Three novel genes tied to mandibular prognathism in eastern Mediterranean families

Pamela G. Genno,[a] Georges M. Nemer,[b] Savo Bou Zein Eddine,[b] Anthony T. Macari,[a] and Joseph G. Ghafari[a]
Beirut, Lebanon

Introduction: Mandibular prognathism (MP) is subject to major polygenic influence and segregates within families in autosomal dominance with variable expressivity and incomplete penetrance. We aimed to identify the inheritance pattern and genes and loci involved in the development of MP in Mediterranean families and to evaluate the dentoskeletal characteristics of affected individuals. **Methods:** Fifty-one eastern Mediterranean families with individuals affected by MP were identified. Data and biospecimens were collected from 14 of the families, including clinical examination, lateral cephalography (on subjects with Class III malocclusion), and 5 mL blood drawn from consenting affected and nonaffected relatives. Next-generation sequencing (NGS) was performed on 8 families (7 Lebanese, 1 Lebanese/Syrian), including large numbers of affected individuals over many generations and severe conditions, with the use of whole-exome sequencing. **Results:** Most pedigrees suggested autosomal-dominant inheritance with an equal number of affected male and female individuals. Affected individuals had macrognathic and prognathic mandibles with dentoalveolar compensation. Genetic screening did not correspond with previously reported MP-linked genes, but yielded 3 novel genes (*C1orf167*, *NBPF8*, *NBPF9*) on chromosome 1 potentially responsible for mandibular development and macrognathism. **Conclusions:** In this first genetic study with the use of NGS on the largest reported number of families with MP, novel genes (C1orf167, NBPF8, NBPF9) were associated with familial MP in the eastern Mediterranean population. (Am J Orthod Dentofacial Orthop 2019;156:104-12)

Mandibular prognathism (MP) is a dentofacial phenotype of skeletal disproportion characterized by mandibular overgrowth with (50%-60%) or without (20%) undergrowth of the maxilla, resulting in a more prominent lower jaw/chin, a concave profile and an anterior crossbite, which represent the traits of Class III malocclusion, or mesiocclusion.[1] Not always evident in early childhood, MP emerges progressively with the growth of the general skeleton, accelerates in puberty, and is completely expressed when the body is fully mature.[2] In severe malocclusions, the combination of orthodontic treatment and orthognathic surgery involving either or both jaws is needed in adulthood for better facial appearance and function.[3]

The complex etiology of Class III with MP involves genetic and environmental factors. The genetic component is supported by familial aggregation.[4] Frequently, the "large lower jaw" phenotype (Habsburg jaw) persists in successive generations of the family with various levels of expressivity (severities). MP has definite patterns of mendelian inheritance and is segregated mainly in an autosomal-dominant manner with incomplete penetrance and an estimated heritability ratio (H^2) of 0.316.[5,6]

Few genetic studies have been conducted in families with a high frequency of MP, underscoring the poorly understood determinants of this trait. The available genetic mapping from different ethnic populations has yielded several susceptibility genes.[7-18] The varying results of these genome-wide linkage studies indicated

[a]Division of Orthodontics and Dentofacial Orthopedics, Department of Otolaryngology and Head and Neck Surgery, American University of Beirut, Beirut, Lebanon.
[b]Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon.
All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
This work was supported by the Medical Practice Plan (grant CUF 22425) and the University Research Board (grant 103185) at the American University of Beirut. The funding sources provided only financial support for the conduct of the research; they had no involvement in the study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.
Address correspondence to: Anthony T. Macari, Associate Professor, Division of Orthodontics and Dentofacial Orthopedics, Department of Otolaryngology and Head and Neck Surgery, American University of Beirut Medical Center, Riad El Solh, Beirut 1107 2020, Lebanon; e-mail, am43@aub.edu.lb.
Submitted, March 2018; revised and accepted, August 2018.
0889-5406/$36.00

https://doi.org/10.1016/j.ajodo.2018.08.020

that MP is a complex condition with a polygenetic causation, locus heterogeneity in its development, and correlation with the ethnicity of the population. The results also disclosed the absence of investigation in the Mediterranean population.

Our aims in the present study were (1) to explore the inheritance pattern and identify the candidate genes and loci involved in the development and familial transmission of MP in Mediterranean families, and (2) to evaluate the skeletal and dentoalveolar cephalometric characteristics of affected individuals. The underlying hypothesis was that specific candidate loci and genes have an etiologic role in the susceptibility to MP and that inheritance is related more to mandibular macrognathism.

MATERIAL AND METHODS

Fifty-one eastern Mediterranean families known to include subjects diagnosed with MP during previous or ongoing treatment of some members (at our institutional orthodontic clinic) were first approached and their pedigrees drawn. Fourteen families (12 Lebanese and 2 Lebanese/Syrian) including 81 subjects (42 male, 39 female; 40 affected, 41 nonaffected) agreed to participate. The study was approved by the Institutional Review Board of the American University of Beirut, and all patients signed the informed consents approved by the Institutional Review Board before the research initiation.

The data and biospecimen collection process consisted of a clinical examination, taking a lateral cephalometric radiograph (of only 36 subjects, 18 male and 18 female, on whom Class III features were noted clinically), and drawing 5 mL blood from all 81 subjects, with the use of needles and capillary blood collection tubes with purple tops. Because of budgetary limitation, the genetic analysis was performed on 49 members (24 male, 25 female; 27 affected, 22 nonaffected) of 8 families (7 Lebanese, 1 Lebanese/Syrian), selected for the following considerations: The 8 initial patients had the most severe conditions; 7 families had the largest number of affected individuals over many generations; 6 families had an autosomal dominant mode of transmission; 1 family and 1 individual without a family pedigree of MP had the recessive mode. The reason for this mix was to cover all variations of inheritance that occurred in our population. The sole individual was selected because of his severe phenotype despite the fact that he was the only affected individual in his family. In fact, the recessive mode of inheritance may induce a more severe affection status because subjects must be homozygous, having 2 copies of the same disease/trait allele. Lateral cephalographs were obtained on 22 of the 27 affected subjects (13 male and 9 female; Table I). Each of the 49 subjects was assigned a family code (A-H) followed by a number (1-13; Fig 1).

For inclusion in the study, the families had to be of Mediterranean identity with several affected individuals over many generations, based on the following criteria: ANB angle $\leq 0^\circ$ and/or a negative Wits appraisal of at least -2.0 mm, increased mandibular length (Co-Gn above 1 standard deviation of the norm for their age), normal to increased maxillary length (ANS-PNS), and Class III malocclusion (anterior teeth in crossbite or at least edge to edge). Excluded were subjects with short maxillary length and congenital disorders (eg, cleft lip or palate) or a general physical disease.

The cephalographs were obtained at our department in the same machine (Instrumentarium, Tuusula, Finland). The patient was positioned in natural head position with the jaws in centric occlusion and lips in gentle touch. The cephalograms were digitized by 1 investigator (P.G.) with the use of the Dolphin Imaging program (version 11.5; La Jolla, Calif). Angular and linear measurements (Supplementary Table I; Supplementary Tables I-IV are available at www.ajodo.org) gauged the characteristics of the cranial base and each jaw, as well as the vertical and sagittal relationships among these structures (eg, facial heights, dentoalveolar angulations). The measurements were compared between 2 groups: group 1 comprising 22 affected individuals included in the genetic analysis, and group 2 comprising 45 affected subjects not included in the genetic analysis. The malocclusion is illustrated in Figure 2 in 2 subjects from family B.

Genomic DNA was isolated with the use of the Qiagen Blood-Midi kit (Qiagen Science, Germantown, Md) according to the manufacturer's recommendations. DNA was quantified and assessed for quality with the use of Nanodrop at our institution's molecular core facility. The experimental genetic procedure, performed by Macrogen Laboratory (South Korea; dna.macrogen.com), included 4 main steps: captured library preparation, cluster generation by a unique isothermal "bridged" amplification, whole-exome sequencing (WES) with the use of a HiSeq2000/2500 Illumina platform to determine the exact sequence of nucleotides, and data analysis with the use of several computer programs (GWA, Picard, GATK, and Snpeff).

During the first stringent filtering, only the passed variants having a high putative impact and a coverage read depth >40 were analyzed. In family E, which had a recessive inheritance pattern, the selected variants were also homozygous. During the second filtering, individuals of the same family were compared in an attempt to find common genes between the affected members that were not observed in the nonaffected members. The analysis was repeated by selecting only

Table I. Summary of the demographic characteristics of the 8 selected families

			Blood collection (n)				*Lateral cephalograph (n, affected)*	
Family	*Ethnicity*	*Mode of inheritance*	*Male*	*Female*	*Affected*	*Nonaffected*	*Male*	*Female*
A	L	AD	3	5	5	3	1	3
B	L/S	AD	2	1	2	1	2	0
C	L	AD	4	4	5	3	2	1
D	L	AD	3	4	4	3	1	2
E	L	R	1	3	2	2	1	1
F	L	AD	8	5	5	8	3	1
G	L	AD	2	3	3	2	2	1
H	L	R	1	-	1	-	1	-
Total			24	25	27	22	13	9
Total			49		49		22	

AD, autosomal-dominant; *L*, Lebanese; *R*, recessive; *S*, Syrian.

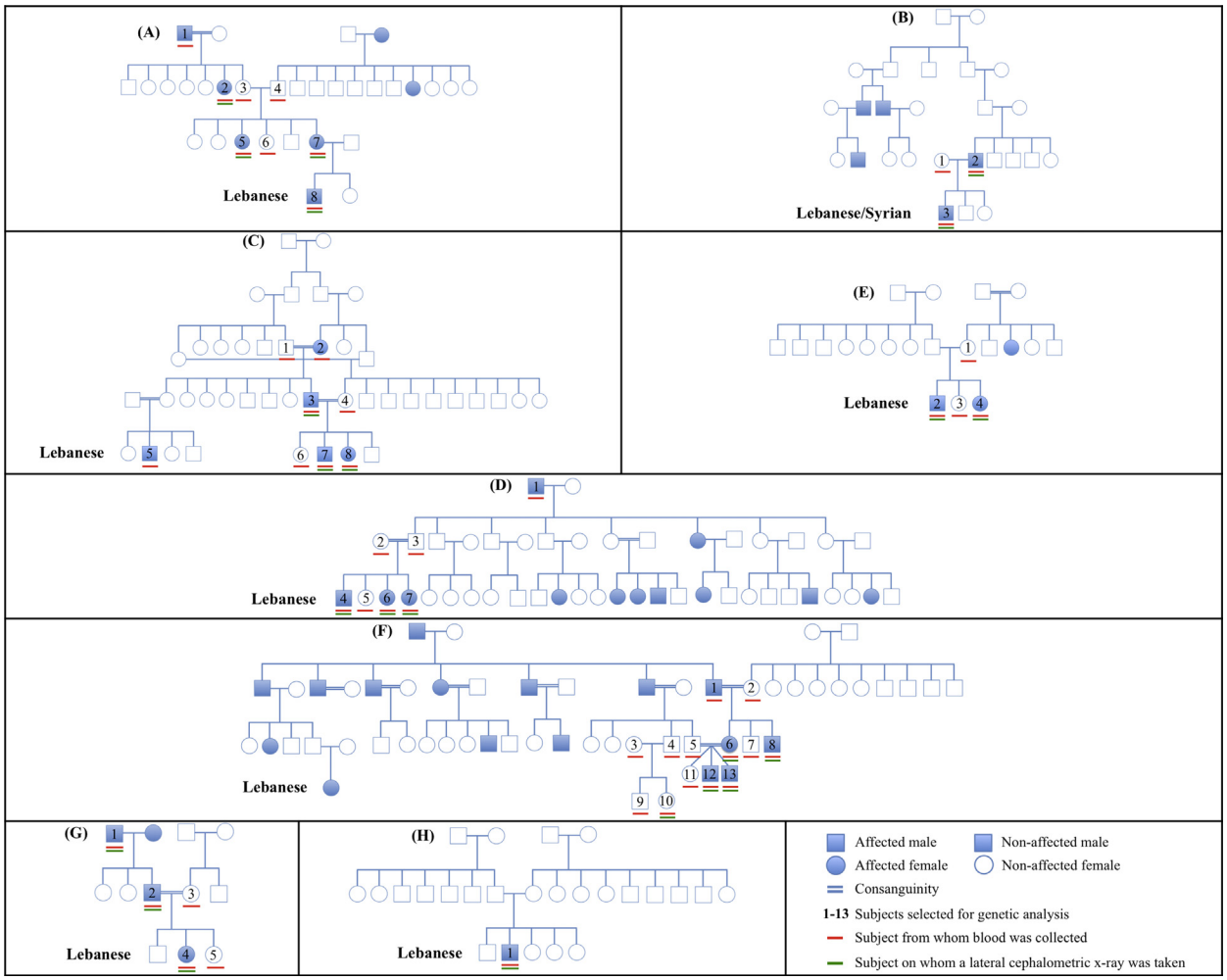

Fig 1. Pedigrees of the selected families (8 of the original 14, 49 of the original 81 subjects). Families **A, B, C, D, F,** and **G** follow the autosomal-dominant pattern, with families **B** and **C** having partial penetrance leading to a failure of the trait expression in some individuals who carry the condition's allele. Families **E** and **H** follow the autosomal-recessive pattern. DNA sequencing and analysis were not performed on subjects D4 and F9 owing to poor blood sample quality. H1 was selected because of his severe phenotype despite the fact that he is the only affected individual in his family.

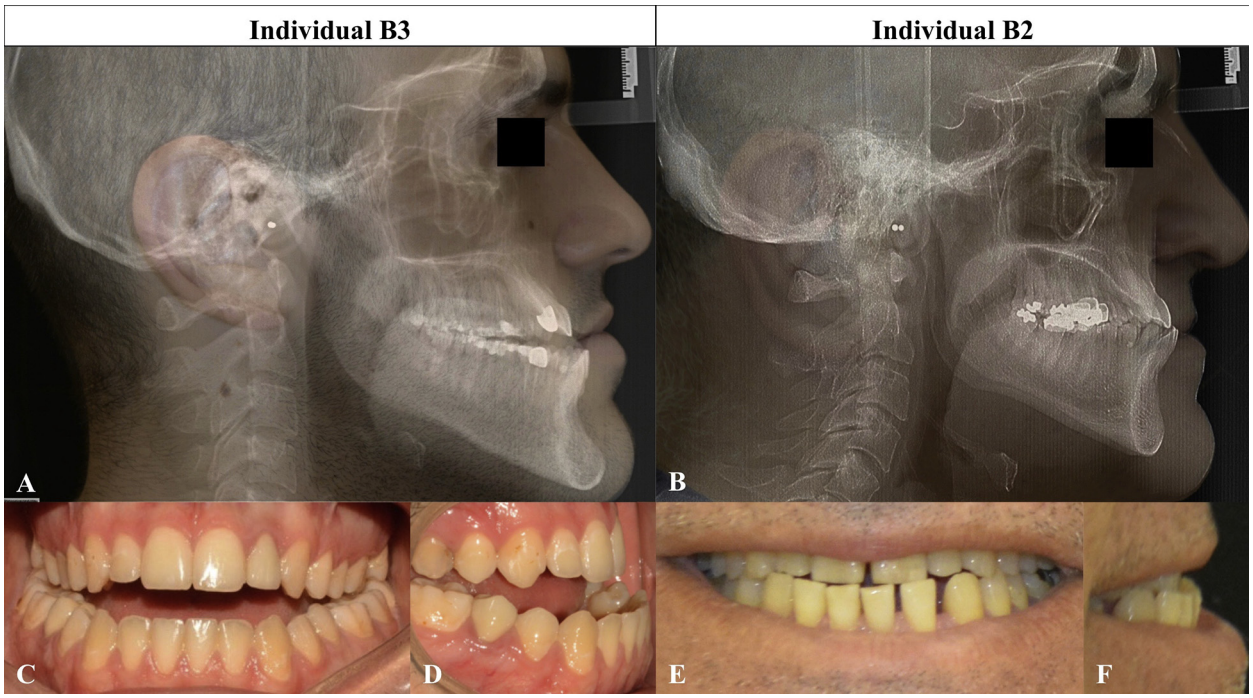

Fig 2. Superimposition of the facial profile photograph on the lateral cephalometric radiograph of **A,** subject B3, age 24.4 years, and **B,** his father (subject B2), age 54.10 years, revealing mandibular macrognathism. The corresponding front and right lateral occlusal photographs **(C-D** and **E-F)** reveal the more severe anterior crossbite of the son.

the passed variants having a moderate putative impact and a minor allele frequency (MAF) ≤0.001 (0.1%) in the normal population; a comparison was also accomplished between individuals of the same family for common gene identification.

In families B and G, where a large number of common genes were noted, 2 subfilterings were performed to reduce the number of genes: the first consisted of keeping only the genes that had a MAF equal to 0; the second was a complimentary biochemical interface carried out to prove that the results obtained by sequencing affect protein function. The effects of the filtered variants on protein function were assessed in silico with the use of both Polyphen2 and SIFT software. During this second subfiltering, which is applicable only on the variants with 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 included 1 affected individual, only the passed variants having a high putative impact and a MAF ≤0.01 (1%) were preserved. Then, the genes having several mutations were filtered out and the potential genes highlighted.

After the filtering steps, a reduced number of possible candidate genes emerged. Depending on their function, some of these were filtered out and the others were considered as candidate genes for MP. A search followed to verify if the potential candidate genes were present in individuals among the 7 other selected families. Also, the candidate genes were compared with those discovered in previously published studies to determine if common genes were observed.

Statistical analysis

A 2-sample independent *t* test was made to assess the differences in cephalometric measurements between groups 1 and 2. Statistical significance was set at $P \leq 0.05$. Intraexaminer reliability for cephalometric measurements was assessed by means of Spearman intraclass correlation after randomly selecting, redigitizing, and analyzing 20% of the total sample (n = 7) 5 months after the initial digitization.

RESULTS

Most of the pedigrees of the 51 families suggested a mendelian inheritance pattern and segregation in an autosomal dominant pattern. The following findings were observed (Tables II and III): equal number of reported generations per family (n = 3); equal average

number of reported affected males and females (n = 2); more families with male predominance (56.9%) and with at least 3 affected males (37.3%); more families with no affected females (29.4%); 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 was noted in those 4 families). Out of the 8 selected families, 6 had consanguinity and an autosomal dominant mode of inheritance and 2 had a recessive inheritance pattern.

The intraclass correlation coefficients computed for intraexaminer reliability ranged from 0.92 to 1. Among the 8 selected families, the average ANB angle (−1.96°), Wits appraisal (AO-BO −9.13), and incisal crossbite (−2.64 mm) reflected a skeletal Class III malocclusion. On average, mandibular macrognathism (Co-Gn 120.65 mm, Co-Pog 117.41 mm) and prognathism (SNB 83.05°) were observed; maxillary length (ANS-PNS 51.28 mm) and position (81.10°) were normal; MP/SN (37.54°) denoted a tendency to hyperdivergence; proclined maxillary and retroclined mandibular incisors revealed dentoalveolar compensation (Supplementary Table II). These measurements were more severe than those within the total 51 families. The average *Z*-score (2.42) of mandibular length (Co-Gn) corresponding to the 8 selected families was more than 2 standard deviations above the norm, indicating mandibular macrognathism.

In general, measurements were greater in group 1 than group 2, with statistically significance observed for differences in mandible-related features, particularly greater mandibular length (Co-Pog, Co-Gn) and more severe anterior crossbite in group 1 (Table IV).

After the first stringent filtering, 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. After the second filtering, many genes were found to be common between the affected individuals except in families A and D, where no shared gene was noted. In the remaining families, the number of common variants in specific genes among the affected individuals was 93 in family B, 1 in family C, 14 in family E, and 14 in family G (Supplementary Table III).

When the analysis was repeated on the passed variants having a moderate putative impact and an MAF ≤0.001 (0.1%), 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 was 132 in family B, 3 in family C, 1 in family D, 4 in family F, and 12 in family G (Supplementary Table IV).

Table II. Numbers and averages of generations and affected male and female individuals in the 51 Middle Eastern families

Parameter	*Generations*	*Affected female*	*Affected male*
Total number	165	110	119
Average	3.59	2.39	2.59

Following the first subfiltering in 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 in family G. For the variants having a moderate putative impact, the reduction was to 104 in family B and 8 in family G. After the second subfiltering, the number of common variants in specific genes was reduced to 4 in family B and 1 in family G. In family H, the number of variants having a high putative impact and a MAF ≤0.01 (1%) was 127.

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* was one of the 14 common genes between affected individuals of family G, having a high putative impact, and the 3 last genes (*NBPF9*, *OBSCN*, and *PPIG*) are common between affected individuals of family B, having a high putative impact.

In family H, after filtering out the genes having several mutations, 7 potential candidate genes (*NBPF8*, *PRR21*, *ALS2CL*, *MAML3*, *GRIFIN*, *RBBP6*, and *TEX13A*) were highlighted and their functions checked. All of the potential genes, except *C1orf167*, *NBPF8*, and *NBPF9*, were not considered as candidate genes for MP because they do not have a known role in the formation of the jaws or their MAF indicated a frequent occurrence for the minor allele in the normal healthy population. *C1orf167* (located on chromosome 1, locus 1p36.22, variant position 11,844,520) was considered to be a potential candidate gene for MP. *NBPF8* (located on chromosome 1, locus 1p11.2, variant position 144,828,764) and *NBPF9* (located on chromosome 1, locus 1q21.2, variant position 145,368,525), which are part of the same *NBPF* family, also were considered to be potential candidate genes for MP.

DISCUSSION

This study stands out as the genetic investigation with the largest number of families with MP and the first

Table III. Pedigree analysis of the 51 Middle Eastern families

Parameter	*n*	*%*	*Parameter*	*n*	*%*
Families with more affected females	19	37.3	Families with more affected males	29	56.9
Families with ≥3 affected females	12	23.6	Families with ≥3 affected males	19	37.3
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	7.8

among a Mediterranean population. Furthermore, the selection of Class III was based mainly on mandibular macrognathism, another distinction from most previous reports. In the only other study referring to MP, Perillo et al (2015) related the body of the mandible (Go-Pog), rather than its length (Co-Pog), to the anterior cranial base (SN), compared with the absolute size (>1 SD) considered in our study. In addition, although we used common cephalometric measurements to define Class III, the cutoff values for inclusion were stringent.

The 3 potentially novel genes *C1orf167*, *NBPF8*, and *NBPF9* that segregated with the phenotype could be implicated in mandibular development. *C1orf167* was associated with MP because its function is still not discovered although it has an open reading frame (ORF) and 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 *NBPF9* were linked with MP because they play a role in human evolution and their frequencies are rare in the normal population. The *NBPF* gene family was shown to reflect the continuous evolution of primate genomes,[19] and human evolution shows a reduction in facial prognathism over time.[20] Although little or no biologic rationale exists for the involvement of these DNA variants in the development of MP at this point, our genetic analyses included genotyping of all families' members and are suggestive of critical roles for these genes in the underlying phenotypes.

The 3 genes share the same characteristics: location on chromosome 1, heterozygosity, stop gained effect, high putative impact, coding transcript biotype, and MAF equal to 0. They differ by the reference and variant alleles and the positions of the mutation and amino acid (Table V). The genes were not found to be associated with MP in previous studies and are not present in the normal database of the whole globe, which includes 6000 healthy individuals for WES and 100,000 healthy individuals for whole-genome sequencing.

Genetic analysis revealed the absence of a common gene, loci, or variant being associated with MP between the present report and the results of previous studies undertaken in different ethnic populations. This finding underscores the polygenicity and locus heterogeneity in the development of MP along with the likely correlation of this feature with the ethnicity of the population. Previous genetic studies identified 7 susceptibility loci on chromosome 1, increasing to 9 with our findings, compared with 5 loci reported on chromosome 12 and 1-2 loci on the other chromosomes (Table VI). In addition, some reports correlated with MP the susceptibility loci 1p35.2, 1p35.3, and 1p36.12, which are close to the locus 1p36.22 that harbors the novel gene *C1orf167*.[7-9] The combined results indicate that chromosome 1, specifically the region 1p35-36, is potentially highly linked to MP.

The observed mendelian inheritance pattern, mainly the autosomal-dominant type, in our families is aligned with findings from previous reports that are strongly supportive of a genetic cause for the condition.[4-6,21,22] Our finding of no sex differences in the incidence of MP corroborates some reports[23,24] but contradicts other reports of greater incidence in males[25] or females,[26] reinforcing the absence of conclusive findings on sex. Consanguinity, noted in 6 of the 8 selected families, could be related to the familial transmission of MP, supporting previous findings that MP has been passed on and exaggerated over time through intermarriage.[5]

Cephalometric analysis indicated that the affected subjects have a macrognathic mandible and a tendency to a hyperdivergent facial pattern that underlie the Class III malocclusion typically defined by a prognathic mandible and dentoalveolar compensatory inclinations. The previously reported hyperdivergent facial pattern was suggested as a compensation of the underlying skeletal Class III.[27,28]

We 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 a recent report, the condyles of patients with unilateral condylar hyperplasia were similar in volume to the condyles of patients with MP and statistically significantly different from nonhyperplastic condyles.[29,30] Research is needed to test this hypothesis.

Table IV. Comparison between measurements (means) of the subjects included (group 1) and excluded (group 2) from the genetic analysis

Category	*Measurement*	*Group 1*	*Group 2*	P
Cranial base	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
Mandible	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
Symphysis	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*
Chin	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
Maxilla	ANS-PNS	51.40	49.21	0.078
Facial height	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
Vertical relationship between the jaws (facial divergence)	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*
Sagittal relationship between the jaws	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
Dentoalveolar relationships	1/NA	28.54	27.31	0.560
	1-NA	4.08	4.82	0.324
	1/SN	109.44	107.77	0.487
	1/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
	1/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 pedigrees but not included in the genetic analysis).
*$P \leq 0.05$ (statistically significant).

The WES technique, which is part of next-generation sequencing (NGS), provided an unbiased analysis of variations in human coding sequences, allowing the identification of a number of true candidate genes through reliable results.[31,32] The method was applied in other studies,[14,16,17] but only 1 family was used in each previous study compared with the 8 families that underwent massive parallel sequencing in our investigation. The inclusion of families with affected individuals over 3-4 generations demonstrated the segregation of the phenotype across generations.

The function of the novel susceptibility gene *C1orf167* was not discovered, because a transgenic study would be needed, a limitation in this study related

Table V. Summary of the characteristics of the 3 novel genes

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

HET, heterozygous; *MAF*, minor allele frequency.

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

Chromosome number	Locus	References	Number of loci reported on each chromosome
1	1p22.1	Frazier-Bowers et al 2009	9 (1 locus similar in separate studies)
	1p22.3, 1q32.2	Ikuno et al 2014	
	1p35.2, 1p36.12	Yamaguchi et al 2005	
	1p35.2	Jang et al 2010	
	1p35.3	Xue et al 2010	
	1p36.22, 1p11.2, 1q21.2	Genno et al (present study)	
3	3q26.2	Frazier-Bowers et al 2009	1
4	4p16.1	Li et al 2010	1
5	5p12, 5p13	Yamaguchi et al 2005	2
6	6q25	Yamaguchi et al 2005, Cruz et al 2011	1
10	10p12.1, 10p12.3	Perillo et al 2015	2
11	11q22.2, 11q22.3	Frazier-Bowers et al 2009	2
12	12q13.11	Xue et al 2014	5 (2 loci similar in separate studies)
	12q13.13, 12q23	Frazier-Bowers et al 2009	
	12q22, 12q23	Nikopensius et al 2013	
	12q24.11	Tassopoulou-Fishell et al 2012	
	12q24.11	Cruz et al 2017	
14	14q24.3, 14q31.2	Li et al 2011	2
19	19p13.2	Yamaguchi et al 2005	1
21	21q21.3	Guan et al 2015	1

to budgetary constraints. New genetic mapping studies with the use of larger sample sizes and WGS technology would allow a better understanding of the genetic determinants of facial development in general and MP in particular. Genetic studies are also indicated to test the involvement of the *C1orf167*, *NBPF8*, and *NBPF9* genes in other ethnic populations or to discover common genes in different ethnic groups. In family H, future sequencing on the parents of the severely affected individual H1 would be helpful in an attempt to identify the heterozygous allele pairs that led to the homozygosity in this subject.

The results here can help in estimating the genetic susceptibility to MP in families with affected individuals and contribute to improved comprehension of the molecular mechanisms of jaw development and possibly treatment of associated malocclusions. Early forecast (through blood test) of the condition might lead to earlier treatment that would reduce malocclusion severity and possible avoidance of later orthognathic surgery or to foregoing earlier interventions in favor of a later orthognathic surgery when true MP is genetically determined as a "certainty." Such personalized approach would avoid the side-effects of long treatments.

In this context, genetic profile analysis could enhance the accuracy of predictive models of treatment outcome. Prediction studies have underscored the high frequency of unsatisfactory orthopedic outcome and greater possibility for relapse.[33] As the needed development of genetic analysis of MP proceeds, the addition of DNA information to the lateral cephalometric and clinical measurements usually used to develop the predictive equations might strengthen those models.

CONCLUSION

In this first genetic study on a large number of families with MP, NGS was used to better understand the variations and the risks for MP. WES was performed on 8 eastern Mediterranean families including 49 individuals; 3 novel genes on chromosome 1 (*C1orf167*, *NBPF8*, and *NBPF9*) were found that could cause

familial MP. Further studies are needed to determine whether these candidate genes are relevant in other populations. Functional studies of the novel genes in bone and muscle development are necessary to better understand the etiology of MP.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the families who participated in this study, the laboratory technicians who helped in the routine procedures, Dr. Antoine Hanna and Dr. Mouhannad Khandakji for the statistical analysis, and Mr. Tony Bou Abboud for his help in data management.

REFERENCES

1. Proffit W, Phillips C, Dann C. Who seeks surgical-orthodontic treatment? Int J Adult Orthodon Orthognath Surg 1990;5:153-60.
2. Shira R, Neuner O. Surgical correction of mandibular prognathism. Oral Surg Oral Med Oral Pathol 1976;42:415-30.
3. Sperry TP, Speidel TM, Isaacson RJ, Worms FW. The role of dental compensations in the orthodontic treatment of mandibular prognathism. Angle Orthod 1977;47:293-9.
4. Kraus B, Wise W, Frie R. Heredity and the craniofacial complex. Am J Orthod Dentofacial Orthop 1959;45:172-217.
5. Wolff G, Wienker T, Sander H. On the genetics of mandibular prognathism: analysis of large European noble families. J Med Genet 1993;30:112-6.
6. El-Gheriani AA, Maher BS, El-Gheriani AS, Sciote JJ, Abu-Shahba FA, Al-Azemi R, et al. Segregation analysis of mandibular prognathism in Libya. J Dent Res 2003;82:523-7.
7. Yamaguchi T, Park S, Narita A, Maki K, Inoue I. Genome-wide linkage analysis of mandibular prognathism in Korean and Japanese patients. J Dent Res 2005;84:255-9.
8. Jang JY, Park EK, Ryoo HM, Shin HI, Kim TH, Jang JS, et al. Polymorphisms in the Matrilin-1 gene and risk of mandibular prognathism in Koreans. J Dent Res 2010;89:1203-7.
9. Xue F, Wong R, Rabie A. Identification of SNP markers on 1p36 and association analysis of EPB41 with mandibular prognathism in a Chinese population. Arch Oral Biol 2010;55:867-72.
10. Xue F, Rabie A, Luo G. Analysis of the association of COL2A1 and IGF-1 with mandibular prognathism in a Chinese population. Orthod Craniofac Res 2014;17:144-9.
11. Li Q, Zhang F, Li X, Chen F. Genome scan for locus involved in mandibular prognathism in pedigrees from China. PLoS One 2010;5:e12678.
12. Li Q, Li X, Zhang F, Chen F. The identification of a novel locus for mandibular prognathism in the Han Chinese population. J Dent Res 2011;90:53-7.
13. Tassopoulou-Fishell M, Deeley K, Harvey E, Sciote J, Vieira A. Genetic variation in Myosin 1H contributes to mandibular prognathism. Am J Orthod Dentofacial Orthop 2012;141:51-9.
14. Nikopensius T, Saag M, Jagomägi T, Annilo T, Kals M, Kivistik PA, et al. A missense mutation in DUSP6 is associated with Class III malocclusion. J Dent Res 2013;92:893-8.
15. Ikuno K, Kajii T, Oka A, Inoko H, Ishikawa H, Lida J. Microsatellite genome-wide association study for mandibular prognathism. Am J Orthod Dentofacial Orthop 2014;145:757-62.
16. Guan X, Song Y, Ott J, Zhang Y, Li C, Xin T, et al. The ADAMTS1 gene is associated with familial mandibular prognathism. J Dent Res 2015;94:1196-201.
17. Perillo L, Monsurrò A, Bonci E, Torella A, Mutarelli M, Nigro V. Genetic association of ARHGAP21 gene variant with mandibular prognathism. J Dent Res 2015;94:569-76.
18. Cruz CV, Mattos CT, Maia JC, Granjeiro JM, Reis MF, Mucha JN, et al. Genetic polymorphisms underlying the skeletal Class III phenotype. Am J Orthod Dentofacial Orthop 2017;151:700-7.
19. Vandepoele K, Van Roy N, Staes K, Speleman F, Van Roy F. A novel gene family NBPF: intricate structure generated by gene duplications during primate evolution. Mol Biol Evol 2005;22:2265-74.
20. Mills JRE. Occlusion and malocclusion of the teeth of primates. In: Brothwell DR, editor. Dental anthropology. Oxford: Pergamond Press; 1963. p. 29-51.
21. Cruz R, Krieger H, Ferreira R, Mah J, Hartsfield JJ, Oliveira S. Major gene and multifactorial inheritance of mandibular prognathism. Am J Med Genet A 2008;146:71-7.
22. Frazier-Bowers S, Rincon-Rodriguez R, Zhou J, Alexander K, Lange E. Evidence of linkage in a Hispanic cohort with a Class III dentofacial phenotype. J Dent Res 2009;88:56-60.
23. Solow B, Helm S. A method for tabulation and statistical evaluation of epidemiologic malocclusion data. Acta Odont Scand 1968;26(1-2):63-88.
24. Joshia N, Hamdanb A, Fakhouri W. Skeletal malocclusion: a developmental disorder with a life-long morbidity. J Clin Med Res 2014;6:399-408.
25. El-Mangoury N, Mostafa Y. Epidemiologic panorama of dental occlusion. Angle Orthod 1990;60:207-14.
26. Wood B. Malocclusion in the modern Alaskan Eskimo. Am J Orthod Dentofacial Orthop 1971;60:344-54.
27. Stapf WC. A cephalometric roentgenographic appraisal of the facial pattern in Class III malocclusions. Angle Orthod 1948;18:20-3.
28. Ellis E, McNamara JA. Components of adult Class III malocclusion. J Oral Maxillofac Surg 1984;42:295-305.
29. Goulart DR, Muñoz P, Olate S, de Moraes M, Fariña R. No differences in morphological characteristics between hyperplastic condyle and class III condyle. Int J Oral Maxillofac Surg 2015;44:1281-6.
30. Goulart DR, Muñoz P, López MGC, de Moraes M, Olate S. Comparative evaluation of condylar volume between patients with unilateral condylar hyperplasia and Class III dentofacial deformity. J Oral Maxillofac Surg 2017;75:180-8.
31. Macrogen. Available at: http://dna.macrogen.com/eng/support/ces/guide/ces_sample_submission.jsp.
32. Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, et al. Exome sequencing as a tool for mendelian disease gene discovery. Nat Rev Genet 2011;12:745-55.
33. Fudalej P, Dragan M, Wedrychowska-Szulc B. Prediction of the outcome of orthodontic treatment of Class III malocclusions—a systematic review. Eur J Orthod 2010;33:190-7.

APPENDIX

Supplementary Table I. Definitions of angular and linear cephalometric measurements

Measurement	*Definition*	*Category*
SN	Length of the anterior cranial base	Cranial base
S-Ar	Length of the posterior cranial base	
SN/H	Angle between the anterior cranial base and true horizontal	
N-S-Ar	Saddle angle	
Ar-Go-Gn	Angle of the mandible between the ramus and mandibular plane	Mandible
Ar-Go-Me	Angle of the mandible between the ramus and mandibular plane	
Co-Go-Me	Angle of the mandible between the ramus and mandibular plane	
Ar-Gn	Length of the mandible	
Co-Gn	Length of the mandible	
Co-Pog	Length of the mandible	
Co-Go	Length of the ramus of the mandible	
Ar-Go	Length of the ramus of the mandible	
Go-Me	Length of the body of the mandible	
Go-Gn	Length of the body of the mandible	
Go-Pog	Length of the body of the mandible	
Chin width at apex of i	Line through the apex, parallel to the horizontal, intersecting anterior and posterior contours of the symphysis	Symphysis
Chin width at level of D	Line through D parallel to the horizontal, intersecting anterior and posterior contours of the symphysis	
D-i apex	Distance between point D and mandibular incisor apex	
D-Me	Distance between point D and menton	
Ant slope of the chin/V	Angle between the anterior slope of the chin and the vertical	Chin
Post slope of the chin/V	Angle between the posterior slope of the chin and the vertical	
Angle ant/post slopes	Angle between the anterior slope plane and the posterior slope plane	
ANS-PNS	Maxillary length	Maxilla
N-ANS	Linear measurement between nasion and anterior nasal spine	Facial heights
ANS-Me (AFH)	Anterior facial height	
PFH	Posterior facial height	
LFH/TFH	Ratio between lower facial height and total facial height	
AFH/PFH	Facial height index: ratio between ant and post facial heights	
MP/SN	Angle between cranial base cant and mandibular plane (MP)	Vertical relationship between the jaws (facial divergence)
MP/H	Angle between mandibular plane (MP) and the true horizontal	
PP/MP	Angle between palatal plane (PP) and mandibular plane (MP)	
PP/H	Angle between palatal plane (PP) and the true horizontal	
SNA	Angle between anterior cranial base cant and point A	Sagittal relationship between the jaws
SNB	Angle between anterior cranial base cant and point B	
ANB	Angle between points A and B	
AOBO	Distance between perpendiculars from A and B to the occlusal plane	
1/NA	Angle between maxillary incisor long axis and line joining nasion-A	Dentoalveolar relationships
1-NA	Distance between maxillary incisor long axis and line joining nasion-A	
1/SN	Angle between maxillary incisor long axis and anterior cranial base	
1/PP	Angle between maxillary incisor long axis and palatal plane	
i/NB	Angle between mandibular incisor long axis and line joining nasion-B	
i-NB	Distance between mandibular incisor long axis and line joining nasion-B	
i/MP	Angle between mandibular incisor long axis and mandibular plane (MP)	
1/i	Angle between maxillary and mandibular incisors long axes	
OJ	Horizontal distance between the incisal edges of maxillary and mandibular incisors	

Supplementary Table II. Measurements related to the affected individuals that are part of the 8 selected families (n = 22)

Measurement	*Mean*	*SD*	*Max*	*Min*
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
1/NA	28.52	9.36	46.8	5.3
1-NA	4.03	3.38	10.1	−3.5
1/SN	109.70	10.29	128.4	87.9
1/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
1/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

Supplementary Table III. Summary of the common variants in specific genes between affected individuals of each family after the first and second filterings (high putative impact)

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

Supplementary Table IV. Summary of the shared variants in specific genes between affected individuals of each family after the second filtering (moderate putative impact)

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