

The ADAMTS9 gene is associated with mandibular retrusion in a Chinese population

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Abstract

Background: Many studies suggest that genetics plays an important role in mandibular retrusion. In this study, we hypothesized that single nucleotide polymorphisms (SNPs) of ADAMTS9 gene is associated with mandibular retrusion in a Han Chinese population.

Methods: Saliva samples from 60 patients undergoing orthodontic for correction of malocclusion were collected. 130 SNPs genotyping of ADAMTS9 was used to assess the association of polymorphisms with the mandibular retrusion. The general linear model using age, gender and ANB as covariates weighed the relationship between SNP and mandibular retrusion. Additionally we leveraged the generalized multifactor dimensionality reduction (GMDR) method to investigate SNP-SNP interactions. The significance level was set at $P < 0.05$ in this study.

Results: The general linear model results showed that four SNPs (rs1014640, rs7648540, rs75839462 and rs4605539) in the ADAMTS9 gene may be related to the occurrence of mandibular retrusion, even after Bonferroni correction. In addition, we further found that the interaction between the ADAMTS9 rs75839462 and ADAMTS9 rs80118777 promoted the occurrence of mandibular retrusion.

Conclusion: Our findings suggest that the ADAMTS9 gene may cause mandibular retrusion independently and through SNP-SNP interactions.

Background

Mandibular retrusion is one of the common facial developmental deformities, which not only affects the facial beauty but also causes obstructive sleep apnea in severe cases¹⁻³. There is growing evidence that genetics play an important role in mandibular retrusion. Many genes are considered pathogenic genes for mandibular retrusion such as IGF, KAT6B, HDAC4, GHR, and LTBP2⁴⁻⁸. However, no clear pathogenic gene has been found so far. Dohmoto found two significant Quantitative trait locus (QTL) within the regions 13 cM and 16 cM in chromosome 11 may be responsible for mandibular length. It indicated that some major genes are responsible for mandibular length⁹.

ADAMTS family proteins are widely involved in cartilage growth and development. Among them, ADAMTS1, 4, 5, 9 have been proven to have cartilage aggrecanase activity, which is mainly responsible for the degradation of cartilage extracellular matrix aggrecan during the cartilage ossification process¹⁰⁻¹¹. The ADAMTS-9 protein also plays a key role in organ shape and the inhibition of angiogenesis¹². Some studies have suggested that ADAMTS9 may play an important role in bone growth, the deletion of ADAMTS9 would hinder growth and development¹³⁻¹⁴. These studies suggest that ADAMTS9 may play a potent regulatory role during bone remodeling. To our knowledge, no one has studied the relationship between ADAMTS9 and mandible development.

Thus, we hypothesized that the ADAMTS9 may be associated with mandibular retrusion.

Methods

The study protocol approved by the Institutional Review Board at the School and Hospital of Stomatology, Wenzhou Medical University. Each subject signed the approved informed consent form.

Study population

Sixty subjects undergoing orthodontic for correction of malocclusion were recruited from the Department of Orthodontics, School and Hospital of Stomatology, Wenzhou Medical University. Patients with systemic disease or developmental abnormalities were excluded.

Craniofacial Measurements

WinCeph software was used to measure lateral cephalograms, detail See Fig. 1 .

We used condyliongonion (Co-Go) , gonion gnathion (Go-Gn) and condylion-gnathion (Co-Gn), represent for mandibular ramus height, mandibular corpus length, and condylion-gnathion, respectively. Each cephalogram was traced by the primary examiner (CY) and verified by another (ZY); All cephalograms will be remeasured at an interval of 3 weeks. Measurement error was estimated according to Dahlberg's formula (Dahlberg, 1940), and the errors of the linear measurements was ranged from 0.2 to 0.5 mm.

Genotyping

DNA was extracted from saliva samples using Oragene kits following the manufacturer's instructions (DNA Genotek, Ontario, CA, USA). The 141 SNPs in ADAMTS9 were selected for genotyping on all subject using the Axiom Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA, USA). In further analyses, due to failure to achieve Hardy-Weinberg equilibrium ($P < 0.05$) or due to a genotyping call rate < 0.95 , eleven SNPs were excluded.

Statistical analysis

In this study, we using general linear model using age, gender, ANB as covariates were performed using Stata (StataCorp 2011, College Station, TX, USA) to assess the association of the investigated SNP with mandibular retrusion. The Bonferroni correction was used to adjust the multiple testing. The significance level was set at $P < 0.05$ in this study.

The generalized multifactor dimensionality reduction (GMDR) method was used to investigate SNP-SNP interactions. We tested two-way up to three-way interactions using 10-fold cross-validation. The GMDR software will provided some output parameters, including the testing accuracy and empirical P values, to assess each selected interaction. Additionally, the fact such as age, gender and ANB were as covariates in our interaction analyses. Permutation testing obtained empirical P values of prediction accuracy as a benchmark based on 1,000 shuffles.

Results

The demographic and cephalogram measure was described in Table 1. First, we investigated the association between mandibular retrusion and ADAMTS9 genes. There were 17 tag SNPs significant association ($P < 0.05$) with mandibular. After applying Bonferroni correction ($P < 0.05/130 = 0.0003$), only four key SNPs: rs1014640 ($P = 1.2 \times 10^{-12}$), rs4605539 ($P = 1.4 \times 10^{-15}$), rs7648540 ($P = 0.0001$), and rs75839462 ($P = 1.5 \times 10^{-14}$) remained significant association with mandibular retrusion, as shown in Table 2.

The analysis of SNP-SNP interaction which employed categorized ANB scores (normal: $ANB < 4$; mandibular retrusion: $ANB > 4$) as an outcome using GMDR analysis. As shown in Table 3, there was a significant difference in two SNPs (rs75839462 and rs80118777) in two-way model, three SNPs (rs4605539, rs11921149, and rs13434166) in three-way model, indicating a potential SNP-SNP interaction among these SNPs in causing mandibular retrusion.

Discussion

To our best knowledge, this is the first study to assess whether the ADAMTS9 gene is associated with the risk of mandibular retrusion and/or through SNP-SNP interactions among Chinese individuals. It is the first time found that the ADAMTS9 gene may play an important role in a Chinese mandibular retrusion population. Four SNPs were considered as mandibular retrusion variation after correcting for multiple testing. In addition, we found that SNP-SNP interactions between SNPs of the ADAMTS9 gene may play an important role in the etiology of mandibular retrusion.

The main findings indicate that four key SNPs (rs73832338, rs9985304, rs4317088, and rs9831846) within the ADAMTS9 gene may contribute to susceptibility to mandibular retrusion even after applying Bonferroni correction. ADAMTS9 is extremely important for the growth of organisms. Deletion of the ADAMTS9 gene can cause death in mice¹⁵. ADAMTS9 mutations can cause a variety of diseases, such as obesity, type 2 diabetes, age-related macular degeneration, and Alzheimer's disease¹⁶⁻¹⁷. But, the relationship between mandibular retrusion and ADAMTS9 has not been reported. Kanae et al.¹⁸ found that ADAMTS9 can maintain the growth and proliferation of chondrocytes through the degradation of the extracellular matrix of cartilage, thereby promoting the cartilage ossification. Therefore, we speculate that inactivating mutations in ADAMTS9 may disrupt the growth balance of the mandible and then cause abnormalities in the mandible. However, we need further research to determine why the ADAMTS9 mutant phenotype manifests as mandibular hypoplasia.

Recognizing the interactions between SNPs had great significance in explaining the pathogenesis in complex diseases. More and more theoretical and experimental evidence shows that SNP interaction is one of the important genetic foundations for the complex diseases¹⁹⁻²¹. Identifying these SNP interactions and identifying related genes is one of the important ways to analyze the pathogenesis of complex diseases. However, few studies conduct this analysis to weigh SNP-SNP interactions. We found

that ADAMTS9 rs9985304 and ADAMTS9 rs76346246 on causing mandibular retrusion by using the GMDR approach. It indicated that ADAMTS9 gene may promote mandibular retrusion through SNP-SNP interaction. No exact genes have been found for mandibular retrusion till now. It suggestion that the interaction of SNP with other SNPs may be a key cause of mandibular retrusion.

A main weakness of our study is only included chinese han population. In future study,we need included more other ethnic populations to evaluate the association and interactions of the ADAMTS9 with mandibular retrusion. Second,the animal model of pathogenic gene mutations has not been constructed, the link between genotype and animal phenotype can not be proved. Therefore, whether the ADAMTS9 gene mutation is widely present in the mandibular retrusion population and its exactly mechanism in the pathogenesis of mandibular retrusion remains to be further studied.

Conclusions

In summary, the ADAMTS9 gene associated with the risk of mandibular retrusion and/or through SNP-SNP interactions among Chinese individuals. More samples will need to be included in the future to further demonstrate the role of the ADAMTS9 gene identified in this study.

Abbreviations

Single nucleotide polymorphisms: SNPs ; Generalized multifactor dimensionality reduction: GMDR;
Quantitative trait locus: (QTL)

Declarations

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Authors' contributions

Y. Cai and W.T. Chen contributed to data acquisition

and analysis, and manuscript drafting. Y.Zhou and Z.Y. Ni were responsible for the conception and design of the study and critically revised the manuscript. All authors gave final approval of the submitted version and agreed to be accountable for all aspects of the work.

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Availability of data and materials

Data will be available upon request by email.

Ethics approval and consent to participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Research Ethics Committee of UFVJM, 2174074) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All subjects signed a written informed consent form before the beginning of this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1 Clinical characteristics of study subjects.

Characteristic	Overall
No. of subjects, n	65
Mean age+SD, year	25.4+5
Male, %	53.85
ANB+SD, degree	5.26+2.1
ondyliogonion (Co-Go) , mm	42.36+5.6
gonion gnathion (Go-Gn) , mm	70.68+4.5
condylion-gnathion (Co-Gn), mm	110.23+4.2

Table2 Linear regression models of associations between 17 tag SNPs and mandibular retrusion.

Gene	CHR	SNP	A1	A2	MAF	P	BETA	SE
ADAMST9	3	rs17070967	C	T	0.077	0.8721	0.05	0.68
		rs894744	G	A	0.308	0.6075	0.21	0.29
		rs1036920	A	G	0.229	0.5681	0.17	0.31
		rs1014640	T	C	0.441	1.2×10^{-12}	-0.25	0.15
		rs17726803	T	C	0.335	0.8212	0.68	0.43
		rs73832332	T	C	0.236	0.4352	0.28	0.35
		rs13318141	T	C	0.315	0.0299	0.54	0.24
		rs7648540	C	A	0.245	0.0001	-0.24	0.15
		rs75839462	C	T	0.069	1.5×10^{-14}	-0.15	0.15
		rs4371513	A	G	0.419	0.0105	0.36	0.08
		rs4605539	T	C	0.073	1.4×10^{-15}	-0.28	0.15
		rs9836710	G	A	0.117	0.5223	0.78	0.50
		rs11921149	A	G	0.365	0.1825	1.01	0.31
		rs58650552	T	C	0.186	0.0904	0.87	0.29
		rs6776363	C	G	0.347	0.0572	1.02	0.35
		rs12492549	G	C	0.447	0.0061	0.65	0.41
		rs73124286	T	G	0.408	0.0187	0.24	0.29

A1 = minor allele, A2 = major allele, BETA = Beta coefficients, Chr = chromosome, SE = standard error. Analysis was obtained after adjustment for covariates including age, gender, and ANB. P values of < 0.0003 are shown in bold.

Table 3. SNP-SNP interaction models identified by the GMDR method.

Best interaction model	Testing accuracy (%)	P value
rs75839462 and rs80118777	72.34	P<0.0001
rs4605539, rs11921149, and rs13434166	70.68	P<0.0001

Figures

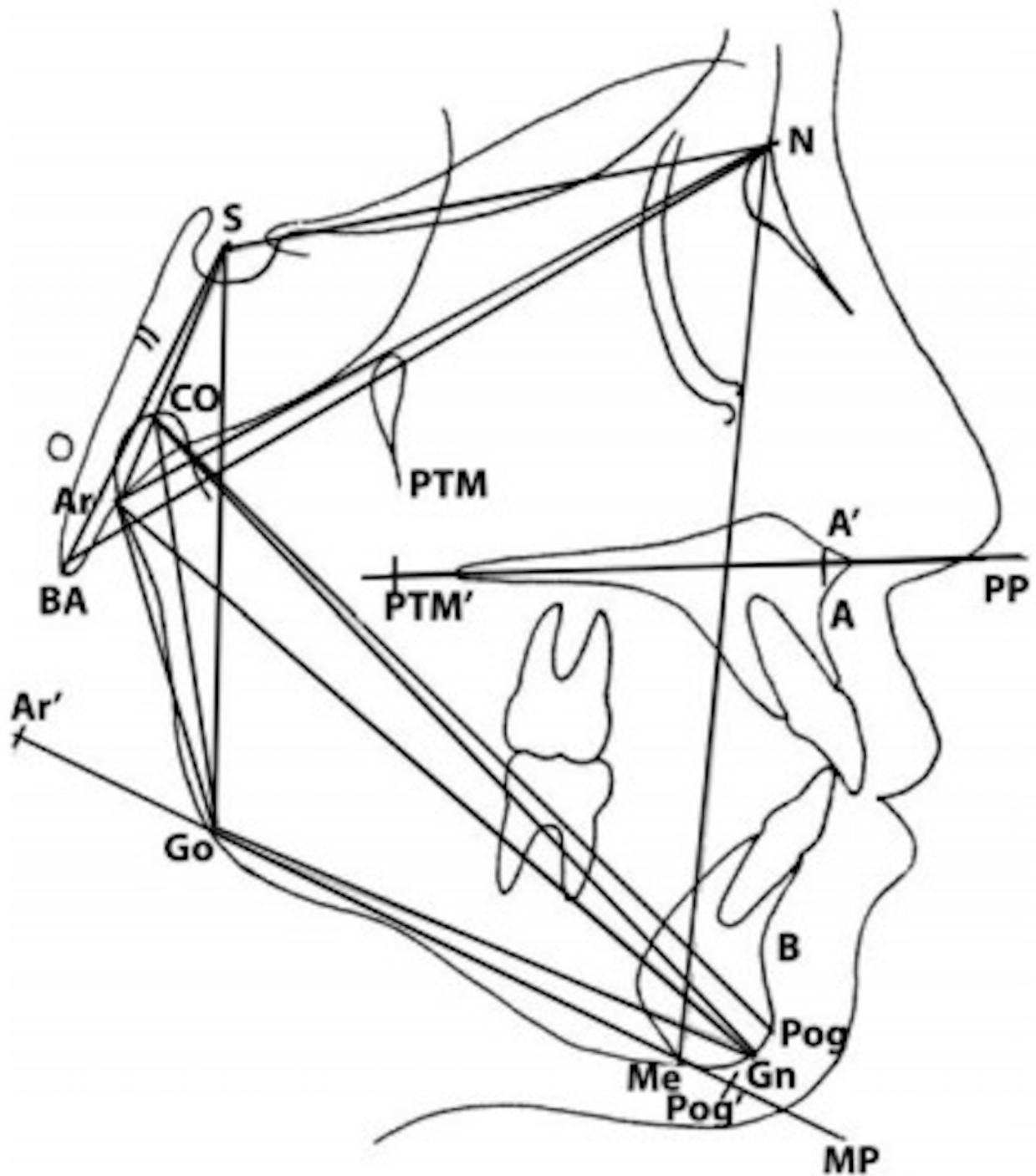


Figure 1

Lateral cephalometric measurements studied. Points: , gonion (Go), gnathion(Gn), nasion (N), A perpendicular to palate plane (A), condylion(Co). Lines and planes: mandibular ramus length (Co-Go), mandibular corpus length(Go-Gn), overall mandibular length (Co-Gn). (J Dent Res 84(11)2005 Growth Hormone Receptor and Mandibular Height)