Novel genes linked to Class II Division 1 malocclusion with mandibular micrognathism

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Introduction: Mandibular micrognathism (MM) is an underdeveloped mandible resulting from complex interactions between genetic and environmental factors. Prior research focused mainly on the genetic determinants of mandibular retrognathism, not necessarily reflecting micrognathism, thus supporting the need to study MM. This study aimed to explore the inheritance pattern and identify the candidate genes involved in the development and familial transmission of MM. **Methods:** Diagnosing probands with MM was based on clinical and lateral cephalometric data. The pedigrees were drawn for 11 identified families, 5 of whom accepted to undergo detailed data and biospecimen collection. These families included 15 MM and 13 non-MM subjects over 2-3 generations. The procedure involved the withdrawal of 5 mL of blood. Genomic DNA was isolated from blood cells to investigate protein-coding regions via whole exome sequencing. Standardized filtering steps were employed, and candidate genes were identified. **Results:** Most of the pedigrees suggested a Mendelian inheritance pattern and segregated in an autosomal-dominant manner. One of the families, which also underwent biospecimen, displayed an X-linked inheritance pattern of the trait. Genetic screening disclosed 8 potentially novel genes (*GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7,* and *WNT6*). None of the recognized exonic regions were previously reported. **Conclusions:** Eight novel genes were identified in association with MM in the largest number of families reported to date. The genes were X-linked in 1 family, a finding previously not observed in mandibular genetics. (Am J Orthod Dentofacial Orthop 2023;163:667-76)

Mandibular micrognathism (MM) indicates a significant reduction in the mandible size associated with a reduced or absent chin projection in a convex facial profile. The short recessed mandible has been linked with respiratory problems, including obstructive sleep apnea,[1] with genetic syndromes such as Pierre Robin and Treacher-Collins, and environmental insults such as the ankylosis of the temporomandibular joint consequent to trauma or severe infection in the joint area that lead to motion-impeding scarring.[2] In nonsyndromic or pathologic conditions, MM is compatible with the description of skeletal Class II Division 1 (CII/1) malocclusion but with a distinctly smaller-than-average or dwarfed mandible.[3] Although a precise quantification of such dwarfing is lacking, the CII/1 with MM may be considered a potential subphenotype of this malocclusion with more specific genetic characteristics.

The etiology of Class II malocclusions is predominantly genetic, with a variable influence of environmental factors expressed over time.[4] CII/1 and 2 malocclusions have been related to polygenic inheritance patterns.[5] The etiologic complexity of CII/2 may be traced to the classification scope of this malocclusion, which includes a CII/2 variation.[6] The CII/1 genetic studies are mainly based on the association with mandibular retrognathism, the most common phenotype of CII/1,[7] which may or may not be accompanied by micrognathism. To our knowledge, only 1 linkage study of a genome-wide family-based MM has been performed in a Columbian population.[8] The results revealed a single nucleotide polymorphism in the affected

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and nonaffected subjects, thus not confirming the association with MM, although it was not based on whole exome sequencing.

Several chromosomal regions or loci that harbor susceptibility genes for CIII/1 have been identified through genetic mapping conducted in different ethnic populations (Supplementary Table). The genetic determinants of MM remain ambiguous and need focused research separate from retrognathism, which can be associated with the normal or near-average mandibular size.

From a clinical perspective, knowledge of a patient's inheritance of MM would help determine the limitation of orthopedic interventions at an early age, particularly when complemented with clinical and radiographic predictors of growth outcome. Such information would assist the clinician and parents in deciding on the timing and modality of treatment.

We hypothesized that micrognathic mandibles, being more severely reduced than retrognathic mandibles, might be subject to more genetic than environmental influences, thus warranting distinct genetic assessment. Accordingly, we aimed to explore the inheritance pattern and identify the candidate genes and loci involved in the development and familial transmission of MM in CIII/1.

MATERIAL AND METHODS

The Institutional Review Board at the American University of Beirut approved the protocol of the study (no. BIO.2018.0289) before its initiation, and consent was obtained from all participants. Eleven families of Eastern Mediterranean heritage and Caucasian features were approached in whom characteristics of MM have been identified over at least 2 generations during the treatment of 1 member of the younger generation at the Division of Orthodontics and Dentofacial Orthopedics of the American University of Beirut Medical Center. Excluded were subjects with congenital disorders (eg, cleft lip or palate) and reported illness and chronic ailment.

Two steps of recruitment were followed. At the initial step, the patients with MM were confirmed on the basis of clinical examination and cephalometric measurements: an ANB angle $\geq 4.5^{\circ}$ and a positive Wits appraisal ≥ 2.0 mm accompanied with a reduced mandibular length, measured as the distance condylion-gnathion (Co-Gn) at a minimum of 1 standard deviation below the norm for the subject's age.[9] Correspondingly, a bilateral dental CIII malocclusion was mandated, with full molar distocclusion at least on 1 side, a minimum of end/on the relationship on the other, and an increased overjet. Other family members were initially classified on the basis of clinical examination, with the presumed members with MM exhibiting facial and occlusal characteristics similar to those of the identified patients and the non-MM members having either normal occlusion (Class I) or mild to moderate Class I or Class II malocclusion with straight or slightly convex profiles. Pedigrees were drawn on all families. The numbers of males and females with MM in these families were equal (n = 26).

In the second step, only 5 families (4 Lebanese and 1 Syrian/Jordanian), including 28 subjects (15 with MM, 13 non-MM), agreed to proceed with the genetic analysis that required a blood draw. Each subject was assigned a family code (A-E) followed by a number (Fig 1). Non-MM family members were included in the biospecimen collection as controls. The recruitment process, including blood withdrawal, spanned nearly 5 months.

Including the 5 probands, cephalograms were obtained on the 15 MM participants (10 males and 5 females) using the same machine (Instrumentarium, Tuusula, Finland). The subjects were positioned in natural head position with the jaws in centric occlusion (most unstrained retruded position of the mandible). Cephalometric x-rays were not taken on the non-MM participants because they were expected to be of different ages (range, 8.8-73.9 years; mean 37.91 $\pm$ 22.67 years of growing and adult subjects including grandparents), thus limiting valuable comparison between groups. Accordingly, we matched for gender and age the MM subjects with patients from our university clinical pool who had CIII/1 malocclusion with retrognathic but not micrognathic mandibles (mandibular length not below the standard deviation for their age/gender norm). The groups were compared with a *t* test.

The cephalograms were digitized and analyzed by 1 investigator (M.C) using the Dolphin Imaging program (version 11.5; Dolphin Imaging and Management Solutions, Chatsworth, Calif). Angular and linear cephalometric measurements were made to gauge the characteristics and relationships among the cranial base and the jaws. The cephalographs of subjects with MM in 3 generations from family A, including the proband depicting MM, are shown in Figure 2. Cephalometric measurements of sagittal and vertical jaw relations along with mandibular components are displayed in Table I. Z scores demonstrating the deviation of mandibular length (Co-Pog) of the probands and other family members with MM are presented in Figure 3.

Biospecimen collection for genetic analysis consisted of 5 cc of blood withdrawn at our medical center from both MM and non-MM participants by the institutional staff. Genomic DNA was isolated using the Qiagen Blood-Midi kit (Qiagen Sciences Inc, Germantown, Md)

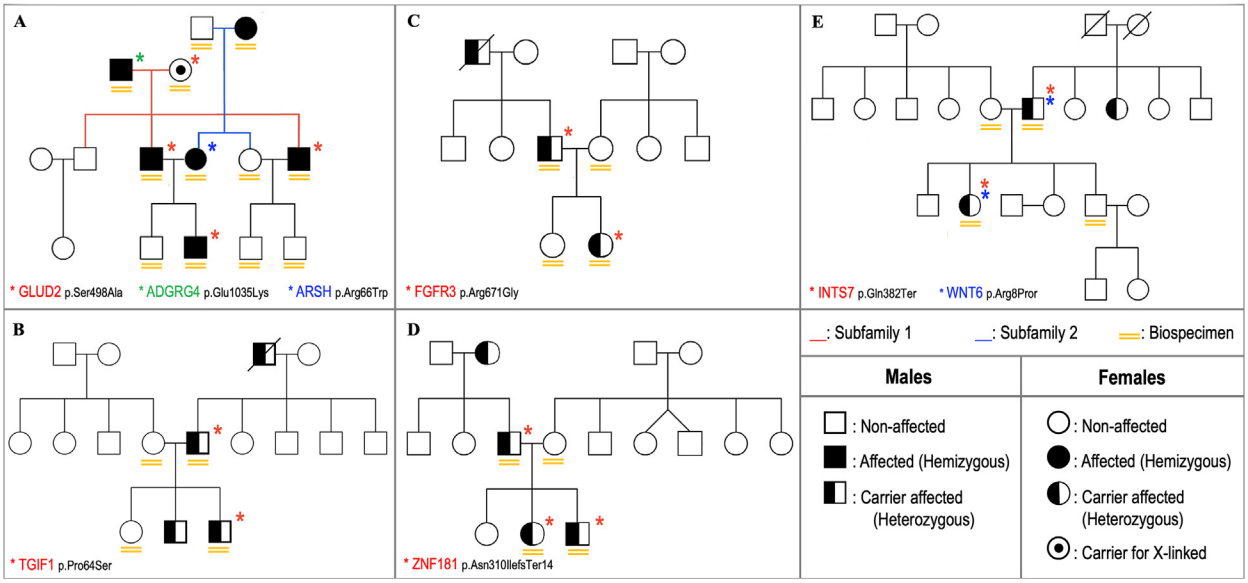

Fig 1. Pedigrees of the 5 families unveiling the mode of transmission of the candidate genes: **A,** In family A, subfamily 1 displays an X-linked recessive pattern of inheritance, whereas subfamily 2 reveals an X-linked dominant inheritance. **B-E,** Families B, C, D, and E exhibit an autosomal-dominant mode of inheritance. Note. The members with MM who participated in the genetic analysis (asterisk near geometric identifier) totaled 15. The X-linked carrier was not affected.

as per the manufacturer recommendations in the genetic unit of the Department of Molecular Biology and Genetics at our university. DNA quantity and quality were assessed using the bioanalyzer at the institutional department of Biochemistry and Molecular Genetics. Whole exome sequencing (WES) was employed to efficiently identify coding variants in the genome by the NovaSeq 6000 Illumina platform (San Diego, Calif). This procedure was conducted at Macrogen Laboratory (Seoul, South Korea; dna.macrogen.com) using SureSelect Human All Exon V6 r2 as a capture method with 101 bp read length at an average depth of 41× (median, 30×). Quality check on raw sequencing reads was performed using FastQC, followed by reads trimming and mapping to the human genome hg19 assembly using the Burrows-Wheeler Aligner as previously described.[10] We adopted a Phred scaled quality score PSQ>30. Aligned reads were marked for duplication, followed by local realignment using the Genome Analysis Toolkit (GATK4). Annotation was performed using the Illumina Variant Studio, and variants were classified into the following: Frame_Shift_Del, Missense_Mutation, Nonsense_Mutation, Frame_Shift_Ins, In_Frame_Ins, Splice_Site_Acc, Splice_Site_Don, Splice_Reg, In_Frame_Del, and Synonymous_Mutation.

The software includes 2 in silico predictive tools for missense variants: SIFT and Polyphen2, which were used in the second filtering round. Further analysis, when needed, was carried out using the Varsome (www.varsome.com) and CADD (www.cadd.gs.washington.edu) platforms to score the variants and update their minor allele frequencies in different populations.

Initial filtering covered a comparison between the results of this study and those of available reports in the literature to screen common genes (corresponding references are listed in Supplementary Table) (Fig 4). Subsequently and during the first round of stringent filtering, the only variants analyzed were the passed variants having a high putative impact (either missense mutations or disruptive mutations: frameshift or stop codon inside the coding region) and a coverage read >20. All the nonsynonymous variants were included. The intronic regions and variants in the noncoding regions and synonymous variants were excluded. The variants with a minor allele frequency[11] in the gnomAD database of less than 5% were included against the normal population.

During the second round of stringent filtering, subjects of the same family were compared with find, if any, common genes among the MM members that were not observed in the genetic material of the non-MM members. A function check was carried out on the possible candidate genes that had materialized from the several filtering steps. The genes that did not have a role in the formation

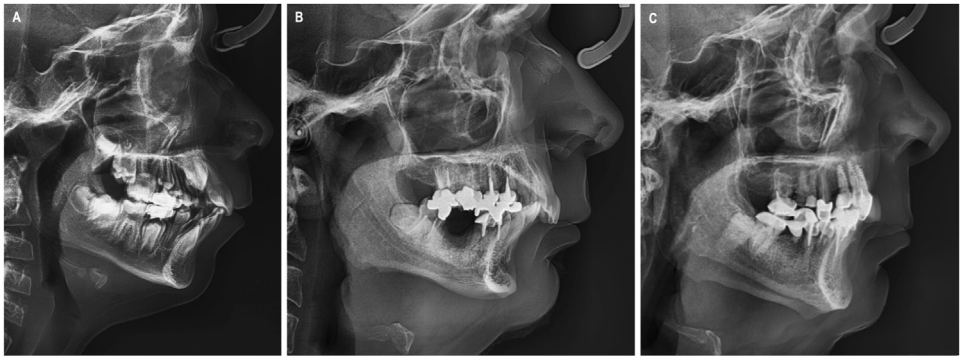

Fig 2. Lateral cephalometric views of 3 subjects with MM from 3 generations (family A): **A,** Proband; **B,** Paternal uncle, second generation; **C,** Paternal grandfather, third generation.

Table I. Cephalometric measurements of the probands in the 5 participating families

				Sagittal and vertical measurements				*Mandibular measurements (mm)*		
Family	*Gender*	*Age (y)*		*SNA°*	*SNB°*	*ANB°*	*MP-SN°*	*Co-Pog*	*Co-Go*	*Go-Pog*
A	Male	10.5	P	90.0	83.0	6.3	30.9	95.0	46.2	68.3
			N	80.8 ± 3.9	77.8 ± 3.5	2.9 ± 2.1	34.3 ± 4.7	99.2 ± 3.5	47.5 ± 2.8	66.5 ± 3.4
B	Male	12.8	P	77.0	70.3	7.3	33.6	97.0	46.5	66.5
			N	81.5 ± 4.4	78.8 ± 4	2.7 ± 2.2	33.2 ± 5	106.3 ± 3.8	51.2 ± 3.7	70.7 ± 3.6
C	Male	48.6	P	74.0	69.4	4.7	36.5	109.9	61.9	52.8
			N	83.0 ± 4.6	81.5 ± 4.4	1.5 ± 2.6	29 ± 6.9	116.7 ± 3.7	60 ± 4.1	78.9 ± 3.8
D	Female	12.4	P	80.0	73.0	7.5	39.2	94.7	44.6	64.1
			N	80.2 ± 4.1	77.3 ± 4.2	2.8 ± 2.3	34.9 ± 5.5	100.6 ± 2.3	49.8 ± 3.4	67.6 ± 3.4
E	Female	23	P	75.0	65.0	11.0	53.5	94.8	50.9	59.5
			N	80.8 ± 4.9	78.7 ± 5.2	2.1 ± 2.4	32.8 ± 7.3	106.0 ± 2.7	54.0 ± 3.9	72.0 ± 3.7

Note. Source: Bhatia and Leighton.[9]
P, Proband; *N*, Norm according to age and gender.

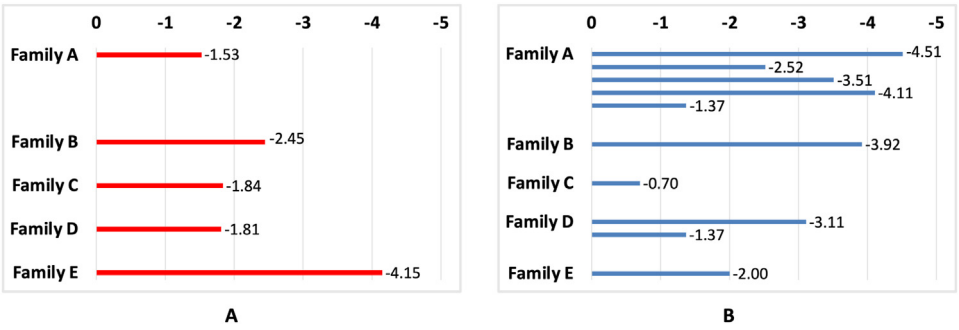

Fig 3. Z scores of mandibular length (Co-Pog) for probands **(A)** and other affected family members **(B)**. The z-score of the nonproband MM family member (daughter of the proband) was less than −1 (although her z-score for Co-Gn was −1). After the second round of stringent filtering and the function check, the mutation in *FGFR3* was only present in her and her father and absent in the genetic material of the nonaffected mother and sister. *FGFR3* has been directly involved in mandibular morphogenesis, specifically in the elongation of Meckel's cartilage.

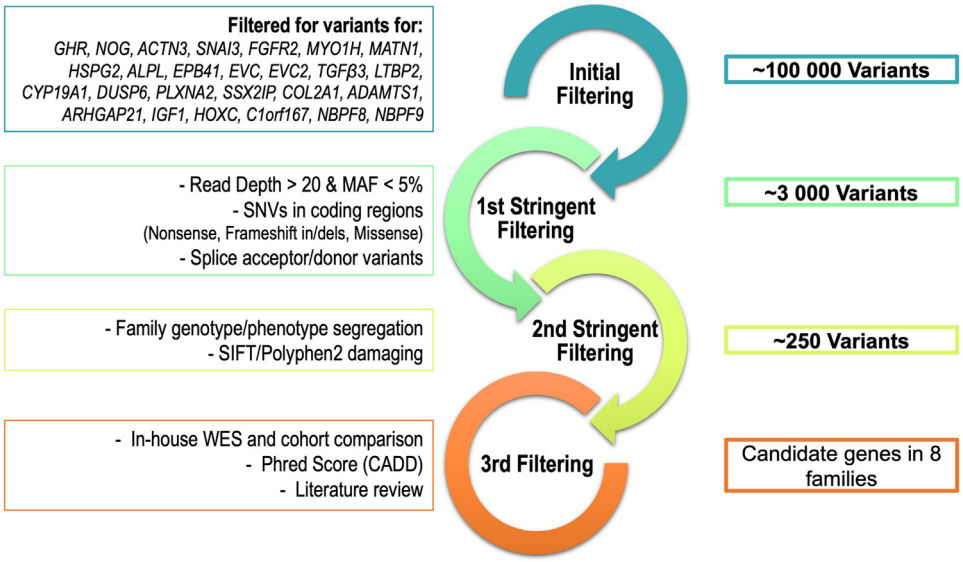

Fig 4. Flow chart of the various filtering steps.

of the bones or jaws were filtered out; the remaining were considered candidate genes for MM.

Statistical analysis was not needed because the data assessment was a genotype-phenotype linkage based on the results of the WES, not a linkage analysis that requires SNP genotyping across the genome. The WES accounts for all the variables (eg, level of inbreeding) because it allows the analysis of the genotypes with a hypothesis-free strategy whereby any variant(s) would be assessed across the family as being linked to the phenotype.

RESULTS

The pedigrees of the 11 families with affected subjects disclosed 3 reported generations per family and suggested a Mendelian inheritance pattern, whereby MM segregated in an autosomal-dominant manner. Consanguinity was not reported in any of the families. In the 5 committed families, the genetic variants in exonic regions of the 28 affected and nonaffected subjects were identified through the WES. The initial filtering comparisons with published data on candidate genes implicated in craniofacial development revealed that the variations in these candidate genes, loci, or variants were either intronic or synonymous. They were found in both affected and nonaffected subjects, indicating that none of the variations could be disease-causing or associated with MM, as defined in our study.

After the first stringent filtering, the number of variants was reduced from 100,000 to 2000-3000 in all families (Fig 4). The second stringent filtering disclosed many genes common among the affected subjects. After the function check, 8 potential family candidate variants in the following specific genes were found to be shared among those subjects and to segregate with the phenotype: *GLUD2* p.Ser498Ala, *ADGRG4* p.Glu1035Lys, *ARSH* p.Arg66Trp, *TGIF1* p.Pro64Ser, *FGFR3* p.Arg671Gly, *ZNF181*p.Asn310IlefsTer14, *INTS7*p.Gln384Ter and *WNT6* p.Arg8Pro (Table III).

The 8 genes were located on different chromosomes. In family A, the 3 candidate genes (*GLUD2*, *ARSH,* and *ADGRG4*) were on chromosome X. Considering the presence of 2 subfamilies in family A, *GLUD2* was located on the chromosome X of the male affected off-springs in subfamily 1 and was heterozygous recessive in the mother. The ARSH mutation was inherited as heterozygous dominant in subfamily 2. The *ADGRG4* mutation was found in only 1 subject in subfamily 1, after an additional sorting of the variants. Reports are not available on this mutation. The candidate genes in families B (*TG1F1*), C (*FGFR3*), and D (*ZNF181*) were on chromosomes 18, 4, and 19, respectively. In family E, 2 genes (*INTS7* and *WNT6*) were identified on 2 different chromosomes (1 and 2, respectively) that may or may not segregate in a digenic pattern. Families B, C, D, and E displayed an autosomal-dominant mode of inheritance for the corresponding variants (Fig 1).

The comparison of the cephalometric variables of the MM subjects with the matched non-MM controls revealed statistically significant differences related only to mandibular measurements and the total and lower

Table II. Cephalometric measurements of the MM participants compared with matched CIII/1 subjects with no MM

Variables	*MM (n = 15)*	*Control (n = 15)*	P *value*
Co-Pog, mm	100.11 ± 5.26	110.75 ± 6.18	<0.0001
Co-Gn, mm	102.90 ± 5.70	113.31 ± 7.03	<0.0001
Co-Go, mm	52.67 ± 6.10	59.25 ± 7.91	<0.001
Go-Pog, mm	64.08 ± 11.12	71.07 ± 5.58	0.020
Go-Gn, mm	70.09 ± 3.58	75.67 ± 5.36	0.003
Ar-Gn, mm	103.78 ± 5.75	110.03 ± 7.55	0.004
PP/H, °	-3.74 ± 3.60	2.59 ± 3.22	<0.0001
N-ANS (TFH), mm	50.83 ± 3.51	53.58 ± 4.37	0.040
ANS-Me (AFH), mm	63.36 ± 6.78	69.50 ± 7.48	0.025
Ar-Go (PFH), mm	44.64 ± 5.11	47.60 ± 6.58	0.030
AFH/PFH, %	134.18 ± 14.74	147.77 ± 18.18	0.044

Note. Only statistically significantly different variables among 40 studied are displayed.
TFH, total facial height; *AFH*, anterior facial height; *PFT*, posterior facial height.

Table III. Summary of the characteristics of the shared variants among MM subjects (that segregate with the phenotype) after the second filtering (high putative impact) and function check in all families

Family	*Gene*	*Chr*	*Positions*	*HGVSp*	*dbSNP*	*Variant effect predictor*	*gnomAD*	*CADD*	*SIFT*	*Polyphen*
A	*GLUD2*	X	120183030	p.Ser498Ala	rs9697983	Missense	0.0266	5.402	Tolerated (0.95)	Benign (0.001)
	ADGRG4	X	135428968	p.Glu1035Lys	rs149243520		0	7.894	Deleterious (0)	Benign (0.087)
	ARSH	X	2928174	p.Arg66Trp	rs148749736		0.000109	9.535	Deleterious (0)	probably damaging (0.982)
B	*TGIF1*	18	3452167	p.Pro64Ser	–	Missense	0	10.12	Deleterious, low confidence (0)	Benign (0)
C	*FGFR3*	4	1808029	p.Arg671Gly	–	Missense	0.00000403	28.0	Deleterious (0.01)	Benign (0.121)
D	*ZNF181*	19	35232206	p.Asn310IlefsTer14	–	Frameshift	0	–	–	–
E	*INTS7*	1	212154515	p.Gln384Ter	–	Stop-gained	0	39.0	–	–
	WNT6	2	219724783	p.Arg8Pro	–	Missense	0	15.17	Deleterious, low confidence (0.03)	Unknown (0)

Note. All variants are heterozygous.
Chr, chromosome.

face height distances (all smaller in the MM subjects) (Table II). The palatal plane was significantly tipped down posteriorly in the MM group.

DISCUSSION

We focused in this study on nonsyndromic micrognathism associated with CIII/1 and was not limited to its predominant association with mandibular retrognathism.[7] Most cephalometric variables differed between the MM subjects and the non-MM CIII/1 matched-control group related to mandibular morphology. This finding reflects the rigor of our selection process and supports the reference to the MM patients as representing a distinguishable variation or subphenotype of CIII/1.

New genetic definitions were determined in 5 families, the greatest number to be analyzed in affected subjects and encompassing 2-3 generations, demonstrating the segregation of the phenotype across generations. Most pedigree studies attribute to CIII/1 a polygenic mode of inheritance.[4] In contrast, the analysis of our MM subjects suggests Mendelian inheritance in all families, 4 of whom displayed autosomal dominance and 1 an X-linked pattern, a unique finding to date. The disparity may be related to sample definition, notably the inclusion of CIII/1 with MM in our sample vs predominantly mandibular retrognathism in other studies. Environmental factors and other genes may modify the clinical expression but do not inhibit the development of a Mendelian trait.[4] This potential supports the presence of a mutation or a series of mutations responsible for the phenotype.

We set stringent inclusion criteria for the definition of micrognathism in the probands below at least 1 standard deviation of the norm for their age. This approach was an attempt at sorting out CIII/1 conditions of retrognathism

Table IV. Summary of the 8 family candidate genes with their corresponding reported function and tissue expression

Family	Gene	Chr	Craniofacial tissues	Other tissues	Functional processes
A	*GLUD2*	X		Human steroidogenic tissues[13]	Human brain development (strengthening cortical excitatory transmission)[14]
	ARSH†	X		Bone and cartilage[15]	Hormone biosynthesis, modulation of cell signaling, and degradation of macromolecules[15]
	ADGRG4‡	X	Jaw muscles		
B	*TGIF1*†	18			Bone remodeling (osteoblasts differentiation impairment)[16]
C	*FGRF3*‡	4	• Meckel's cartilage • Mandibular bones morphogenesis[17]		• Elongation of Meckel's cartilage • Intramembranous ossification[17]
D	*ZNF181*‡	19	• Dysmorphic facial features • Mandible (syndromes)[18]		Transcription factor (cell function)[18]
E	*INTS7*‡	1	Abnormal mandibular arch skeleton[19]	• Gastrointestinal • OCPD[19]	3' end processing of snRNAs (U1 and U2)[19]
	WNT6‡	2	• Lower jaw-specific defects[20] • Condylar perichondrium[21] • Aberrant TMJ development[21]		• Oncogenesis[20] • Cell fate and patterning regulation (embryogenesis)[20] • Upstream regulators of Bmp and Edn1 signaling[20] • Epithelial remodeling and epithelial-mesenchymal transformation[22]

Chr, chromosome; *OPCD*, obsessive-compulsive personality disorder; *TMJ*, temporomandibular joint; *snRNAs*, small nuclear RNAs.
†Linked to cranio-facial and mandibular morphogenesis; ‡Linked to bone and cartilage.

with micrognathism from those with closer to average-size mandibles. However, this distinction does not rule out the possibility of nonmicrognathic jaws presenting with equally or more severe retrognathism than the micrognathic mandibles. Previous investigations have focused more on prognathism and macrognathism, probably because it is the most inherited malocclusion.[5] The scarcity of genetic analysis on micrognathism limits the interpretation of the findings on the candidate genes.

The 8 potentially novel variants found in this study were recognized to segregate with the phenotype and could be implicated in mandibular development. The variants are either absent or present at a very low minor allele frequency (Table III) in the whole globe normal database, which includes 125,748 healthy subjects for WES and 15,708 healthy subjects for whole-genome sequencing.[12] Five of the encoding genes harboring these variants (*ADGRG4, FGFR3, ZNF181, INTS7,* and *WNT6*) were recognized as having a direct link to mandibular and craniofacial morphogenesis, involved in either the endochondral (condyle) or the intramembranous (remodeling) ossification (Table IV).

More specifically, *GLUD2* has been suggested to contribute to human brain development. The association of this hominoid gene with the MM phenotype is plausible because it is exclusively a human evolutionary acquired gene (not expressed in mice), and its implication in organ developments other than the brain remains unknown.[14] *ARSH* belongs to the family of sulfatases and is clustered with *ARSE*, whereby evolutionary analyses suggested that *ARSE* was the ancestor gene of the cluster. Importantly, mutations in *ARSE* are responsible for X-linked CDPX (chondrodysplasia punctata 1), a congenital disease characterized by abnormalities in cartilage and bone development with variable degrees of severity.[15] Mutation in *ADGRG4* was discovered in 1 MM individual of family A. Its frequency in the normal population is null, and its transcription results in the GPR112 protein, which is identified through a targeted antibody and finds a high expression in the muscles of the jaws, whereby the suggested association with MM.

The reason for linking *TGIF1* with MM is its identification as a novel regulator of bone remodeling and an essential component of the parathyroid hormone anabolic action in a mouse model.[16] *FGFR3* has been involved in the morphogenesis of mandibular bones, whereby *FGFR3* signaling is required for the elongation of Meckel's cartilage. *FGFR2* and *FGFR3* are also involved during the intramembranous ossification of mandibular bones.[17] *ZNF181* falls within the category of transcription factors and is located at 19q13.1; microdeletions in this location have been associated with syndromes exhibiting dysmorphic facial features that entailed the mandible.[18]

INTS7, which encodes a subunit of the Integrator complex, has been allied with abnormal mandibular arch skeleton shape in a zebrafish model.[19] *WNT6* is a member of the WNT family, which consists of structurally related genes that encode secreted signaling proteins. WNT members are regulated by Gli2 (transcriptional activator). Mice deficient in Gli2 displayed aberrant temporomandibular joint development in which the condyle loses its growth-plate-like cellular organization and the disk is not formed. In addition, the condyle was reported to be significantly smaller in size.[21]

The traceability of the recognized candidate genes to mandibular components reflects the association of mandibular development, favorable or deficient, with different parts of the mandible in different subjects. Moss recognized 5 skeletal units within the mandible, the condyle, the coronoid, the ramus, the body, and the chin while emphasizing the concert of genomic and epigenetic processes and mechanisms to provide the necessary and sufficient causes of growth and development.[23] Whether and how much each of these units is linked to various genes and later to variable environmental influences are questions to be explored in uncounted research projects.

The influence of environmental/epigenetic factors in restricting (eg, respiratory function) or enhancing (eg, orthopedic/orthodontic treatment) the expression of the genetic blueprint and the possibility of pathway connections among the candidate genes should also be investigated. In addition, even though the distinction of MM within the CIII/1 phenotype is supported by the findings, future research to sort out the frequency and particularities of micrognathism vs retrognathism only will require a larger number of pedigrees in different populations. Moreover, maxillary prognathism, not the prevalent etiology in CIII/1, warrants a separate study.

In comparative assessment with previous studies, the equal number of affected males and females joins prior findings of no gender differences.[5] Sex-linked or sex-influenced inheritance of CIII/1 was not reported previously.[4] We identified in 1 of the 5 families 3 genes located on chromosome X, the first such finding regarding, to the best of our knowledge, nonsyndromic malocclusions. Indeed only syndromic MM has been reported on chromosome X (eg, chondrodysplasia punctata 1,[15] lissencephaly2,[24] and X-linked mental retardation syndrome[25]).

The genetic screening did not show any aberration in the reported genes linked to Class II mandibular morphology that were revealed in the earlier studies of mostly mandibular retrognathism (Supplementary Table). Chromosomal regions or loci other than those found in our study have been identified that harbor susceptibility genes for CIII malocclusion and mandibular retrognathism (Supplementary Table). In the publication on MM in 4 Columbian families, the authors aimed to determine the presence of the NOG gene polymorphisms linked with anomalies in the skeletal development of mice.[8] A single nucleotide polymorphism was found in both affected and nonaffected subjects, but it may not be related to MM as it could be a population polymorphism. In addition, the cephalometric definition and severity of micrognathism were not assessed using the norms of corresponding age.

A comparative display of the location of candidate genes influencing mandibular morphology reveals a wider spread of chromosomes linked to retrognathism and micrognathism than their counterparts' prognathism and macrognathism in Class III malocclusion (Supplementary Fig). Five identified chromosomes (1, 12, 4, 10, 15) carry candidate genes for both conditions.

Clinical implications of the findings relate to the association of genetic susceptibility to MM with the timing and duration of treatment. Longitudinal studies of untreated CIII/1 have indicated a lack of self-resolution with growth.[26] Clinical investigations revealed variable success in normalizing the underlying skeletal dysplasia with orthopedic treatment.[7] If the malocclusion is not resolved, the early intervention would minimize its severity and enhance self-perception, but orthognathic surgery may still be required later. This outlook puts in question the longer treatment duration, particularly considering the frequency of unsatisfactory orthopedic outcomes and the possibility for relapse, mainly in severe malocclusions.[7,27,28]

Anticipation of MM through blood testing might suggest earlier intervention, possibly in the deciduous dentition when the CIII/1 is identified in association with a short recessed mandible (particularly in the presence of a familial pattern) or omitting earlier treatment in favor of later orthognathic surgery to avoid the side effects and the uncertainty of protracted orthopedic and orthodontic procedures. Bridging genetic knowledge, when perfected, with clinical practice will require sequential investigations. Moreover, prediction models of CIII/1 treatment outcome could be strengthened by integrating the genetic analysis with the clinical and cephalometric assessments to set apart the various subphenotypes associated with this malocclusion and determine which have more potential for orthopedic correction.

CONCLUSIONS

The study of the genetic basis for mandibular micrognathism, explored with the application of WES in 5 families (15 subjects with MM and 13 without MM),

resulted in the identification of 8 new candidate genes (*GLUD2, ADGRG4, ARSH, TGIF1, FGFR3, ZNF181, INTS7,* and *WNT6*). *GLUD2, ARSH,* and *ADGRG4* were located on the X chromosome in 1 family, a novel finding regarding mandibular genetics because X-linked genes were related only to syndromic mandibular dysmorphologies. The genes *TGIF1, FGFR3, ZNF181, INTS7,* and *WNT6* had been recognized with associations to mandibular development, although not specifically micrognathism.

AUTHOR CREDIT STATEMENT

Michelle R. El Chekie contributed to data curation, investigation, methodology, project administration, and manuscript review and editing; Georges Nemer contributed to data curation, formal analysis, funding acquisition, and manuscript review and editing; Athar Khalil contributed to formal analysis and methodology; Anthony T. Macari contributed to project administration, funding acquisition, visualization, and manuscript review and editing; and Joseph G. Ghafari contributed to conceptualization, funding acquisition, investigation, methodology, original draft manuscript review and editing, and supervision.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ajodo.2022.04.020.

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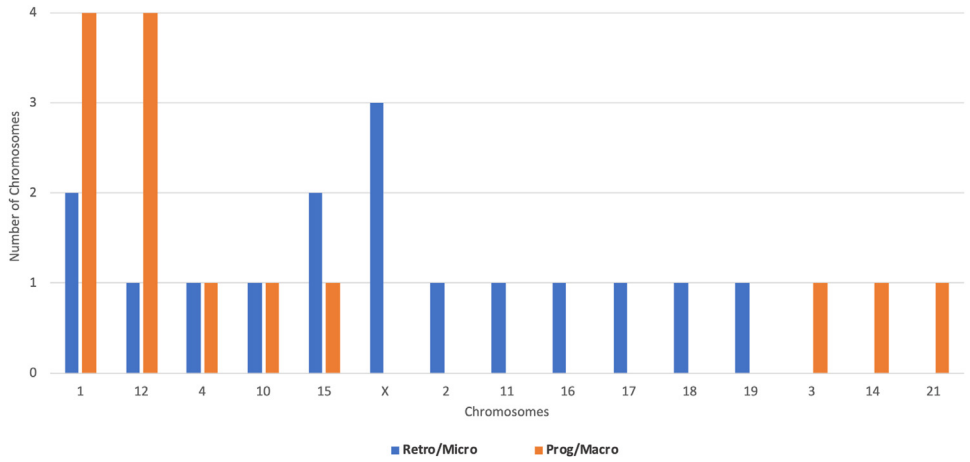

Supplementary Fig. Graph summarizing the location of candidate genes related to retroganthism and micrognathism (Retro/Micro; *blue*) and those related to prognathism and macrognathism (Prog/Macro; *orange*) on the respective chromosomes in the present and published studies (Supplementary Table). *1,* Researchers have identified a wider spread of chromosomes carrying Retro/Micro than Prog/Macro; *2,* Chromosomes 1, 12, 4, 10 and 15 have been reported to carry candidate genes for both conditions (Retro/Micro and Prog/Macro); *3,* More candidate genes for Prog/Macro have been reported on chromosomes 1 and 12. The statistics include the findings of the present study.

Supplementary Table. Summary of the susceptibility genes implicated in craniofacial development in different ethnic populations in previous studies

Gene	*Chr*	*Effect*	*Population*	*Reference*
Mandibular retrognathism and micrognathism				
GHR P561T	15	Shorter mandibular ramus (Co-Go)	Japanese	Yamaguchi et al[1]
GHR P561T	15	Negative impact on mandibular growth	Japanese	Sasaki et al[2]
NOG rs1348322	17	MM	Hispanic	Gutiérrez et al[3]
ACTN3 rs1815739	11	Class II	French	Zebrick et al[4]
SNAI3 rs28755	16	Class II, accentuated convex profile	Caucasian	Fontoura et al[5]
FGFR2 rs11200014	10	Increased risk of Cl II	Caucasian	Fontoura et al[5]
MYO1H rs3825393	12	MR	Indian	Arun et al[6]
MATN1 rs1149042	1	MR	South Indian	Balkhande et al[7]
Mandibular prorognathism and macrognathism				
HSPG2, MATN1and ALPL	1	MP	Korean/Japanese	Yamaguchi et al[8]
MATN1	1	MP	Korean	Jang et al[9]
EPB41	1	MP	Chinese	Xue et al[10]
EVC and EVC2	4	MP	Chinese Han	Li et al[11]
TGFβ3 and LTBP2	14	MP	Chinese Han	Li et al[12]
CYP19A1 (rs2470144)	15	Increased pubertal sagittal mandibular growth in males	Chinese	He et al[13]
MYO1H (rs10850110)	12	MP	White, African American, Hispanic, Asian	Tassopoulou-Fishell et al[14]
DUSP6	12	MP, MD	Estonian	Nikopensius et al[15]
PLXNA2 and SSX2IP	1, 3	MP	Japanese	Ikuno et al[16]
COL2A1	12	MP	Chinese	Xue et al[10]
ADAMTS1	21	MP	Chinese	Guan et al[17]
Gly1121Ser variant in ARHGAP21	10	MP	Italian	Perillo et al[18]
IGF1, HOXC and COL2A1	12	MD	Hispanic	Frazier-Bowers et al[19]
C1orf167, NBPF8, NBPF9	1	MP	Eastern Mediterranean (Caucasian)	Genno et al[10]

Ch, chromosome; *MM*, mandibular micrognathism; *MR*, mandibular retrognathism; *MP*, mandibular prognathism; *MD*, maxillary deficiency.

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