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Article

Genes involved in the maintenance of vitamin D levels might be associated with mandibular retrognathism

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Abstract: In this study we evaluated, if single nucleotide polymorphisms (SNPs) in the genes encoding *PTH, VDR, CYP24A1* and *CYP27B1* are associated with Mandibular Retrognathism (MR). Samples from biologically-unrelated patients receiving orthodontic treatment were included in this study. Pre-orthodontic lateral cephalograms were used to determine the phenotype. Patients having a retrognathic mandible (SNB<78º) were selected as cases and those with an orthognathic mandible (SNB=78º–82º) were selected as controls. Genomic DNA was used for genotyping analysis of SNPs in *PTH* (rs694, rs6256 and rs307247), *VDR* (rs7975232), *CYP24A1* (rs464653) and *CYP27B1* (rs927650). Chi-squared or Fisher's tests were used to compare genotype and allele distribution among groups. Haplotype analysis was performed for the SNPs in *PTH*. The established alpha was p<0.05. Multifactor dimensionality reduction (MDR) was used to identify SNP-SNP interactions. A total of 48 MR and 43 controls were included. In the genotype and allele distribution analysis, the SNPs rs694, rs307247 and rs464653 in were associated with MR (p<0.05). MDR analyses predicted the best interaction model for MR was rs694-rs927650, followed by rs307247-rs464653-rs927650. Some haplotypes in the *PTH* gene presented statistical significance. Our results suggest that SNPs in *PTH*, *VDR*, *CYP24A1* and *CYP27B1* genes are associated with the presence of mandibular retrognathism.

Keywords: mandible; retrognathism; gene; polymorphism

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1. Introduction

Skeletal malocclusions are a set of human craniofacial morphologic characteristics that result in an improper skeletal relationship of the jaws and specific facial patterns. In a classical study, cephalometric radiographs of several families were analyzed to evaluate facial morphologic differences among family members. The authors observed a high correlation between parents and their offspring and concluded that a strong familial tendency for skeletal malocclusions exists supporting the impact of genetic background on face morphology [1]. More recently, many studies in different populations have been performed and demonstrated that genes involved in a variety of functions are associated with skeletal malocclusions and morphological patterns of the face [2,3]. Mandibular Retrognathism (MR) is a common skeletal malocclusion in humans. It refers to a retruded

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position of the mandible as a consequence of an anomaly of the skeletal jaw-cranial base relationship [4].

Single Nucleotide Polymorphisms (SNPs) are a type of genetic variant involving variation of a single base pair in the genome. This is the most common type of genetic variation in humans, and it has been stated that these variants could explain differences in individual predisposition to present complex traits [5]. Previous studies showed that MR was associated with SNPs in various genes, such as *Myosin 1H* (*MYO1H*) [6], *Matrilin 1* (*MATN1*) [4], *ADAM Metallopeptidase with Thrombospondin Type 1 Motif 9* (*ADAMTS9*) [7] and *Bone Morphogenic Protein 2* (*BMP2*) [8] genes. In addition, SNPs in the gene encoding Growth Hormone Receptor (GHR) have been associated with variations in the mandibular pattern, including prognathism [2,9], supporting that genes coding for hormones, hormone receptors, hormone precursors and molecules involved in hormonal synthesis could also be involved in the etiology of other phenotypes.

Two important hormones that play a crucial role in bone development are Parathyroid Hormone (PTH) and Vitamin D (a secosteroid hormone). These are major regulators of mineral metabolism involved in calcium and phosphate homeostasis as well as in bone growth and development [10]. The biological actions of vitamin D are exerted by binding to the nuclear vitamin D receptor (VDR) [11]. VDR is expressed in the parathyroid glands acting as sensors for the detection and maintenance of adequate vitamin D levels, regulating PTH synthesis and release [12], which among other tissues affects the periodontal ligament [13]. Additionally, other molecules such as the vitamin D 24-hydroxylase (CYP24A1) and 1-hydroxylase (CYP27B1) enzymes participate in related processes, being considered as pivotal determinants of the local concentration of active vitamin D [14].

Nutrition and biomechanical factors can affect the facial pattern, however, the most important factor related with craniofacial growth and development seem to be hormones, genetic and molecular mechanisms, as well as the interplay among them [15]. Therefore, in the present study we evaluated, if SNPs the in genes encoding *PTH, VDR, CYP24A1* and *CYP27B1* as well as the interplay among them are associated with MR. A tightly controlled connection between vitamin D, serum calcium, and genes involved in the maintenance of vitamin D levels orchestrates mineral homeostasis and development.

2. Materials and Methods

Sample

This nested case-control study was previously approved by the Human Ethics Committee of the University of São Paulo - Ribeirão Preto Dental School (# 01451418.3.0000.5419). Informed consent/assent was obtained from all participants and/or their legal guardians age-appropriate. This project was performed according to the Helsinki Declaration and its amendments. The Strengthening the Reporting of Genetic Association study (STREGA) statement checklist [16] was followed to develop and report the results of this study.

Patients undergoing orthodontic treatment at the University of São Paulo were recruited and consecutively included in this study from 2015 to 2017. Patients with syndromes, congenital alterations, hormonal and/or metabolic disorders or those with previous orthodontic and/or orthopaedic treatments were not included. All patients included were biologically-unrelated and self-reported as Caucasian.

Phenotypes definition

Pre-orthodontic lateral cephalograms with the mandible in centric relationship were used and digital cephalometric tracings performed by a calibrated orthodontist using the software Dolphin Imaging Version 8.0 (Dolphin Imaging, Chatsworth, CA, USA). The following landmarks were used to determine the phenotype: point A, point B, sella (S), and nasion (N) and, therefore, the angular measurements SNB and ANB were calculated. Patients having a retrognathic mandible (SNB<78º) were selected as cases, and those with

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an orthognathic mandible (SNB=78º–82º) were selected as controls. Patients with mandibular prognathism (SNB>82º) were excluded.

Also, the linear measurements associated with mandibular size (mandibular length, Co-Gn; length of mandibular base, Go-Pg; and mandibular ramus height, Co-Go) were measured in millimetres (mm) and compared between the patients with retrognathic mandible and the patients with orthognathic mandible.

Allelic discrimination

Genomic DNA extracted from saliva was used for genotyping analysis. Briefly, for saliva collection, saline mouth solution to rinse in the mouth for 60 seconds was used. Therefore, the genomic DNA was extracted from buccal epithelial cells from saliva samples as previously described [17]. Quantification of the concentration and purity of the DNA was determined by spectrophotometry (Nanodrop 1000; Thermo Scientific, Wilmington, DE, USA).

Six SNPs were evaluated in the present study and are reported in Table 1. The genotyping was blindly performed using the Taqman™ method for real-time PCR in the StepOnePlusTM sequence detection system, Applied Biosystems™ (Foster City, CA, USA) or in the Mastercycler® ep realplex-S thermocycler, Eppendorf AG (Hamburg, Germany). Additionally, 10% of the sample was genotyped twice and an agreement of 100% was observed. The reaction was previously described [18].

Table 1. Characteristics of the studied SNPs.

Gene	SNP	Base change	Type of alteration/region
	rs694	C>T	Intron
PTH	rs6256*	G>T	Arg115Ter, R (Arg) >(Ter)
	rs307247	G>A	3' UTR
VDR	rs7975232#	C>A	Intron
CYP27B1	rs464653	A>G	Intron
CYP24A1	rs927650	C>T	Intron

Note: *Stop gain. #known as ApaI. Information was obtained in https://www.ncbi.nlm.nih.gov/snp/.

Statistical Analysis

Chi-squared test was used to estimate the Hardy-Weinberg equilibrium (https://wpcalc.com/en/equilibrium-hardy-weinberg/).

Chi-squared or Fisher's exact tests were used to compare gender, genotypic and allelic distribution among groups. Mann-Whitney U test was applied for the comparison of continuous data among the groups after evaluate the normality by Shapiro–Wilk test and use Levene's test to assess homogeneity of variance. These analyses were performed by GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, USA).

Haplotype analysis was performed for the SNPs in PTH by PLINK version 1.06 (https://zzz.bwh.harvard.edu/plink/ld.shtml). The established alpha for these analyses was $p<0.05$.

Multifactor dimensionality reduction (MDR) was done to identify SNP-SNP interactions using gender and age as co-variables as previously reported [8] A 10-fold cross-validation was calculated and the 1000 permutation test determined statistical significance of the models. Models with the cross-validation consistency of 9/10 or 10/10 and the Testing Balancing Accuracy (TBA) > 0.55 and $p \leq 0.05$ were considered best models. Entropy values were calculated according to a formula [19] and MDR created dendrograms and interaction graphs using these values. MDR analysis can be performed freely in a JAVA language software (https://sourceforge.net/projects/mdr/).

3. Results

A total of 91 patients were included. Figure 1 presents the flow diagram of the study participants and genotype success rate for each SNP. The sample characteristics and

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mandibular parameters according to the groups (MR and control) are shown in Table 2. There was no significant difference between the groups according to age and gender distribution ($p>0.05$). The participants in the MR group had significantly lower linear measurements in the mandible than the control group ($p < 0.05$). Furthermore, the SNB angle was significantly lower and the ANB angle significantly higher in the retrognathic subjects ($p<0.001$)

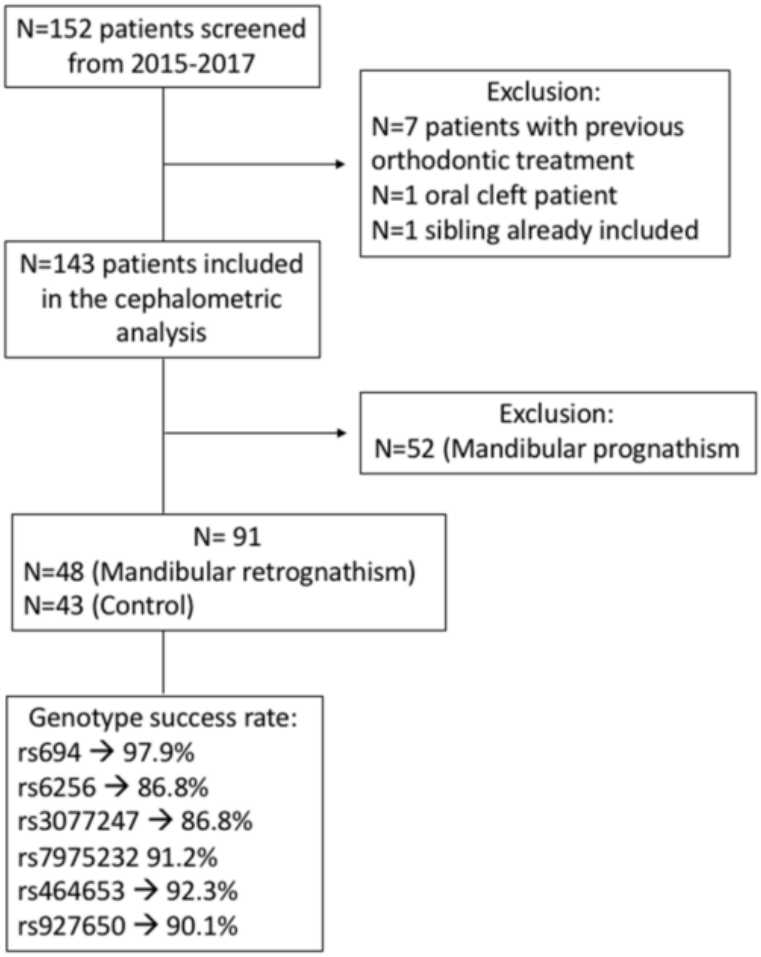

Figure 1. Flow chart of patients' selection.

All the SNPs assessed were within the Hardy-Weinberg equilibrium. Genotype and allele distributions are demonstrated in Table 3. The SNPs rs694 and rs307247 in *PTH*, and the SNP rs464653 in *CYP27B1* were significantly associated with mandibular retrognathism ($p<0.05$).

The best models for MDR analyses were rs694 (PTH), rs927650 (CYP24A1), rs307247 (PTH) - rs464653 (CYP27B1) - rs927650 (CYP24A1) and rs694 (PTH), rs6256 (PTH), rs307247 (PTH), rs7975232 (VDR), rs464653 (CYP27B1), rs927650 (CYP24A1) with a cross-validation consistency of 10 out of 10. Table 4 shows MDR-predicted interaction models.

Figure 2 shows the interactions between SNPs (dendrogram and interaction map). The strongest synergism interaction effect was between rs307247 (*PTH*) and rs927650 (*CYP24A1*), followed by the interaction between rs6256 (*PTH*) and rs464653 (*CYP27B1)*.

The haplotype analysis for the SNPs in the *PTH* gene is presented in Table 5. Some haplotypes were associated with mandibular retrognathism.

Table 2. Comparison of cephalometric variables between mandibular retrognathism and control groups.

Variables	MR	Control	p-value

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Gender n (%)			
Male	22 (45.8)	17 (39.5)	0.672
Female	26 (54.2)	26 (60.1)	
Age			
Median (IQR)	12.0 (4.0)	12.0 (4.5)	0.809
SNB (º)			
Median (IQR)	76.1 (3.5)	80.0 (2.0)	**< 0.001***
ANB (º)			
Mean (SD)	4.2 (2.3)	2.4 (2.3)	**< 0.001***
Co-Gn (mm)			
Mean (SD)	110 (8.0)	115 (9.4)	**0.008***
Go-Pg (mm)			
Median (IQR)	64.4 (6.9)	68.2 (5.5)	**0.014***
Co-Go (mm)			
Median (IQR)	54.0 (8.8)	56.8 (7.4)	**0.044***

Note: IQR means interquartile range; SD means standard deviation. MR means mandibular retrognathism. * means statistically significant difference ($p<0.05$).

4. Discussion

Disorders of the face and dental jaws, such as skeletal malocclusions, are very common developmental disorder in all ethnic populations [20]. Skeletal malocclusion affects dental and facial tissues [20]. Mandibular retrognathism is a condition that not only affects facial aesthetics, but is also associated with problems such as temporomandibular disorders [21] and alterations in the respiratory pattern and normal sleep [20,22]. The prevalence of skeletal malocclusion ranges according to the studied population [20]. In the orthodontic population studied here, skeletal class II malocclusion affects about of 30% the sample [8,23] and mandibular retrognathism affects 35% of the sample [8]. Retrognathism is a much more frequent condition than mandibular prognathism; however, its etiology has been the subject of only few studies. Most studies on the genetic background of skeletal malocclusion have been focusing on prognathism and Class III malocclusion, which is less frequent in the general population. Studies on genes involved in skeletal Class II malocclusion, retrognathism and micrognathia are rarer [2] and only few genes and SNPs were evaluated so far [4,6-8,24]. Therefore, the present study aimed to explore the role of some SNPs, as well as their interaction, in the mandibular retrognathism phenotype in humans.

Sequence variation in human genes is largely confined to SNPs and is valuable in tests of association with common traits, such as retrognathism. Uncovering the SNPs and genes responsible for the regulation of facial morphology is not a trivial task. Human facial development is a complex multistep process, implicating several signaling cascades of factors. The mechanisms involved in this process include the expression of innumerous genes and proteins translation. These events are precisely timed and are under hormonal control [25]. Therefore, in the present study we evaluated, if common SNPs in genes involved in hormonal synthesis and metabolisms were associated with mandibular retrognathism.

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Table 3. Genotype and allele distribution among the groups.

Gene	*SNP*	*Frequency- n (%)*			*p-value*	*OR (CI 95%)*
		Genotype/Allele	*Control*	*MR*		
PTH	rs694	Genotype **TT**	4 (10.8)	19 (44.2)	Reference	-
		Genotype CT	22 (59.5)	19 (44.2)	**0.004***	0.18 (0.06 – 0.59)
		Genotype CC	11 (29.7)	5 (11.6)	**0.001***	0.09 (0.02 – 0.44)
		Allele T	30 (40.5)	57 (59.4)	Reference	-
		Allele C	44 (59.5)	39 (40.6)	**0.014***	0.46 (0.25 – 0.87)
	rs6256	Genotype GG	30 (81.1)	32 (76.2)	Reference	
		Genotype GT	7 (18.9)	9 (21.4)	0.740	1.20 (0.39 – 3.39)
		Genotype TT	0 (0.0)	1 (2.4)	>0.999*	-
		Allele G	67 (90.5)	73 (86.9)	Reference	-
		Allele T	7 (9.5)	11 (13.1)	0.472	1.44 (0.51 – 3.71)
	rs307247	Genotype GG	12 (33.3)	26 (60.5)	Reference	
		Genotype AG	14 (38.9)	12 (27.9)	0.074	0.39 (0.14 – 1.16)
		Genotype AA	10 (27.8)	5 (11.6)	**0.019***	0.23 (0.06 – 0.78)
		Allele G	38 (52.8)	64 (75.0)	Reference	
		Allele A	34 (47.2)	22 (25.0)	**0.003***	0.37 (0.19 – 0.73)
VDR	rs7975232	Genotype AA	14 (35.0)	18 (41.9)	Reference	
		Genotype AC	17 (42.5)	21 (48.8)	0.934	0.96 (0.37 – 2.44)
		Genotype CC	9 (22.5)	4 (9.3)	0.121	0.34 (0.10 – 1.31)
		Allele A	45 (56.2)	57 (66.3)	Reference	-
		Allele C	35 (43.8)	29 (33.7)	0.184	0.65 (0.34 – 1.20)
CYP27B1	rs464653	Genotype AA	13 (33.3)	25 (55.6)	Reference	-
		Genotype AG	21 (53.9)	15 (33.3)	**0.037***	0.37 (0.13 – 0.93)
		Genotype GG	5 (12.8)	5 (11.1)	0.358	0.52 (0.12 – 2.09)
		Allele A	47 (60.3)	45 (64.3)	Reference	-
		Allele G	31 (39.7)	25 (35.7)	0.613	0.84 (0.44 – 1.68)
CYP24A1	rs927650	Genotype CC	17 (44.7)	15 (34.1)	Reference	-
		Genotype CT	16 (42.1)	26 (59.1)	0.197	1.84 (0.75 – 4.69)
		Genotype TT	5 (13.2)	3 (6.8)	0.633	0.68 (0.16 – 3.45)
		Allele C	50 (65.8)	56 (63.6)	Reference	-
		Allele T	26 (34.2)	32 (36.4)	0.773	1.09 (0.57 – 2.12)

Note: * means statistically significant difference ($p<0.05$).

PTH is an 84-amino acid peptide hormone synthesized in the cells of the parathyroid glands. This hormone is a major mediator of bone remodeling and plays a crucial role in calcium homeostasis, showing several effects on the bone remodeling process, resulting in anabolic activity (bone formation) and catabolic activity (bone resorption) [26,27]. PTH promotes calcium release at bone level, in which a hypocalcemic signal will lead to a higher release and synthesis of PTH restoring the serum calcium to normal [28]. In our study, two SNPs in the gene encoding PTH were associated with retrognathism in the univariate analysis: the intronic SNP rs694 and the SNP rs307247 which is located in a 3' untranslated region (3'UTR). One important aspect in genetic association studies is that the majority of traits were associated with traits in non-coding regions (intronic and intergenic), called regulatory SNPs [29]. Intronic variants can impact alternative splicing by interfering with splice site recognition [30]. Also, 3'UTRs can modify gene expression by controlling the mRNA nuclear export, cytoplasmic localization and stability or by affecting translational efficiency. These gene fragments are targeted by microRNA as well as regulatory molecules [31]. However, one aspect to be highlighted for future studies designs is that a redundancy was observed between rs694 and rs307247 in the interaction map.

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Table 4. Summary of MDR analysis results.

Locus number	Best Combination	Cross-validation consistency	Testing Balanced Accuracy	P-value
2	rs694 (*PTH*), rs927650 (*CYP24A1*)	10/10	0.6742	**0.040***
3	rs307247 (*PTH*), rs464653 (*CYP27B1*), rs927650 (*CYP24A1*)	10/10	0.7651	**<0.001***
4	rs307247 (*PTH*), rs7975232 (*VDR*), rs464653 (*CYP27B1*), rs927650 (*CYP24A1*)	8/10	0.7016	**0.009***
5	rs694 (*PTH*), rs307247 (*PTH*), rs7975232 (*VDR*), rs464653 (*CYP27B1*), rs927650 (*CYP24A1*)	8/10	0.6832	**0.026***
6	rs694 (*PTH*), rs6256 (*PTH*), rs307247 (*PTH*), rs7975232 (*VDR*), rs464653 (*CYP27B1*), rs927650 (*CYP24A1*)	10/10	0.7085	**0.008***

Note: * means statistically significant difference (p<0.05). P-values were based on a 1000 permutations test.

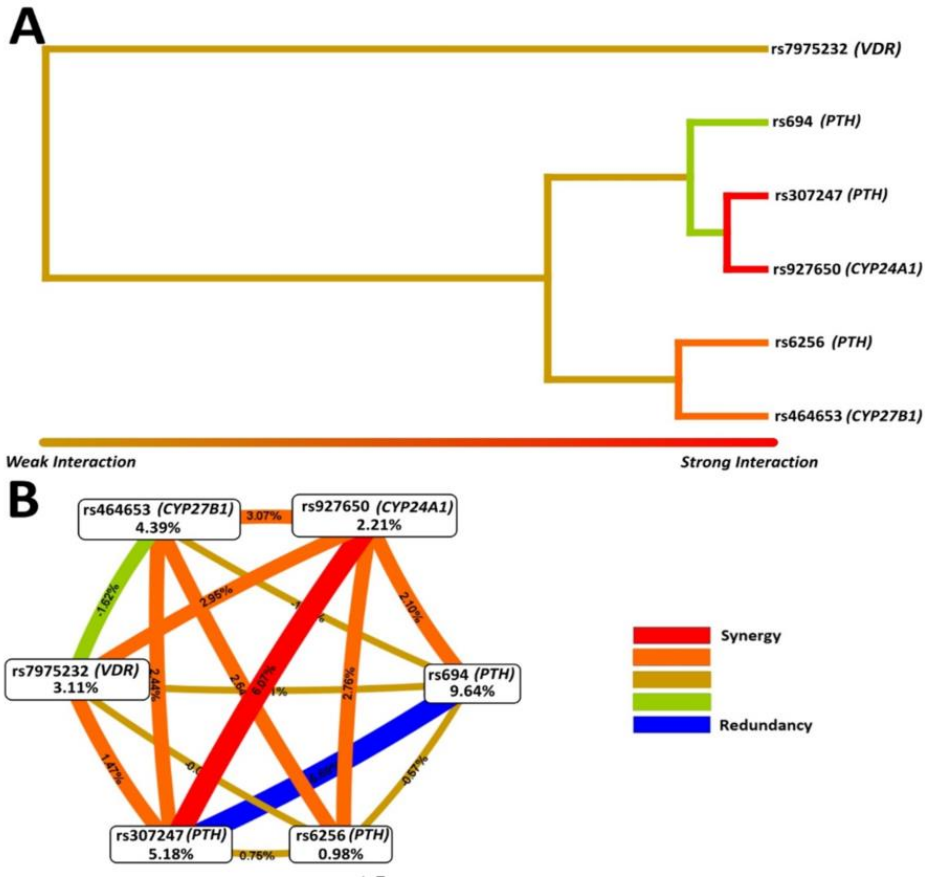

Figure 2. Interaction analysis for the studied SNPs by MDR: A- Interaction dendrogram; B- Interaction entropy graph.

Table 5. Haplotype association analysis of SNPs in PTH gene.

SNPS	Haplotype	MR	Control	*p-value*
rs694 - rs6256 - rs307247	C-G-A	0.25	0.47	**0.004***
	C-T-G	0.01	0.03	0.510
	T-T-G	0.11	0.06	0.231
	C-G-G	0.06	0.09	0.530
	T-G-G	0.54	0.34	**0.010***
rs694 - rs6256	T-T	0.13	0.09	0.472
	C-G	0.33	0.59	**0.001***
	T-G	0.53	0.31	**0.004***
rs694 - rs307247	C-A	0.25	0.47	**0.004***
	C-G	0.08	0.12	0.365

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	T-G	0.66	0.40	**0.001***
	A-G	0.26	0.47	**0.006***
rs307247 - rs6256	G-T	0.13	0.09	0.510
	G-G	0.60	0.43	**0.027***

Note: * means statistically significant difference.

In our study, we also evaluated the SNP rs6256 that is located in exon 3 of the PTH gene. This synonymous variant might contribute to the altered gene expression. A previous study demonstrated that serum PTH levels were higher in individuals carrying the rs6256 AA genotype [32]. We observed that this SNP was associated with MR the MDR analysis and in the haplotype analysis.

It is well known that vitamin D requires the involvement of several key proteins in a closely regulated process. The vitamin D produced in the skin, in response to UVB light or from exogenous supplements, is sequentially hydroxylated into the active metabolite 1,25(OH)2D (the biologically active form of vitamin D) in the kidney and other tissues via the enzyme CYP27B1 [33]. Also 1,25(OH)2D concentrations are regulated by CYP24A1[34]. In the genotype distribution, as well as in the MDR analysis, the intronic SNP rs464653 in CYP27B1 was associated with mandibular retrognathism. A strong interaction and synergism were observed between the SNP rs307247 in PTH and rs927650 in CYP24A1. Parathyroid glands contain CYP27B1 and CYP24A1 and the expression levels of these both enzymes are transcriptionally regulated in a highly reciprocal manner by PTH [35]. Therefore, our results suggest that SNPs in genes involved in this highly regulated interaction are involved in mandibular morphology in humans.

A study evaluating animals fed with the low calcium and vitamin D-deficient diet observed that this diet caused alterations in craniofacial morphology, including reduced mandibular dimensions [36]. The biological effects of vitamin D are mediated by binding to its receptor, a member of the nuclear receptor superfamily, codified by the gene VDR [11]. Although the studied SNP rs7975232 in VDR seems to have only a small role in the mandibular retrognathism phenotype, this result should be interpreted with caution. In our analysis, only one SNP was evaluated in VDR, which is a gene with some well-known SNPs. Therefore, the coverage of the gene was a limitation of our study. It is possible that other SNPs in VDR as well as their interaction are also involved in the retrognathism phenotype. Additionally, generalizability of results to populations of other ancestries is unknown. Further studies are necessary to evaluate the role of these SNPs in skeletal malocclusions in different populations.

5. Conclusions

Briefly, MR is a polygenic trait. SNPs in coding and regulatory regions can lead to different gene activities and possible contributions to interindividual variability, including variability in facial morphology. Our results support that genes involved in the maintenance of vitamin D levels are involved in the etiology of human MR.

Supplementary Materials: The data generated in the present study are included within the manuscript.

Author Contributions: Conceptualization, E.C.K., P.N.F, M.N.M., P.P. and C.K.; resources, E.C.K., P.P., P.N.F. and C.K.; supervision, M.N.M. and M.B.S.; project E.C.K. and C.K.; sample collection, G.A.M.V., M.N.M. and M.B.S.; phenotype analysis, G.A.M.V., M.N.M. and M.B.S.; statistical analysis, M.A.H.M.O and C.L.B.R; data interpretation, E.C.K., C.L.B.R, G.A.M.V. and C.K.; funding acquisition E.C.K. and C.K.; writing—original draft preparation, E.C.K. C.L.B.R, G.A.M.V. and C.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Human Ethics Committee of the University of São Paulo - Ribeirão Preto Dental School (# 01451418.3.0000.5419).

Informed Consent Statement: Informed consent/assent was obtained from all participants and/or their legal guardians age-appropriate.

Data Availability Statement: The data generated in the present study are included within the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nakasima, A.; Ichinose, M.; Nakata, S.; Takahama, Y. Hereditary factors in the craniofacial morphology of Angle's Class II and Class III malocclusions. *Am J Orthod* **1982,** *82*, 150-156.
2. Uribe, L.M.M.; Miller, S.F. Genetics of the dentofacial variation in human malocclusion. *Orthod Craniofac Res* **2015,** *18*, 91–99.
3. Dehesa-Santos, A.; Iber-Diaz, P.; Iglesias-Linares, A. Genetic factors contributing to skeletal class III malocclusion: a systematic review and meta-analysis. *Clin Oral Invest* in press.
4. Balkhande, P.B.; Lakkakula, B.V.K.S.; Chitharanjan, A.B. Relationship between matrilin-1 gene polymorphisms and mandibular retrognathism. *Am J Orthod Dentofacial Orthop*. **2018**, *153*, 255-261.
5. Teama, S. DNA polymorphisms: DNA-based molecular markers and their application in medicine. In *Genetic Diversity and Disease Susceptibility*, 1nd ed.; Liu Y. IntechOpen: London, England, 2018, Volume 25.
6. Arun, R.M.; Lakkakula, B.V.; Chitharanjan, A.B. Role of myosin 1H gene p morphisms in mandibular retrognathism. *Am J Orthod Dentofacial Orthop* **2016** *149*, 699–704.
7. Wang, C.; Ni, Z.; Cai, Y.; Zhou, Y.; Chen, W. Association of Polymorphism rs67920064 in ADAMTS9 Gene with Mandibular Retrognathism in a Chinese Population. *Med Sci Monit.* **2020** *30*, e925965.
8. Küchler, E.C.; Reis, C.L.B.; Carelli, J.; Scariot, R.; Nelson-Filho, P.; Coletta, R.D.; Paza, A.O.; Matsumoto, M.A.N.; Proff, P.; Kirschneck, C. Potential interactions among single nucleotide polymorphisms in bone- and cartilage-related genes in skeletal malocclusions. *Orthod Craniofac Res* in press.
9. Nascimento, M.A.; Oliveira, D.S.B.; Reis, C.L.B.; Wambier, L.M.; Horta, K.C.; Romano, F.L.; ... and Kuchler, E.C. Association between P561T polymorphism in growth hormone receptor gene and mandibular prognathism: systematic review and metaanalysis. Rio de Janeiro Dental J. **2019**, *4*, 2-11.
10. Khundmiri, S.J.; Murray, R.; Lederer, E. PTH and Vitamin D. *Compr Physiol.* **2016**, *15*, 561-601.
11. Van der Velden, U.; Kuzmanova, D.; Chapple, I.L.C. Micronutritional approaches to periodontal therapy. *Journal of clinical periodontology* **2011**, *38*, 142-158.
12. Beckerman, P.; Silver, J. Vitamin D and the parathyroid. *Am J Med Sci.* **1999,** *317*, 363-369.
13. Wolf, M.; Lossdörfer, S.; Marciniak, J.; Römer, P.; Kirschneck, C.; Craveiro, R.; Deschner, J.; Jäger, A. CD8+ T cells mediate the regenerative PTH effect in hPDL cells via Wnt10b signaling. *Innate Immun.* **2016**, *22*, 674-681.
14. Zhalehjoo, N.; Shakiba, Y.; Panjehpour, M. Gene expression profiles of CYP24A1 and CYP27B1 in malignant and normal breast tissues. *Mol Med Rep.* **2017**, *15*, 467-473.
15. Williams, S.E.; Slice, D.E. Regional shape change in adult facial bone curvature with age. *Am J Phys Anthropol* **2010,** *143*, 437-447.
16. Little, J.; Higgins, J.P.; Ioannidis, J.P.; Moher, D.; Gagnon, F.; Von Elm, E.; ... and Birkett, N. STrengthening the REporting of Genetic Association Studies (STREGA)—an extension of the STROBE statement. *Genetic Epidemiology: The Official Publication of the International Genetic Epidemiology Society* **2009**, *33*, 581-598.
17. Küchler, E.C.; Tannure, P.N.; Falagan-Lotsch, P.; Lopes, T.S.; Granjeiro, J.M.; Amorim, L.M.F. Buccal cells DNA extraction to obtain high quality human genomic DNA suitable for polymorphism genotyping by PCR-RFLP and Real-Time PCR. Journal of *Applied Oral Science* **2012**, *20*, 467-471.
18. Cunha, A.S.; Dos Santos, L.V.; Marañón-Vásquez, G.A.; Kirschneck, C.; Gerber, J.T.; Stuani, M.B.; ... and Küchler, E.C. Genetic variants in tooth agenesis–related genes might be also involved in tooth size variations. *Clinical Oral Investigations* **2021**, *25*, 1307-1318.
19. Jakulin, A., & Bratko, I. Quantifying and visualizing attribute interactions. *arXiv* 2004, cs/0308002v3.
20. Joshi, N.; Hamdan, A.M.; Fakhouri, W.D. Skeletal malocclusion: a developmental disorder with a life-long morbidity. *Journal of clinical medicine research*, **2014**, *6*, 399-408.
21. Miller, J.R.; Burgess, J.A.; Critchlow, C.W. Association between mandibular retrognathia and TMJ disorders in adult females. *Journal of public health dentistry* **2004**, *64*, 157-163.
22. Solow, B.; Siersbæk-Nielsen, S.; Greve, E. Airway adequacy, head posture, and craniofacial morphology. *American journal of orthodontics* **1984,** *86*, 214-223.
23. Costa, A.M.G.; Trevizan, M.; Matsumoto, M.A.N.; da Silva, R.A.B.; da Silva, L.A.B.; Horta, K.C.; ... and Küchler, E.C. Association between tooth agenesis and skeletal malocclusions. *Journal of oral & maxillofacial research*, **2017**, *8*, e3.
24. Gutierrez, S.J.; Gomez, M.; Rey, J.A.; Ochoa, M.; Gutierrez, S.M.; Prieto, J.C. Polymorphisms of the noggin gene and mandibular micrognathia: a first approximation. *Acta Odontol Latinoam* **2010,** *23*, 13–9.

25. Barash, M.; Bayer, P.E.; van Daal, A. Identification of the Single Nucleotide Polymorphisms Affecting Normal Phenotypic Variability in Human Craniofacial Morphology Using Candidate Gene Approach. *J Genet Genome Res*, **2018,** *5*, 041.
26. Barros, S.P.; Silva, M.A.D.; Somerman, M.J.; Nociti Jr, F.H. Parathyroid hormone protects against periodontitis-associated bone loss. *Journal of dental research* **2013,** *82*, 791-795.
27. Tokunaga, K.; Seto, H.; Ohba, H.; Mihara, C.; Hama, H.; Horibe, M.; Yoneda, S.; Nagata, T. Topical and intermittent application of parathyroid hormone recovers alveolar bone loss in rat experimental periodontitis. *J Periodont Res* **2011,** *46*, 655–662.
28. Silva, B.C.; Costa, A.G.; Cusano N. E.; Kousteni, S.; Bilexikian, J.P. Catabolic and anabolic actions of parathyroid hormone on the skeleton. *Journal of Endocrinological Investigation* **2011**, *34*, 801–810.
29. Hindorff, L.A.; Sethupathy, P.; Junkins, H.A.; Ramos, E.M.; Mehta, J.P.; Collins, F.S.; Manolio, T.A. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci U S A* **2009**, *106*, 9362–9367.
30. Lin, H.; Hargreaves, K.A.; Li, R.; Reiter, J. L.; Wang, Y.; Mort, M.; ... and Liu, Y. RegSNPs-intron: a computational framework for predicting pathogenic impact of intronic single nucleotide variants. *Genome biology*, **2019**, *20*, 1-16.
31. Hughes, T.A. Regulation of gene expression by alternative untranslated regions. *Trends Genet.* **2006**, *22*, 119–122.
32. Kanzawa, M.; Sugimoto, T.; Kobayashi, T.; Kobayashi, A.; Chihara, K. Parathyroid hormone gene polymorphisms in primary hyperparathyroidism. *Clin Endocrinol* **1999,** *50*, 583–588.
33. Lehmann, B., Meurer, M. Vitamin D metabolism. *Dermatologic therapy* **2010**, *23*, 2-12.
34. Tashiro, K.; Abe, T.; Oue, N.; Yasui, W.; Ryoji, M. Characterization of vitamin D-mediated induction of the CYP 24 transcription. *Molecular and cellular endocrinology* **2004**, *226*, 27-32.
35. Segersten, U.; Björklund, P.; Hellman, P.; Åkerström, G.; Westin, G. Potentiating effects of nonactive/active vitamin D analogues and ketoconazole in parathyroid cells. *Clinical endocrinology* **2007,** *66*, 399-404.
36. Engström, C.; Linde, A.; Thilander, B. Craniofacial morphology and growth in the rat. Cephalometric analysis of the effects of a low calcium and vitamin D-deficient diet. *Journal of Anatomy*, **1982,** 299-314.