3, CLCA2, DNTTIP2, PTPN7* and *AHCTF1*).

After the 2nd subfiltering done on families B and G for the variants having a moderate putative impact, the number of common variants in specific genes was reduced to 4 (*PADI3, KLHDC7A, ANXA9* and *FAM89A*) in family B and 1 (*CLCA2*) in family G.

In family H, the number of variants having a high putative impact and a Minor Allele Frequency (MAF) ≤ 0.01 is 127. After filtering out the genes having several mutations, 7 genes (*NBPF8, PRR21, ALS2CL, MAML3, GRIFIN, RBBP6* and *TEX13A*) were highlighted (Table IV.39).

Table IV.39: Summary of the characteristics of the 7 highlighted genes in family H (high putative impact).

Gene Name	Chromosome	Position	Zygosity	Effect	MAF
NBPF8	1	144,828,764	HET	stop_gained	.
PRR21	2	240,982,059	HOM	frameshift_variant	.
ALS2CL	3	46,729,697	HET	stop_gained	0.0016
MAML3	4	140,811,083	HOM	frameshift_variant	.
GRIFIN	7	2,515,382	HOM	frameshift_variant	.
RBBP6	16	24,564,879	HET	frameshift_variant	.
TEX13A	X	104,464,281	HOM	splice_acceptor_variant &splice_donor_variant &intron variant	.

Subsequent to the several steps of filtering in families A-F, 8 potential candidate genes (*MPL, SLFNL1, SKI, C1orf167*, *UPP2, NBPF9, OBSCN* and *PPIG*) that segregate with the phenotype were highlighted and their functions checked. The first 4 genes are common between affected individuals of family F (*C1orf167* having a high putative impact; *MPL, SLFNL1* and *SKI* having a moderate putative impact). *UPP2* is one of the 14 common genes between affected individuals of family G, having a high putative impact and the 3 last genes are common between affected individuals of family B, having a high putative impact. In family H, 7 potential candidate genes (*NBPF8, PRR21, ALS2CL, MAML3, GRIFIN, RBBP6* and *TEX13A*) were highlighted and their functions checked.

All the potential genes, except *C1orf167, NBPF8* and *NBPF9,* were not considered as candidate genes for MP because they do not have a role in the formation of the jaws and/or their MAF indicate a frequent occurrence for the minor allele in the normal healthy population.

C1orf167 was considered a potential candidate gene for MP because its function is still not discovered although it has an ORF and since its frequency is rare in the normal population. An ORF is defined as a continuous stretch of codons that do not contain a stop codon and that has the potential to be translated into a protein.

NBPF8 and *NBPF89,* that are part of the same *NBPF* family, were also considered as potential candidate genes for MP because they play a role in human evolution and since their frequencies are rare in the normal population.

Therefore, the 3 potentially novel genes *C1orf167, NBPF8* and *NBPF9* that segregate with the phenotype, could be implicated in mandibular development. They share the same characteristics: location on chromosome 1, heterozygosity, stop gained

effect, high putative impact, coding transcript biotype and MAF equal to ".". However, they differ by the locus and positions of the mutation and amino acid (Table IV.40). The amino acid position refers to the site of the stop codon relative to the length of the protein. Those 3 novel genes were not found to be associated with MP in previous genetic studies.

Table IV.40: Summary of the characteristics of the 3 novel genes and their variants.

Gene Name	Chromosome	Locus	Position of the variant	Zygosity	Effect of the variant	MAF	Amino acid position	Amino acid length
C1orf167	1	1p36.22	11,844,520	HET	stop_gained	.	1,099	1,449
NBPF8	1	1p11.2	144,828,764	HET	stop_gained	.	936	941
NBPF9	1	1q21.2	145,368,525	HET	stop_gained	.	1,058	1,110

CHAPTER V
DISCUSSION

A. Summary

The genetic component plays a substantial role in the etiology of MP, as supported by the observation of familial aggregation. Sequencing of the human genome has been completed and researchers are making inroads into understanding the genetic basis of many common human traits and diseases. The use of Genome Wide Association Studies (GWAS) has emerged, enabling genetic variants at specific loci to be associated with particular traits and diseases. Therefore, genetic association data are now providing new routes to understanding the etiology of conditions, predicting a patient's risk to the trait or treatment response as well as improving personalized prevention and treatment.

The technology and statistical methods for completing whole genome tagging of variants and GWAS has developed rapidly over the last decade. This recent progress has allowed the investigations of susceptibility genes that are responsible of the development of MP through the previous few genetic mapping studies that were performed in different ethnic populations: Korean, Japanese, Chinese, African-American, Hispanic, Estonian and Italian. Those studies identified several chromosomal regions or loci that harbor susceptibility genes for MP, which strongly support heterogeneity in the development of MP and the fact that the genes involved in its etiology may be correlated with the ethnicity of the population.

In the present study, Whole Exome Sequencing (WES) was performed on 8 Mediterranean families including 49 individuals (affected and non-affected) of different generations. The genetic objectives of this research project were met since it explored

the inheritance pattern of MP in the Mediterranean population and identified 3 new candidate loci and genes (*C1orf167, NBPF8* and *NBPF9*) responsible of the development and familial transmission of this condition in this specific population. *NBPF8* and *NBPF9* genes contributed also to the evolution of primates, including man. The cephalometric aim of this study was also achieved, since it evaluated the skeletal and dento-alveolar characteristics of individuals affected by MP across the available generations.

B. Discussion of major findings

Pedigree analysis showed that most pedigrees suggest a Mendelian inheritance pattern and segregate in an autosomal dominant manner, which support several previous genetic studies (Cruz et al., 2008; El-Gheriani et al., 2003; Frazier-Bowers et al., 2009; Kraus et al., 1959; Wolff, Wienker, & Sander, 1993). The strong Mendelian inheritance pattern argues for a stronger genetic defect that in other conditions or diseases is not as obvious, and increases confidence in finding a mutation or a series of mutations. 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) per family, and more families with male predominance. Given the equal number of affected males and females, we can hypothesize that in Middle Eastern families with female predominance, the number of affected females is greater.

The results of this study support previous reports of no gender differences related to the incidence of MP (Joshia et al., 2014; Solow & Helm, 1968) and contradict others that found an increased incidence in males (El-Mangoury & Mostafa, 1990) or females (Wood, 1971). While disparity underscores the absence of conclusive findings

concerning the gender similarities or differences related to the incidence of MP, it may be related to sampling or population differences. Moreover, consanguinity, which was noted in 6 out of the 8 selected families, could be related to the familial transmission of MP. This premise supports previous findings stating that MP has been passed on and exaggerated over time through intermarriage, which caused acute inbreeding (Wolff, Wienker, & Sander, 1993).

Cephalometric analysis indicated that individuals from the 8 selected families have more severe facial, skeletal and dento-alveolar characteristics than the remaining approached families, as demonstrated by greater values for the majority of the cephalometric measurements: mandibular lengths (Co-Gn and Co-Pog), facial divergence angular measurements indicating a tendency to a hyperdivergent pattern, SNB angle, ANB angle and linear measurement AO-BO (referring to a more severe skeletal Class III malocclusion underlined by a more prognathic mandible) and dento-alveolar compensation denoted by a more severe anterior crossbite. The latter is in agreement with other studies stating that individuals with mandibular prognathia typically include more pronounced dento-alveolar compensation, that is, proclination of maxillary and retroclination of mandibular incisors (Spalj, Mestrovic, Lapter Varga & Slaj, 2008). These characteristics justify the selection of the individuals to undergo the genetic analysis.

The average hyperdivergent facial pattern, also noted in 2 out of the 8 selected families, along with a short posterior cranial base length or a short ramus height, also intersects with previous studies that noted a hyperdivergent facial pattern and excessive lower facial height as common characteristics of skeletal Class III patients compared to other malocclusions (Ellis & McNamara, 1984; Siriwat & Jarabak, 1985; Stapf, 1948).

In fact, a hyperdivergent facial pattern could represent a compensation of the underlying skeletal Class III. In contrast, some studies found that the hypodivergent pattern is the most common facial type in class III malocclusions (Mouakeh, 2001), while others described a normal vertical growth pattern (Spalj, Mestrovic, Lapter Varga & Slaj, 2008). The vertical facial pattern is not only related to bone development leading to the different malocclusions, but also to muscle development. In fact, the skeletal growth and form depends 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. In hyperdivergent subjects, the weaker forces possibly produced by passive stretching of hypofunctional muscles result in more eruption of the upper molars and less inhibition of periosteal bone apposition in the angular region, thus leading to vertical growth (Bresin, 1986 & Petrovic, 1982). 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).

Across the 8 selected families, the most severe features are noted in family B and to a lesser extent family H, as confirmed by mandibular length and Z score analyses. This finding and severity of the Z score in general, confirm our hypothesis that inheritance is mainly related to mandibular macrognathism.

Genetic analysis revealed the absence of a common gene, loci or variant being associated with MP between the present study and the results of previous studies undergone in different ethnic populations. This finding supports the polygenicity and locus heterogeneity in the development of MP in addition to the fact that the genes concerned in the etiology of this feature may be correlated with the ethnicity of the population. In other words, this result supports the strong genetic influence on MP and that each candidate gene would be responsible of a part of the etiology of this trait. Therefore, we cannot search for one universal gene in all affected individuals.

Following the several steps of filtering, a shared variant between affected individuals in family F suggested that the protein-coding gene *C1orf167* (located on chromosome 1, locus 1p36.22, variant position 11,844,520) could be a potential candidate gene for MP because its function is not determined yet, although it has an ORF, and its frequency is rare in the normal population. In addition, the genes *NBPF8* (highlighted in family H; located on chromosome 1, locus 1p11.2, variant position 144,828,764) and *NBPF9* (found in family B; located on chromosome 1, locus 1q21.2, variant position 145,368,525) that are part of the same *NBPF* family, also emerged as potential candidate genes for MP because they play a role in human evolution and their frequencies are rare in the normal population. In fact, *NBPF* gene family reflects the continuous evolution of primate genomes that resulted in large physiological differences. Because primate genomes indicate a recent expansion of the *NBPF* sequences resulting in species-specific genes, it can be speculated that *NBPF* genes played a role in the evolution of primates, including man (Vandepoele, Van Roy N., Staes, Speleman & Van Roy F., 2005). Because of evolution, it has been observed that in man, there is a marked reduction in facial prognathism, an increase in brain size, a

reduction in size of the dental arches and an increased incidence of malocclusion when compared with other primates. Even human evolution shows a reduction in facial prognathism over time, which is supported by comparison of the Habsburg family to facial features of the current population (Brothwell, 2014). *NBPF* gene family seems also to be involved in cancer (e.g. neuroblastoma, lung adenocarcinoma, breast cancer) and in brain and developmental disorders. Copy number variations in the *NBPF* gene have been found in patients with attention deficit hyperactivity disorder, intellectual disability and congenital anomalies, autism and schizophrenia (Alqallaf, Alkoot & Mash'el, 2013; Andries, Vandepoele & Van Roy, 2012). *NBPF8* was highlighted in family H that has a recessive inheritance pattern, because it has a role in human evolution and because another gene of the same *NBPF* family was also found in family B, despite the fact that *NBPF8* is heterozygous.

Therefore, those potentially novel genes *C1orf167, NBPF8* and *NBPF9* that segregate with the phenotype, could be implicated in mandibular development. Families F, H and B, where the 3 novel genes were found, have the most severe skeletal and dento-alveolar MP features (mainly families B and H, with Z scores over 3; 6.47 in family B), which reinforce our conclusive genetic findings. In addition, the fact that family H has an average hyperdivergent pattern suggests that the gene *NBPF8* could be involved in the etiology of this vertical growth pattern.

Previous genetic studies identified 7 susceptibility loci on chromosome 1 but when considering results of the present study, the number increases to 10, compared to 5 loci on chromosome 12 and 1-2 loci on the other chromosomes (Table V.1). In addition, some previous genetic reports correlated with MP the susceptibility loci 1p35.2, 1p35.3 and 1p36.12 (Jang et al., 2010; Xue, Wong & Rabie, 2010; Yamaguchi,

Park, Narita, Maki & Inoue, 2005), which are close to the locus 1p36.22 that harbors the novel gene *C1orf167*. Furthermore, in the study of Ikuno et al. (2014), which is one of the 3 previous reports that applied WES, the loci 1q32.2 and 1p22.3 were considered as susceptibility chromosomal regions. All these findings indicate that the chromosome 1 and specifically the region 1p35-36 is potentially highly linked to MP.

Table V.1: Summary and number of the susceptibility loci reported on each chromosome by previous studies and the present study.

Chromosome Number	Locus	Number of loci reported on each chromosome
1	1p22.1, 1p22.2, 1p22.3, 1p35.2, 1p35.3, 1p36.12, 1q32.2 **1p36.22, 1p11.2, 1q21.2 : present study**	10
3	3q26.2	1
4	4p16.1	1
5	5p12, 5p13	2
6	6q25	1
10	10p12.1, 10p12.3	2
11	11q22.2, 11q22.3	2
12	12q13.11, 12q13.13, 12q22, 12q23, 12q24.11	5
14	14q24.3, 14q31.2	2
19	19p13.2	1
21	21q21.3	1

As previously mentioned, the 3 common genes (*MPL, SLFNL1* and *SKI)* found in family F, *UPP2* gene found in family G, the 2 common genes (*OBSCN* and *PPIG*) found in family B and the 6 highlighted genes in family H *(PRR21, ALS2CL, MAML3, GRIFIN, RBBP6* and *TEX13A)* were not considered as potential candidate genes for MP because their functions are not related to the formation of the jaws and/or their MAF indicate a frequent occurrence for the minor allele in the normal healthy population:

- *MPL* gene: provides instructions for making the thrombopoietin receptor protein, which promotes the growth and proliferation of certain blood cells called megakaryocytes that produce platelets. The thrombopoietin receptor may also play a

role in the maintenance and renewal of hematopoietic stem cells and the regulation of hematopoiesis. Numerous *MPL* mutations have been identified in hematopoietic diseases (blood cancers, thrombocytopenia, familial aplastic anemia) that alter the normal regulatory mechanisms and lead to autonomous activation or signaling deficiencies (Chou & Mulloy, 2011; He, Chen, Jiang, Qiu & Zhao, 2013).

- *SLFNL1* gene: is an inducer of growth arrest in T-lymphocytes and causes a cell cycle arrest in thymocytes and NIH3T3 fibroblasts prior to the G_1/S transition by inhibiting induction of cyclin D1, which impairs thymocyte development. Overexpression of *SLFNL1* suppresses the proliferation and tube formation of endothelial progenitor cells in rats; conversely, knockdown of *SLFNL1* promotes the proliferation and tube formation of those cells. Therefore, this gene has a key role in the regulation of the biological behavior of the endothelial progenitor cells (Brady, Boggan, Bowie & O'Neill, 2005; Kuang, Yang, Zhang, Zhang, & Wu, 2014).
- *SKI* gene: provides instructions for making a protein involved in a signaling pathway that transmits chemical signals from the cell surface to the nucleus. This pathway, called the Transforming Growth Factor Beta (TGF-β) pathway, allows the environment outside the cell to affect how the cell produces other proteins. It helps regulate cell growth and division, the process by which cells mature to carry out special functions (differentiation), cell movement and the self-destruction of cells (apoptosis). The *SKI* protein controls the activity of the TGF-β pathway by binding to a certain group of proteins called SMAD complex, which keeps this complex from entering the nucleus and therefore interrupts signaling through the pathway and the activation of particular genes involved in regulation of various cellular processes. Binding of the *SKI* protein can also occur in the nucleus. In other words,

SKI has been shown to interfere with normal cellular functioning by both directly impeding expression of certain genes inside the nucleus of the cell as well as disrupting signaling proteins that activate genes. This role has been associated with various cancers including human melanomas, esophageal squamous cell carcinoma, cervical cancer and the process of tumor progression. The *SKI* protein is found in many cell types throughout the body and plays a role in the development of many tissues, including the skull, other bones, skin and brain since it is commonly active during development. It has also a role in the regulation of craniofacial, neuronal and skeletal muscle development as well as in the signaling networks underlying osteoblast differentiation and bone formation (Chen, Deng & Li, 2012; Luo, 2003).

- *UPP2* gene: is a protein-coding gene that catalyzes the reversible conversion of uridine and deoxyuridine to uracil and ribose- or deoxyribose-1-phosphate, implicating it in both pyrimidine salvage and regulation of uridine homeostasis. The produced molecules are then utilized as carbon and energy sources or in the rescue of pyrimidine bases for nucleotide synthesis. *UPP2* also plays an important pharmacological role in activating fluoropyrimidine nucleoside chemotherapeutic agents and in redox regulation. It may have additional functions in sensing and initiating cellular responses to oxidative stress. Its activity is usually elevated in various tumor tissues (Roosild, Castronovo, Villoso, Ziemba & Pizzorno, 2011; Russell, Cao, Zhang, Handschumacher & Pizzorno, 2001).
- *OBSCN* gene: its abnormality may be involved in the pathogenesis of hypertrophic and dilated cardiomyopathy phenotype via haploinsufficiency. *OBSCN* mutations have been associated also with several types of cancer and aspirin sensitivity in asthmatics (Arimura et al., 2007; Kim et al., 2012; Marston et al., 2015).

- *PPIG* gene: anchors the phosphatase to glycogen and increases its activity towards the glycogen-bound substrates: glycogen synthase and glycogen phosphorylase (Wera & Hemmings, 1995).
- *ALS2CL* gene: is a novel *ALS2* interacting protein that modulates the *ALS2*-mediated molecular and cellular functions (specifically membrane/endosome trafficking), either directly or indirectly. However, the molecular and cellular functions of *ALS2CL* and its functional relationship with *ALS2* are still unknown. The products of *ALS2CL* gene and other genes regulate biologic processes critical to, among others, autoimmunity, Alzheimer's disease, amyotrophic lateral sclerosis and coronary artery disease (Damani, 2011; Suzuki-Utsunomiya et al., 2007).
- *PRR21* gene: is a promoter probe vector for determining the presence of promoter regions in DNA segments and which segments permit microbiological expression of genetic information (Raveendra, Bhat R.S., Bhat S. & Kuruvinashetti, 2009).
- *MAML3* gene: is one of the essential positive regulators of the Notch signaling pathway that is involved in cell-cell communications in vivo. It plays also a role in cell death, nervous development and function and cell cycle (Oyama et al., 2011).
- *GRIFIN* gene: is a galectin-related extracellular matrix protein that is highly expressed in the lens and may be structurally important for its development. It is also expressed in vascular smooth muscle cells, playing a role in vascular elasticity and reactivity (Ogden et al., 1998).
- *RBBP6* gene: interacts with both tumor suppressor proteins p53 and Rb, promoting the degradation of p53 and thereby increasing cell proliferation and inhibiting apoptosis in cancer. However, despite its potential as an anti-cancer target and its

apparently close association with transcription and the cell cycle and apoptosis, very little is known about the function of *RBBP6* (Simons et al., 1997).

- *TEX13A* gene: is localized in the nuclei of spermatogenic cells and the redundant nuclear envelope of mature sperm. Its expression is testis- and germ cell-specific. It is involved in testicular development and has a potential role in transcriptional regulation during spermatogenesis (Kwon et al., 2016; Lee et al., 2003).

The last 5 genes were highlighted because they are homozygous and because the mode of inheritance is recessive in family H (1 affected individual only).

The function of the several other common genes was not explored because they have a high frequency in the normal population. Thus, they can't be potentially linked to MP.

Our research group hypothesized that macrognathism might be a form of bilateral condylar hyperplasia (hypercondylosis) particularly in patients where a Class III or macrognathia is not a documented inherited trait in the family. In this instance, the following observations emerge:

1- In line with the evidence regarding the time of occurrence of unilateral condylar hyperplasia, macrognathism would develop in adolescents, and would not be recognized at earlier developmental stages in childhood.

2- Such condition would not include the development of gigantism, which in addition to macrognathism involves more generalized craniofacial development and augmented sizes of limbs.

3- Bilateral condylar hyperplasia would not be genetically determined, unless condylar hyperplasia was. No evidence addresses this particular issue, except one isolated report of unilateral condylar hyperplasia that was reported in a two brothers and their father (Yang, Lignelli & Ruprecht, 2004).

Recent findings intersect our hypothesis, whereby the condyles of patients with unilateral condylar hyperplasia were similar in volume to the condyles of patients with MP, and presented statistical differences when compared with the non-hyperplastic condyles (Goulart, Muñoz, Olate, de Moraes & Fariña, 2015; Goulart, Muñoz, López, de Moraes & Olate, 2017). Much research should be invested in this hypothesis to sort out its plausibility.

C. Strengths and limitations

1. Strengths

The most important strength of this study is its novelty because it is the first genetic study on large families with MP worldwide using NGS. Also, no previous genetic study was undergone in the Mediterranean population, indicating that the genetic determinants of MP in this ethnic population were completely unclear. Therefore, identifying the candidate genes responsible of the development of MP in this population is a new contribution, especially that many families with several affected individuals over many generations were observed. Consequently, the genetic determinants of MP and the variation in the risk for MP in this population are now better understood.

This result was possible through the application of the revolutionizing sequencing technique "Whole Exome Sequencing (WES)", which is part of the Next Generation Sequencing (NGS) technology that has many advantages over the traditional sequencing method (see chapter II, section A.5.b, page 18). In fact, WES was preferred in this study as it covers most coding variations, including missense, nonsense, splice site and small deletion or insertion mutations. WES can also reveal the frequency of

shared variations in the population and their possible effects on the phenotype, allowing the identification of a number of true candidate genes. Thus, WES approach provides an unbiased analysis of variations in human coding sequences. In this study, sequencing was performed by Macrogen Laboratory, which has served 18 years in the sequencing field using the cutting edge technology and is known as a world leading genetic service provider that delivers fast and reliable results (Bamshad et al., 2011; Macrogen, n.d.; Majewski, Schwartzentruber, Lalonde, Montpetit, & Jabado, 2011). WES using NGS technology was applied on families in the studies of Guan et al. (2015), Nikopensius et al. (2013) and Perillo et al. (2015); however, they included only 1 Chinese family, 1 Estonian family and 1 Italian family respectively, each involving a small number compared to the 8 families that underwent massive parallel sequencing in the present study. In fact, in the 3 earlier studies, WES was only performed on 5 affected individuals (Guan et al., 2015), 4 affected and 1 non-affected individuals (Nikopensius et al., 2013) and 2 affected individuals (Perillo et al., 2015), a combined total of 11 affected and 1 non-affected subjects. In the present study, WES was performed on 26 affected and 21 non-affected individuals.

In addition, genetic screening did not show any aberration in the reported genes linked to MP that were revealed in the earlier genetic studies. Therefore, another strength of this study is the recognition of 3 potentially novel genes (*C1orf167, NBPF8* and *NBPF9)* 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. The identification of those genes was possible through the application of Whole Exome Sequencing (WES), which allowed the exploration of all the genes, variants and chromosomes of each selected individual. This technique was

also applied in other studies (Guan et al., 2015; Ikuno et al., 2014; Nikopensius et al. 2013; Perillo et al., 2015; Yamaguchi et al., 2005). In contrast, some researcher did not explore all the genes of each individual but instead, only ran a search for specific genes or loci that were found by previous studies (Cruz et al., 2017; Jang et al., 2010; Tassopoulou-Fischell et al., 2012; Xue et al., 2010, 2014).

A significant contribution of this study is also in its design, including subjects and families with MP, excluding Class III malocclusions underlined by only maxillary retrognathism. By eliminating a potential confounder presumed to be mainly an environmentally-induced mesioclusion, this differentiation was thought to provide a better genetic delineation of the condition and as such, would provide a guideline to which phenotype is more amenable to orthodontic/orthopedic intervention, and which (the inherited mandibular macrognathism) might only be treated through orthognathic surgery.

Furthermore, the diagnosis of MP in this study was based on several cephalometric measurements: ANB $\leq$ 0 degrees, AOBO $\geq$ -2mm, normal/long ANS-PNS, anterior edge to edge, cross bite or overjet because of severely proclined maxillary incisors and most importantly Co-Gn > 1 standard deviation of the norm for the specific age. This criterion meant that subjects diagnosed as affected had an increased mandibular length (mandibular macrognathism), which was not the case in previous studies. In fact, a limitation of all previous genetic reports is the lack of appropriate definition the MP phenotype that should be based on several morphologic characteristics of the craniofacial complex (mainly increased mandibular length), not only on dental characteristics, facial features or a limited number of cephalometric measurements. In some studies, patients were diagnosed as having MP if they had only

an ANB angle ≤ 0.0 degrees (Guan et al., 2015; Ikuno et al., 2014; Yamaguchi et al., 2005) or ≤ -2.0 degrees (Xue et al., 2010) or an ANB angle ≤ 0.0 degrees and a Wits appraisal ≥ -2.0mm (Frazier-Bowers et al., 2009; Li et al., 2010, 2011; Nikopensius et al., 2013; Xue et al., 2014), in addition to an anterior cross bite, a Class III molars relationship and/or a straight or concave profile (Frazier-Bowers et al., 2009; Guan et al., 2015; Nikopensius et al., 2013; Xue et al., 2014). Only in one study, the diagnosis of MP was based on a mandibular base/cranial base length ratio of ≥ 3.5mm up to 12 years and ≥ 5mm over 12 years (Perillo et al., 2015).

The sample used in this study constitutes by itself a strength because it included several families with affected individuals over 3-4 generations, demonstrating clearly the segregation of the phenotype across generations. This allowed comparisons to find common genes across individuals of the same family and across different families. Other studies had included non-related individuals (Cruz et al., 2017; Ikuno et al., 2014; Tassopoulou-Fischell et al., 2012; Xue et al., 2010, 2014) or only 1-2 families (Guan et al., 2015; Li et al., 2010, 2011; Nikopensius et al., 2013; Perillo et al., 2015).

The present study involved also non-affected individuals of each family that served as controls when comparing the genes and variants across individuals and families, as well as in cephalometric appraisals. This represents a major common advantage to some studies (Cruz et al., 2017; Ikuno et al., 2014; Jang et al., 2010; Li et al., 2010; Xue et al., 2010), compared to others that included only affected individuals (Yamaguchi et al., 2005), which did not allow a comparison of the genetic characteristics of affected and non-affected individuals.

2. Limitations

Some limitations were related to specific families:

1- In family E that has a recessive inheritance pattern, a large number (232) of common genes were found between the 2 affected individuals (non-affected individuals not excluded), but this number was reduced to 14 when the non-affected subjects were filtered out. However, no potential candidate gene for MP could be noted in this family. This is partly due to the fact that blood collection and analysis was done on 3 children and their mother without the inclusion of the father who was deceased, thus constituting a limitation of this study. Had the father been integrated in this research, conclusions may have been drawn on a potential susceptibility gene for MP.

2- Because of the large number of common genes between affected individuals of family B, it was not possible to carefully examine characteristics of all those variants before doing the subfiltering steps.

Another limitation resides in the fact that the function of the novel susceptibility gene *C1orf167* was not discovered. A transgenic study would be needed, which is beyond the scope of the present project in terms of budget.

Finally, 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 3 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 3 novel genes are not present in these databases.

CHAPTER VI
CONCLUSIONS AND RECOMMENDATIONS

A. Conclusions

In both the developmental and clinical contexts, this study represents a significant advance in the fields of orthodontics and dentofacial orthopedics and in oral and maxillofacial surgery. It is the first genetic study on large families with MP worldwide using NGS to better understand the variations and risks for this condition.

A visual inspection of the pedigrees corresponding to the 51 Mediterranean families approached during this study suggests an autosomal dominant mode of inheritance of MP with incomplete penetrance. Analysis indicates an equal number of reported generations per family (n=3), an equal number of reported affected males and females per family (n=2) and more families with male predominance. Analysis also suggests that consanguinity could be related to the familial transmission of this trait.

Skeletal cephalometric measurements of affected individuals indicated increased mandibular length (mandibular macrognathism), normal maxillary length, tendency to a hyperdivergent facial pattern, and skeletal Class III malocclusion underlined by an orthognathic maxilla and a prognathic mandible. Dento-alveolar cephalometric measurements denoted proclined maxillary incisors and retroclined mandibular incisors in compensation for the underlying skeletal discrepancy, with an anterior crossbite. Mandibular length evaluation increased our confidence that inheritance was mainly related to mandibular macrognathism.

Results from the linkage analysis performed on 8 Mediterranean families including 49 individuals, indicated that chromosome 1 (loci 1p36.22, 1p11.2 and

1q21.2), which harbors the novel genes *C1orf167, NBPF8* and *NBPF9* that segregate with the phenotype, is suggestive of linkage to familial MP in the Mediterranean population. The characteristics of those genes include: heterozygosity, stop gained effect, high putative impact, coding transcript biotype and MAF equal to ".". These results are not consistent with previous genetic studies, which show that 3 novel genes were found to be associated with the development and familial transmission of MP in the Mediterranean population. This indicates that if the genes *C1orf167, NBPF8* or *NBPF9,* which were found in families having the most severe skeletal and dento-alveolar MP features, are present in the genotype of an individual, the latter may express the trait. In addition, it was hypothesized that the gene *NBPF8* could be involved in the etiology of the hyperdivergent facial pattern.

Identifying the candidate genes responsible of the development and familial transmission of MP is an important advancement in our field. By better understanding the specific genes contributing to variation in the risk for MP in the Mediterranean population, estimating the genetic susceptibility to this condition in families with affected individuals should be facilitated. This knowledge shall contribute also to improved comprehension of the molecular mechanisms of jaw development and treatment of associated malocclusions and deformities. Early forecast of the condition would lead to 1- earlier treatment that might reduce severity of the condition and possibly avoidance of later orthognathic surgery or 2- probably more realistically, forego earlier interventions in favor of a later orthognathic surgery when true MP is genetically determined as a "certainty." This would allow a more efficient and personalized approach of prevention/treatment, and would avoid the side effects of long treatments.

B. Recommendations

Following the recent advances in molecular genetic studies investigating the candidate genes associated with MP, it would be beneficial in families with a history of affection status to have a blood test and assess the individuals' genes for early forecast the condition, if present. This identification would allow a more rational and effective approach to therapy through a better determination of the borderline between patients who can be treated non-surgically (orthopedically) and those in whom orthognathic surgery is a necessity. Eventually, the genetic test (involving blood collection, DNA extraction and NGS) should become a diagnostic tool that is part of the routine records requested for an accurate diagnosis in orthodontic practice.

In addition, since craniofacial growth, and particularly mandibular growth, is highly variable and is reported to continue into the late developmental stages, when setting up a treatment plan for a patient with affected by MP, a close observation and follow-up of midfacial and mandibular growths during adolescence are essential to ensure stability of the effects of the applied treatment. This would lead to a proper interception of a potential developing MP in patients where excessive forward mandibular growth occurred after treatment completion.

C. Directions for future research

From an educational perspective, little is known about the interaction between genetic and environmental factors in the causation of MP. Therefore, further studies in molecular biology are needed to disclose the gene-environment interactions associated with the phenotypic diversity of MP and the heterogenic developmental mechanisms thought to be responsible for them. Future research studies integrating both factors with

carefully characterized phenotypes will ultimately lead to identification of the etiologic genetic and environmental factors that lead to MP.

In addition, a prospective association study can be designed in the future to conduct the phenotypic and genetic analyses on subjects divided into three groups. These groups could be: individuals affected by true MP, those affected by maxillary retrognathism, and a group with the combination of both MP and maxillary retrognathism.

Furthermore, because of the tremendous complexity of this skeletal jaw disharmony, it is conceivable that in the future, classification by the clinician of this distinct trait would not be based only on its morphology, but primarily on its molecular genetic composition. Accordingly, future studies involving whole genome sequencing (WGS) analysis will need to further characterize the genotype-phenotype relationship to provide a biological basis for prevention and treatment decisions according to the genetic profile. In addition, molecular genetic information could be used to accurately predict long-term growth changes and may ultimately lead to the utilization of gene therapy.

Moreover, function of the novel susceptibility gene *C1orf167* should be discovered using a transgenic study, which is a necessary step to better understand the etiology of MP. It would be interesting also to test the involvement of the 3 candidate genes *C1orf167*, *NBPF8* and *NBPF9* in bone and muscle development.

Additionally, new genetic mapping studies with larger sample size using WGS technology would allow a better understanding of the genetic determinants of facial development in general and MP in particular. It would be interesting to study the heritable patterns of each skeletal morphologic characteristic that may contribute to MP.

For this purpose, the sample of selected families in this study can be enlarged by including in the WES approach the remaining 6 families that underwent the data and biospecimen collection procedure but not the genetic analysis (due to the limited budget).

Genetic studies are also indicated to test the involvement of *C1orf167, NBPF8* and *NBPF9* genes in other ethnic populations. Future studies incorporating individuals of different ethnic populations might also lead to discovery of common genes in different ethnic groups.

Finally, future research on a larger number of pedigrees would lead to more conclusive findings regarding pedigree analysis.

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Appendix I

Invitation to Participate in a Research Study

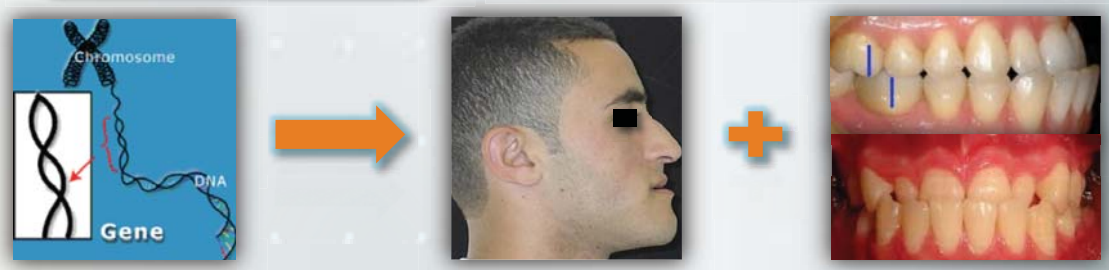
You are invited to participate in a research study entitled *"Association between genes and familial mandibular prognathism in the Mediterranean population"*, conducted by *Dr. Anthony Macari* (Faculty of Medicine - *Division of Orthodontics and Dentofacial Orthopedics at the American University of Beirut*). The conduct of this study will adhere to the IRB approved conditions and terms.

PURPOSE

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

INCLUSION

- Families known to have subjects affected by mandibular prognathism (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 included 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 prognathism in our population,
- the genetic mechanisms that may influence the response of the lower jaw to orthodontic and orthopedic treatment.

- Early prediction of the condition would lead also to variation in the approach of treatment.

COMMITMENT REQUIRED

The study will require you to pass by AUBMC for two visits:

- the first one for 30 minutes.
- the second one for 15 minutes.

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 01/350000 ext 5702.

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 وبروز الفك السفلي العائلي لدى سكان منطقة البحر المتوسط** يجريها الدكتور أنطوني مكاري. ستجرى الدراسة في الجامعة الأميركية في بيروت (قسم تقويم الأسنان وتأهيل الفكّين). تسير مجريات الدراسة وفقاً للشروط والأحكام الموافق عليها من قبل لجنة الاخلاقيات.

أهداف الدراسة

- استكشاف ما إذا كان يتم توريث حالة بروز الفك السفلي (تقدّم حجم ومكان الفكّ السفليّ)
- المساعدة في تحديد الجينات المسؤولة عن تطوير وانتقال هذه الحالة وراثيّاً.

التضمين

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

الفوائد الناتجة عن المشاركة في الدراسة

- لن تحصل على أي مردود مالي مقابل مشاركتك في الدراسة.
- إنها 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 شعبنا.
  - الآليات الوراثية التي تؤثر على تفاعل الفكّ السفليّ مع المعالجة التقويمية و العظمية.
- وقد يؤدي التنبؤ المبكر للحالة إلى تغير في نهج العلاج.

الالتزام المطلوب

سيتطلب هذا الأمر منك أن تقوم بزيارة AUBMC مرّتين:

- الزيارة الاولى مدتها ٣٠ دقيقة.
- الزيارة الثانية مدتها ١٥ دقيقة.

موقع الدراسة

قسم تقويم الأسنان وتأهيل الفكّين، المركز الطبي في الجامعة الأميركية، شارع الحمرا / بيروت، لبنان.

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 انطوني مكاري على الرقم 01350000 مقسم 5702.

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)

ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR PROGNATHISM IN THE MEDITERRANEAN POPULATION	**Participant name:**
Site where the study will be conducted: Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC.	**Principal investigator: Dr. Anthony MACARI** **American University Medical Center** **Hamra Street/Beirut, Lebanon** **Phone: 01350000 ext: 5702**

We want to tell you about a research study we are doing.
A research study is a special way to find out some facts about something.

We are trying to find out more about the genes involved in the development of your lower jaw because it is placed forward, which positions your lower teeth more forward 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 by the study coordinator (Dr. Pamela Genno) who will explain the objectives, procedures, benefits/risks of the study and ask you if you are willing to participate in the research project. If you agree, you will be asked to sign this consent form. After signing it, you will be asked 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 the flyer handed to you by him/her.

1. What is involved in the study?

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

Step	Procedure
1	-The orthodontist will look at your teeth and will take one x-ray of your profile (if features of the condition are noted clinically). -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 forward 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.
2	-A nurse or a 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 forward.

This will require you to come to AUBMC for two visits: the first one (30minutes) to explain the project in details, sign this consent form, have your teeth checked and an X-ray taken (if features of the condition are noted clinically), and the second visit (15minutes) 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. You might get a bruise on your arm. 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 the 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 for 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 am in the study?

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

6. What happens if I get hurt?

The medical team has sufficient knowledge to take care of you, if you get hurt during the study. 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 do not want to do this?

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

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

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

_____	_____
Child's name	**Signature of the child**
_____	_____
Person obtaining assent	**Signature**
	_____
	Date & Time

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

الموافقة للإشتراك في البحث العلمي للمشترك القاصر

قاصرون تتراوح أعمارهم بين 7 سنوات و 12 سنة

الارتباط بين الجينات وبروز الفك السفلي العائلي لدى سكان منطقة 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 تُستخدم لمعرفة حقائق حول شيىء ما.

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

- اذا كنت مريضاً تتلقى العلاج في قسمنا: خلال موعدك الطبّي، ستشرح لك منسقة الدراسة (د. باميلا جنو) أهداف الدراسة، إجراءاتها، وفوائدها / مخاطرها، وستسألك اذا كنت ترغب في المشاركة فيها. اذا وافقت، سيُطلب منك الامضاء على استمارة الموافقة هذه. بعد امضائها، ستُسأل عن تاريخ عائلتك، بما في ذلك حالة تقدم 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	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 متقدّم إلى الأمام 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.
2	ستسحب ممرضة أو طبيب 5 سنتيمتر مكعّب من الدم من ذراعك باستخدام إبرة نظيفة. سيتم ذلك لمعرفة ما هي الجينات التي تتسبب بتقدّم فكك السفلي إلى الأمام.

سيتطلب هذا الأمر منك أن تقوم بزيارة AUBMC مرّتين: الزيارة الأولى (30 دقيقة) لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء استمارة الموافقة هذه، ولمعاينة أسنانك وأخذ صورة بالاشعة السينية جانبية للرأس (في حال وجود علامات الحالة عند معاينة اسنانك). الزيارة الثانية (15 دقيقة) لنأخذ 5 سنتيمتر مكعّب من دمك.

الأحرف الأولى لاسم القاصر:

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. يرجى ملاحظة أنه قد تكون هناك مخاطر غير متوقعة.

3. هل ممكن أن يحصل لي أي شيء جيد ؟

اذا تمكنا من حلّ مشكلتك من خلال العلاج بمشابك الأسنان، فسيكون هذا أفضل ما نقدّمه 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؟

يتمتع الفريق الطبي بالمعرفة الكافية التي تخوّله الاعتناء بك في حال تعرّضت لأي أذى أثناء الدراسة. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جداً.

قبل ان تقول نعم للمشاركة في الدراسة، عليك أن تسأل الدكتور أنطوني مكاري عن جميع الأمور التي لم تفهمها ليشرحها لك.

7. ما هي التكاليف؟

ليس ثمة أي تكاليف مرتبطة بالمشاركة. ستغطي الدراسة تكاليف الاجراءت المطلوبة لتحقيق اهدافها: سحب الدم، التحليل الجيني والأشعة السينية التي ستوخذ 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.
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

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)

ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR PROGNATHISM IN THE MEDITERRANEAN POPULATION	**Participant name:**
Site where the study will be conducted: Division of Orthodontics and Dentofacial Orthopedics, 6th floor, AUBMC.	**Principal investigator: Dr. Anthony MACARI** **American University Medical Center** **Hamra Street/Beirut, Lebanon** **Phone: 01350000 ext: 5702**

We want to tell you about a research study we are doing.
A research study is a special way to find out some facts about something.

We are trying to find out more about the genes involved in the development and familial transmission of mandibular prognathism (advanced lower jaw size and position) 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 by the study coordinator (Dr. Pamela Genno) who will explain the aims, procedures, benefits/risks of the study and ask you if you are willing to participate in the research project. If you agree, you will be asked to sign this consent form. After signing it, you will be asked 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 the flyer handed to you by him/her.

1. What is involved in the study?

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

Step	Procedure
1	-The orthodontist will look at your teeth and will take one x-ray of your profile (if features of the condition are noted clinically). -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 forward 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. ***"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 a 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 forward.

This will require you to pass by AUBMC for two visits: This will require you to pass by for two visits: the first one (30minutes) to explain the project in details, sign this consent form, have a clinical examination and a lateral cephalometric X-ray (if features of the condition are noted clinically), and the second visit (15minutes) 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 radiation 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 prognathism 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 later surgery or

2- more importantly, forego earlier interventions in favor of a later orthognathic surgery when true mandibular prognathism is genetically determined as a "certainty."

4. What other options are there?

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

6. What about confidentiality?

If you agree to participate in this research study, **every reasonable effort will be made to keep your records confidential.**

Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental agencies will have direct access to your medical records.

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

Depending on your request, the results of the study will be disclosed to you and to your family members as soon as the study is completed (approximate date: September 2017). The study doctor can use the study results as long as you cannot be identified.

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

7. What if you are injured in the study?

The medical team has sufficient knowledge to take care of you, if you get hurt during the study. But you should know that this 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.

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 prognathism 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. 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 الأميريكية في بيروت
كلية الطب
شارع بلس
بيروت، لبنان
هاتف: 350000-(01) مقسم: 5445

الموافقة للإشتراك في البحث العلمي للمشترك المراهق

مراهقون تتراوح أعمارهم بين 13 و 17 سنة

الارتباط بين الجينات وبروز الفك السفلي العائلي لدى سكان منطقة 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 تُستخدم لمعرفة حقائق حول شيىء ما.

إننا نحاول معرفة المزيد حول الجينات المعنية بتطوير وانتقال بروز الفك السفلي وراثيّاً (تقدّم حجم ومكان الفكّ السفليّ) لدى سكان منطقة البحر المتوسط. أنت مدعو للانضمام إلى الدراسة:

- اذا كنت مريضاً تتلقى العلاج في قسمنا: خلال موعدك الطبّي، ستشرح لك منسقة الدراسة (د. باميلا جنو) أهداف الدراسة، إجراءاتها، وفوائدها / مخاطرها، وستسألك اذا كنت ترغب في المشاركة فيها. اذا وافقت، سيُطلب منك الامضاء على استمارة الموافقة هذه. بعد امضائها، ستُسأل عن تاريخ عائلتك، بما في ذلك حالة بروز 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	سينظر طبيب تقويم الأسنان إلى أسنانك وسيأخذ صورة بالأشعة السينية ملتقطة من الجانب (اذا تم تدوين خصائص الحالة سريرياً). 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 متقدّم إلى الأمام 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. **"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 بتقدّم فكك السفلي إلى الأمام.

الأحرف الأولى لاسم القاصر:

سيتطلب هذا الأمر منك أن تقوم بزيارة AUBMC مرّتين: الزيارة الأولى (30 دقيقة) لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء استمارة الموافقة هذه، ولتخضع لفحص سريري ولصورة أشعة سينية جانبية لقياسات الرأس (اذا تم تدوين خصائص الحالة سريرياً). الزيارة الثانية (15 دقيقة) لنأخذ 5 سنتيمتر مكعّب 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 1.7 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، اسأل الطبيب المسؤول عن الدراسة. يرجى ملاحظة أنه قد تكون هناك مخاطر غير متوقعة.

3. هل هناك فوائد من 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.

ستسمح لنا هذه الدراسة بأن نفهم أكثر الجينات المعنية التي تساهم في تفاوت خطر بروز 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 بروز الفك السفلي محدد وراثياً على أنه حتميّ.

4. ما هي الخيارات الأخرى؟

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

5. هل يمكن اخراجك من الدراسة بدون موافقتك؟

لن يتم اخراجك من الدراسة من قبل الفريق المختص بالدراسة من دون موافقتك.

6. ماذا عن السريّة؟

اذا وافقت على المشاركة في الدراسة، **سيتم بذل كل جهد معقول للحفاظ على السجلات الخاصة بك بطريقة سرية.**

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 وتُحفظ في بنك الصور الشعاعية الذي تم احداثه وضمّه 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 التخلّص منها في نهاية الدراسة.

بناءً على طلبك، سيتم الكشف عن نتائج الدراسة لك ولأفراد عائلتك بمجرد الانتهاء من الدراسة. (التاريخ المتوقع : أيلول 2017). ويمكن للطبيب المسؤول عن الدراسة استخدام نتائج الدراسة ما دمت غير مُعرّف.

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

الأحرف الأولى لاسم القاصر:

7. ماذا يحصل لو أصابك أذى خلال الدراسة؟

يتمتع الفريق الطبي بالمعرفة الكافية التي تخوّله الاعتناء بك في حال تعرّضت لأي أذى أثناء الدراسة. ولكن يجب أن تعلم أن هذا الخطر ضئيلٌ جداً.

8. ما هي التكاليف؟

ليس ثمة أي تكاليف مرتبطة بالمشاركة. ستغطي الدراسة تكاليف الاجراءت 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 طوعيّ. لست مجبراً على المشاركة في الدراسة اذا لم ترد ذلك، كما أنه يمكنك الانسحاب من الدراسة في أي وقت. قرارك هذا لن يؤدي إلى أي عقوبة أو خسارة المزايا التي تملكها الآن. اذا لديك أسئلة تتعلّق بحقوقك، يمكنك الاتصال بـ:

لجنة الأخلاقيات على 01350000 مقسم: 5445

سيتم إعلامك عن أي معلومات جديدة قد تؤثر على صحتك، عافيتك، 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 وبروز الفك السفلي العائلي لدى سكان منطقة البحر المتوسط" وأنه قد تسنّت لك فرصة طرح أسئلة لتساعدك على فهم ما ستقوم به في هذه الدراسة. كما أن توقيعك يعني أنه قيل لك بأنه يمكنك تغيير رأيك اذا شئت. سيتم اعطاءك نسخة من نموذج الموافقة هذا. وأنك، من خلال توقيع نموذج الموافقة هذا، لا تتخلى عن أي من حقوقك القانونية.

_____	_____
اسم المشترك	السنّ
_____	_____
توقيع المشترك (13 سنة - 17 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 consent to participate in a genetic research study	Participant name:
ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR PROGNATHISM IN THE MEDITERRANEAN POPULATION	**Investigator: Dr. Anthony MACARI** **American University Medical Center** **Hamra Street/Beirut, Lebanon** **Phone: (01) 350 000 ext 5702**

You are being asked to participate in a clinical research study conducted at the American University of Beirut (Division of Orthodontics and Dentofacial Orthopedics; Department of Otolaryngology and Head and Neck Surgery):

- If you are a patient treated in our division: during your orthodontic appointment, you will be approached by the study coordinator (Dr. Pamela Genno) who will explain the aims, procedures, benefits/risks of the study and ask you if you are willing to participate in the research project. If you agree, you will be asked 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 the flyer handed to you by him/her.

Please: 1- take time to read the following information carefully **before you decide whether you want to take part in this study or not.**

2- 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. Aims of the study

The aims of the study are to explore whether the condition of mandibular prognathism (advanced lower jaw size and 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 prognathism. 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	Respond to questions about your family histories and the affection status of other individuals in the family.
2	Have a clinical examination to determine whether your bite corresponds to the condition.
3	If features of the condition are noted clinically, have an x-ray of the face (lateral cephalometric x-ray) taken in our clinic to help us confirm that you are affected by mandibular prognathism, and if needed, impressions of the teeth for dental casts and photographs of the teeth and face. *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.* ***"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	Subsequent to the analysis of the x-ray, a pedigree (a form of family tree) will be drawn to find out who are the subjects in the family affected by mandibular prognathism.
5	5cc of your blood will be collected, which will allow us of isolate the DNA from your whole blood cells. Your DNA will be stored in coded tubes at -80 degrees at the Core Facilities at the American University 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.

All above procedures, including the genetic study, will be conducted in our clinic and facilities within the Medical Center of the American University of Beirut. This will require you to pass by for two visits: the first one (30minutes) to explain the project in details, sign this consent form, have a clinical examination and a lateral cephalometric X-ray (if features of the condition are noted clinically), and the second visit (15minutes) to have 5cc of your blood collected.

2. Risks as a result of participating in the study

Risk	Procedure
Radiation risk	Although there are no proven harmful effects from radiation 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.

Please note that there may be unforeseeable risks.

3. Benefits as a result of participating in the study

There is no direct benefit to you as a person. However, this study has benefits to the group to which you belong and 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 prognathism 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 later surgery or
2- more importantly, forego earlier interventions in favor of a later orthognathic surgery when true mandibular prognathism 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.

4. What other options are there?

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

5. Confidentiality

If you agree to participate in this research study, the information will be kept **confidential**. Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental agencies will have direct access to your medical records.

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

Depending on your request, the results of the study will be disclosed to you and to your family members as soon as the study is completed (approximate date: September 2017).

☐ I want to be informed of the results of the study.

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

6. 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.

7. Will you get paid to be in this study?

You will not be paid to participate in this study.

8. Agreement for the use of samples for genetic testing

Your participation is optional and you can stop it any time you like. **No negative consequences** whatsoever will affect you if you decide not to participate. If you agree to participate, please fill in the following:
I permit coded use of my biologic 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, ☐ 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 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 01-350000 ext. 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time, 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, ☐ legal representative, or ☐ parent/guardian	**Signature**
	Date & Time
Witness's name	**Signature**
	Date & Time

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:
الارتباط بين الجينات وبروز الفك السفلي العائلي لدى سكان منطقة 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):

- اذا كنت مريضاً تتلقى العلاج في قسمنا: خلال موعدك الطبّي، ستشرح لك منسقة الدراسة (د. باميلا جنو) أهداف الدراسة، إجراءاتها، وفوائدها / مخاطرها، وستسألك اذا كنت ترغب في المشاركة فيها. اذا وافقت، سيُطلب منك الامضاء على استمارة الموافقة هذه. بعد امضائها، ستُسأل عن تاريخ عائلتك، بما في ذلك حالة بروز 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.

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 بروز الفك السفلي (تقدّم حجم ومكان الفكّ السفليّ) والمساعدة في تحديد الجينات المسؤولة عن تطوير وانتقال هذه الحالة وراثيّاً. بما أنه من المتوقع أن يكون في لبنان والمنطقة العديد من المشاركين، لقد صنّفنا هؤلاء المشاركين كممثّلي سكان منطقة البحر المتوسط.

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

الخطوة	الإجراءات
1	الإجابة عن أسئلة حول تاريخ عائلتك ومدى تأثّر أفراد آخرين في الأسرة.
2	تخضع للفحص السريري لتحديد ما إذا كانت العضة الخاصة بك تتطابق مع الحالة.
3	اذا تم تدوين خصائص الحالة سريرياً، القيام بصورة بالأشعة السينسة للوجه (صورة جانبية بالأشعة السينية لقياسات الرأس) في عيادتنا لمساعدتنا علىالتأكد من أنك مصاب ببروز الفك السفلي، اضافةً إلى انطباعات الأسنان، اذا لزم الأمر، من أجل قالب الجبس للأسنان وصور للأسنان والوجه. **إذا كنت مريضاً 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".**
4	عقب تحليل الأشعة السينية، سيتم رسم شجرة النسب (شكل من أشكال شجرة العائلة) لمعرفة من هم الأفراد في الأسرة المصابين ببروز الفك السفلي.
5	سيتم أخذ 5 سنتيمتر مكعّب 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 تسلسل الجيل القادم. سيتم جمع البيانات في الجامعة الأميركية في بيروت وتحليلها بعد ذلك بغية اكتشاف أي تغيّرات التي يمكن أن تسبب مرضك.

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 بزيارتنا مرّتين: الزيارة الأولى (30 دقيقة) لنشرح لك المشروع بالتفصيل، لتقوم بإمضاء استمارة الموافقة هذه، ولتخضع لفحص سريري ولصورة أشعة سينية جانبية لقياسات الرأس (اذا تم تدوين خصائص الحالة سريرياً). الزيارة الثانية (15 دقيقة) لنأخذ 5 سنتيمتر مكعّب 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 1.7 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 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 بروز 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 بروز 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 مشاركتك في هذه الدراسة.

5. السرية

اذا وافقت على المشاركة في الدراسة، ستحفظ المعلومات **بسريّة**. 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 وتُحفظ في بنك الصور الشعاعية الذي تم احداثه وضمّه 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 التخلّص منها في نهاية الدراسة.

بناءً على طلبك، سيتم الكشف عن نتائج الدراسة لك ولأفراد عائلتك بمجرد الانتهاء من الدراسة (التاريخ المتوقع:أيلول 2017).

☐ **أريد أن أبلّغ بنتائج الدراسة.**

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

6. ما هي التكاليف؟

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

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

لن تحصل على أي مردود مالي مقابل مشاركتك في الدراسة.

8. 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. 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 لاحقاً 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، ☐ الممثل القانوني، أو ☐ والد، والدة / وصي)،
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 تمّت الإجابة على جميع أسئلتي. وأنا أوافق طوعاً على المشاركة في هذه الدراسة البحثية وأعلم أنه يمكنني الاتصال بالدكتور أنطوني مكاري على الرقم 01350000 مقسم 5702 أو بمن ينوب عنه في الدراسة في حال لديّ أي سؤال.

إذا شعرت أنه لم يتم الاجابة على أسئلتي، يمكنني الاتصال بلجنة الأخلاقيات لحقوق الانسان على الرقم 01350000 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 الموقّعة.

اسم ☐ المريض، ☐ الممثل القانوني، أو ☐ والد، والدة / وصي	**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

Parental consent to participate in a genetic research study	Participant name:
ASSOCIATION BETWEEN GENES AND FAMILIAL MANDIBULAR PROGNATHISM IN THE MEDITERRANEAN POPULATION	**Investigator: Dr. Anthony Macari** **American University Medical Center** **Hamra Street/Beirut, Lebanon** **Phone: (01) 350 000 ext 5702**

Your child is being asked to participate in a clinical research study conducted at the American University of Beirut (Division of Orthodontics and Dentofacial Orthopedics; Department of Otolaryngology Head and Neck Surgery):

- If he/she is a patient treated in our division: during his orthodontic appointment, you will be approached by the study coordinator (Dr. Pamela Genno) who will explain the aims, procedures, benefits/risks of the study and ask you if you are willing to let your child participate in the research project. If you agree, you will be asked 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 the flyer handed to you by him/her.

Please: 1- take time to read the following information carefully **before you decide whether you want him to take part in this study or not.**

2- 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. Aims of the study:

The aims of the study are to explore whether the condition of mandibular prognathism (advanced lower jaw size and 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 prognathism. 30 subjects will be recruited. **Your child's enrolment is not obligatory.** If you let him participate, he will commit to go through the following steps:

Step	Procedure
1	Respond to questions about the family histories, including the affection status of other individuals in the family.
2	Have a clinical examination to determine whether his bite corresponds to the condition.
3	If features of the condition are noted clinically, Have an x-ray of the face (lateral cephalometric x-ray) taken in our clinic to help us confirm that he is affected by mandibular prognathism, and if needed, impressions of the teeth for dental casts and photographs of the teeth and face. ***If he 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. "Your child may not participate in this study if she is pregnant. If she is capable of becoming pregnant, a***

	pregnancy test will be performed before she is exposed to any radiation. You must tell us if she may have become pregnant within the previous 14 days because the pregnancy test is unreliable during that time."
4	Subsequent to the analysis of the x-ray, a pedigree (a form of family tree) will be drawn to find out who are the subjects in the family affected by mandibular prognathism.
5	5cc of his blood will be collected, which will allow us of isolate the DNA from his whole blood cells. His DNA will be stored in coded tubes at -80 degrees at the Core Facilities at the American University of Beirut. Part of his 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.

All above procedures, including the genetic study, will be conducted in our clinic and facilities within the Medical Center of the American University of Beirut. This will require your child to pass by for two visits: the first one (30minutes) to explain the project in details, sign this consent form, have a clinical examination and a lateral cephalometric X-ray (if features of the condition are noted clinically), and the second visit (15minutes) to have 5cc of his/her blood collected.

2. Risks as a result of participating in the study:

Risk	Procedure
Radiation risk	Although there are no proven harmful effects from radiation levels that he will be exposed to during this study, long-term effects on his 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.

Please note that there may be unforeseeable risks.

3. Benefits as a result of participating in the study:

There is no direct benefit to your child as a person. However, this study has benefits to the group to which he belongs and 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 prognathism 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 later surgery or
2- more importantly, forego earlier interventions in favor of a later orthognathic surgery when true mandibular prognathism 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.

4. What other options are there?

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

5. Confidentiality:

If you agree to let your child participate in this research study, the information will be kept **confidential**. Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental agencies will have direct access to his medical records.

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

Depending on your request, the results of the study will be disclosed to you and to your family members as soon as the study is completed (approximate date: September 2017).

☐ I want to be informed of the results of the study.

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

6. What are the costs?

There are no costs associated with participation. The study will cover the cost 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.

7. Will you get paid to have your child be in this study?

You will not be paid to let your child participate in this study.

8. Agreement for the use of samples for genetic testing

The participation of your child is optional and you can make him stop it any time you like. **No negative consequences** whatsoever will affect him if you decide not to let him participate. If you agree that he participates, please fill in the following:

I permit coded use of my child's biologic 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

Child's Participation:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to allow 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 01-350000 ext. 5445. I understand that I am free to withdraw this consent and discontinue participation of my child in this project at any time, even after signing this form, and it will not affect his care. I know that I will receive a copy of this signed informed consent.

_____ _____

Name of
☐ legal representative, or ☐ father/guardian

Signature

_____

Date & Time

_____ _____

Name of
☐ legal representative, or ☐ mother/guardian

Signature

_____

Date & Time

_____ _____

Witness's name

Signature

_____

Date & Time

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

موافقة الأهل على مشاركة أولادهم في دراسة بحثية وراثية	إسم المشترك:
الارتباط بين الجينات وبروز الفك السفلي العائلي لدى سكان منطقة 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 الفكّين):

- اذا هو/هي يتلقون العلاج في قسمنا: خلال الموعد الطبّي، ستشرح لكم منسقة الدراسة (د. باميلا جنو) أهداف الدراسة، إجراءاتها، فوائدها ومخاطرها، وستسألكم اذا كنتم ترغبون مشاركة ولدكم فيها. اذا وافقتم، سيُطلب منكم الامضاء على استمارة الموافقة هذه. بعد امضائها، ستُسألون عن تاريخ عائلتكم، بما في ذلك حالة بروز الفكّ 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 بروز الفك السفلي (تقدّم حجم ومكان الفكّ السفليّ) والمساعدة في تحديد الجينات المسؤولة عن تطوير وانتقال هذه الحالة وراثيّاً. بما أنه من المتوقع أن يكون في لبنان والمنطقة العديد من المشاركين، لقد صنّفنا هؤلاء المشاركين كممثّلي سكان منطقة البحر المتوسط.

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

الخطوة	الإجراءات
1	الإجابة عن أسئلة حول تاريخ العائلة ومدى تأثّر أفراد آخرين في الأسرة.
2	الخضوع للفحص السريري لتحديد ما إذا كانت العضة الخاصة به/بها تتطابق مع الحالة.
3	اذا تم تدوين خصائص الحالة سريرياً، القيام بصورة بالأشعة السينسة للوجه (صورة جانبية بالأشعة السينية لقياسات الرأس) في عيادتنا لمساعدتنا على التأكد من أنه/أنها مصاب(ة) ببروز الفك السفلي، اضافةً إلى انطباعات الأسنان، اذا لزم الأمر، من أجل قالب الجبس للأسنان وصور للأسنان والوجه. **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 ببروز الفك السفلي.
5	سيتم أخذ 5 سنتيمتر مكعّب 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 تسلسل الجيل القادم. سيتم جمع البيانات في الجامعة الأميركية في بيروت وتحليلها بعد ذلك بغية اكتشاف أي تغيّرات التي يمكن أن تسبب مرضه(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 بزيارتنا مرّتين: الزيارة الأولى (30 دقيقة) لنشرح لكم المشروع بالتفصيل، لإمضاء استمارة الموافقة هذه، لخضوعه(ها) لفحص سريري ولصورة أشعة سينية جانبية لقياسات الرأس (اذا تم تدوين خصائص الحالة سريرياً). الزيارة الثانية (15 دقيقة) لنأخذ 5 سنتيمتر مكعّب 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 1.7 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 بروز 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 بروز 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

اذا وافقتم على مشاركة ابنكم/ابنتكم في الدراسة، ستحفظ المعلومات **بسريّة**. 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 وتُحفظ في بنك الصور الشعاعية الذي تم احداثه وضمّه 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 التخلّص منها في نهاية الدراسة.

بناءً على طلبكم، سيتم الكشف عن نتائج الدراسة لكم ولأفراد عائلتكم بمجرد الانتهاء من الدراسة (التاريخ المتوقع:أيلول 2017).

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

6. ما هي التكاليف؟

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

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

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

8. 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.

_____ _____

اسم المحقق أو من ينوب عنه **التوقيع**

_____

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. نعلم أننا سنحصل على نسخة من هذه الموافقة الموقّعة.

اسم ☐ الممثل القانوني، أو ☐ والد/ وصي	**التوقيع**
	التاريخ والساعة
اسم ☐ الممثل القانوني، أو ☐ والدة / وصي	**التوقيع**
	التاريخ والساعة
اسم الشاهد	**التوقيع**
	التاريخ والساعة