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Association of Polymorphism rs67920064 in *ADAMTS9* Gene with Mandibular Retrognathism in a Chinese Population

Authors' Contribution:
Study Design A
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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Mandibular retrognathism is a common oral and maxillofacial deformity that may cause a series of physical and psychological diseases. Many studies indicated that genetic factors play an important role in the occurrence of mandibular retrognathism. In this study, we assess the association between polymorphism rs67920064 in *ADAMTS9* gene and mandibular retrognathism in a Chinese population.

Material/Methods: Sixty participants (20 to 45 y, mean age 32.79 y) were classified into Class I or mandibular retrognathism skeletal-facial profile groups in accordance with cephalometric parameters. Thirty patients with mandibular retrognathism were assigned to the subject group; the others were assigned to the control group. Cephalometric parameters including sella-nasion A point, SN point B, condylion-gnathion (Gn), and gonion-Gn were recorded. Saliva samples from these participants were collected and polymerase chain reaction-restriction fragment length polymorphism was used to distinguish different genotypes of the rs67920064 single nucleotide polymorphisms (SNPs).We evaluated the correlation between mandibular retrognathism and polymorphism rs67920064 in the *ADAMTS9* gene.

Results: The distribution of rs67920064 gene polymorphism in *ADAMST9* gene conforms to Hardy-Weinberg equilibrium. The A point-nasion-B point angle of the participants with the GA genotype of the rs67920064 SNP showed significantly decreased values ($P<0.05$), but there was no difference in length of mandibular body. Beyond that, the chi-square test showed that the GA genotype of rs67920064 SNP was highly associated with mandibular retrognathism ($P<0.05$).

Conclusions: Our research shows that there is an association between polymorphism rs67920064 in the *ADAMTS9* gene and mandibular retrognathism in the Chinese population. Individuals with the GA phenotype are more likely to have mandibular retrognathism.

MeSH Keywords: ADAM Proteins • Retrognathia • Genes, vif

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Background

Mandibular retrognathism is a common oral and maxillofacial deformity that may cause occlusal abnormalities, leading to Class II malocclusion. In addition, it can also cause facial abnormalities, hysteria, and severe obstructive sleep apnea hypopnea syndrome [1].Therefore, mandibular retrognathism not only affects the mental health of patients, but also their quality of life.

Craniofacial morphology is currently thought to be determined by genetic, environmental, mechanical, and epigenetic factors [2]. Nakasima et al. analyzed the lateral cephalometric radiographs of patients with mandibular retrognathism and their parents in several families; these results indicated that the development of a Class II malocclusion has a strong familial tendency [3].

The research of Balkhande et al. has shown that there is a correlation between mandibular retrognathism and *matrilin-1* gene [4]. The *myosin 1H* gene is also thought to be closely related to mandibular retrognathism [5]. In addition, genes such as *KAT6B*, *HDAC4*, and *GHR* are also considered to be related to mandibular retrognathism [6–8]. These studies also suggest that mandibular retrognathism is affected by multiple genes, and genes affecting bone, skeletal muscle, and growth may contribute to the formation of mandibular retrognathism.

The *ADAMTS* gene family contains 19 members that are expressed in a variety of tissues and participate in a variety of physiological and pathological processes. They play important roles in the processes of degradation of extracellular matrix (ECM), angiogenesis, organ generation, hemostatic processes, genetic diseases, cancer, and arthritis [9]. Among them, *ADAMTS9* is the most highly conserved family member [10], and it has also been implicated in several conditions including arthritis, craniofacial development, and eye development [11–13]. Further research shows that *ADAMTS9* is related to chondrocyte proliferation and hypertrophy [14]. These results suggest that *ADAMTS9* may be related to mandibular development. A recent study shows that *ADAMTS9* is associated with patients' mandibular retrognathism in a Chinese population, and the single nucleotide polymorphism (SNP)-SNP interaction may contribute to the formation of mandibular retrognathism [15].

In this study, we evaluated the possible relationship between the *ADAMTS9* gene and mandibular retrognathism by analyzing the distribution of rs67920064 polymorphisms in the *ADAMTS9* gene in a Chinese population with mandibular retrognathism or normal mandible.

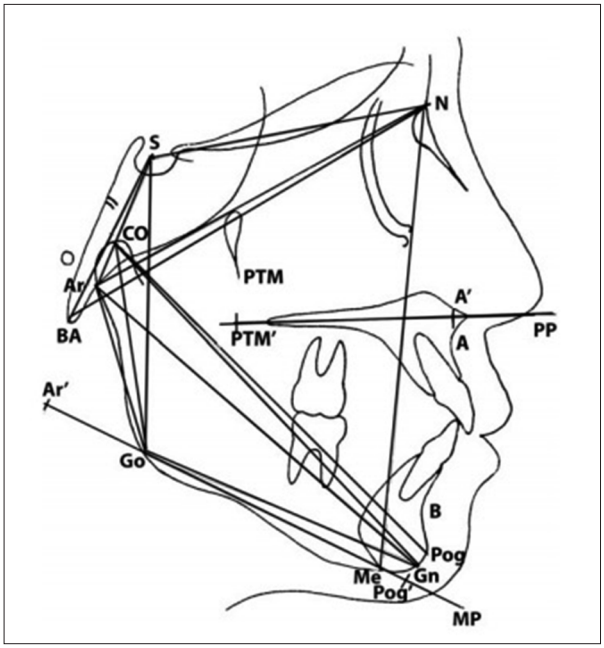

Figure 1. Lateral cephalometric measurements studied. Points: gonion (Go), gnathion (Gn), nasion (N), A perpendicular to palate plane (A).

Material and Methods

The study protocol was approved by the ethics committee of School & Hospital of Stomatology, Wenzhou Medical University (approval 2019011) and followed the Helsinki guidelines on ethics for human research. All adult subjects gave written informed consent. All participants in this study were recruited from the outpatient Department of Orthodontics, Hospital of Stomatology, Wenzhou Medical University. Subjects of both sexes more than 20 years old were included in this study. This study including 30 subjects with mandibular retrognathism and 30 with normal mandible. Patients with a retrognathic mandible (sella-nasion B point [SNB] <78°) were recruited as the subjects, and another 30 patients with a normal mandible (SNB 80±2°) were recruited as the controls. All patients had a normal maxilla (SNA 82±2°). Exclusion criteria were as follow: (1) patients with abnormal maxilla; (2) patients with facial clefting; (3) patients with other systematic diseases.

The measurements were performed by two experienced orthodontists. Landmarks and reference lines are shown in Figure 1 as described by Zhou et al. [16]. Condylion-gonion (Co-Go) together was used to denote the height of mandibular ramus, gonion-gnathion (Go-Gn) as length of mandibular corpus, and condylion-gnathion (Co-Gn) as overall mandibular length. SNA and SNB angles are used to indicate maxillary protrusion and mandibular protrusion respectively. The normal range of SNA is 80–82° and SNB is 78–80°. Angle SNA, angle SNB, angle ANB, angle between Frankfort horizontal (FH) plane and mandibular

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Table 1. Baseline information in the two groups.

Varies	Control group (Mean±SD)	Subject group (Mean±SD)	P-value
Age	32.24±8.52	33.34±7.59	0.862
Gender			
Female	19	20	
Male	11	10	0.905
SNA, °	81.08±1.21	80.78±1.02	0.749
SNB, °	79.89±1.28	74.36±1.39	<0.01
ANB, °	1.19±0.97	6.42±1.34	<0.01
FH-MP, °	27.64±3.25	33.38±4.05	<0.01
(Co-Go), mm	44.26±4.68	42.36±5.61	0.123
(Go-Gn),mm	73.56±5.89	70.68±4.52	0.104
(Co-Gn),mm	117.34±5.24	110.23±4.25	0.268

Go – gonion; Gn – gnathion; SN – sella-nasion line; FH – Frankfort horizontal; MP – mandibular plane.

plane (MP) (FH-MP), and distance from Co-Go, Co-Gn, and Go-Gn were measured and recorded.

To isolate deoxyribonucleic acid, we collected 5 mL of saliva from the participants and placed it in a sterile centrifuge tube (Greiner Bio-one®, Frickenhausen, Germany). Polymorphic sites were determined by polymerase chain reaction-restriction fragment length polymorphism method. Chi-square test is used to detect whether the genotype distribution conforms to the Hardy-Weinberg equilibrium to determine the associations between mandibular retrognathism and *ADAMTS9* gene polymorphisms. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to detect the associations between the rs67920064 polymorphism in *ADAMTS9* gene and mandibular retrognathism. Continuous variables (age, length) in this study were reported as the mean±SD. Gender distribution was expressed using counts, and the distribution in the two groups was expressed as a percent; the difference was tested by chi-square. Different variables were compared between the subject group and the control group. Independent-sample *t* tests were used for measurement data. The chi-square analysis was used for enumeration of data. The level for statistical significance was defined as $P<0.05$. The software SPSS 18.0 (SPSS Inc., Chicago, IL) was used for statistical analysis.

Results

Cephalometric analyses of all participants are in Table 1. Thirty patients with mandibular retrognathism and 30 control individuals participated in this research. The age range was 20–41 years (32.24±8.52 years) in the mandibular retrognathism group and 21–45 years (33.34±7.59 years) in the controls. Nineteen women comprised the subject group, 20 in the control group. There was no significant difference in gender distribution between these two groups. The comparison of the cephalometric analysis between these two groups of patients shows that only the FH-MP measurement has a significant difference ($P<0.01$), with mean values of 33.58° in the subject group and 27.64° in the control group. There is no statistically significant difference in the remaining measurements between these two groups (Table 1). The SNB of the controls was 79.89±1.28°, whereas the SNB of the cases was 74.36±1.39°. There are statistical differences between the two groups ($P<0.01$). As can be seen from this table, the SNA in the control group is 81.08±1.21° and 80.78±1.02°in subject group, with no significant difference between the groups, and both were within the normal range.

When contrasted with the Hardy-Weinberg equilibrium, the distribution of rs67920064 gene polymorphism in *ADAMST9* gene conforms to this rule, suggesting that the genotypes were accordant with the distribution of the general population. Compared with the control group, rs67920064 allele "A" is found at a rather lower rate in the cases. Our results showed that when comparing between mandibular retrognathism cases and controls, a conspicuous discrepancy appeared from statistical analysis ($P=0.014$, OR=0.89, 95% CI=0.12–7.38) (Table 2). In addition, by analyzing the distribution of different genotypes and phenotypes (Table 3), we found that the ANB and SNB angles were not in accordance with different genotypes of rs67920064. The SNB is smaller in heterozygous (GA) genotype individuals than in other genotypes ($P<0.05$).

Discussion

To the best of our knowledge, this is the first study to assess the correlation between rs67920064 polymorphism in *ADAMTS9*

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Table 2. *ADAMTS9* gene rs67920064 single nucleotide polymorphism and mandibular retrognathism.

Genotype	Control group, n (%)	Subject group, n (%)	OR (95% CI)	P value
GG	16 (53)	5 (17)	Reference	
AG	8 (27)	21 (70)	4.62 (1.25–15.36)	
AA	6 (20)	4 (13)	0.89 (0.12–7.38)	0.014
G	42 (70)	45 (75)		
A	18 (30)	15 (25)	1.89 (0.83–4.29)	0.247
HWp	0.156	0.106		

HWp – Hardy-Weinberg P value.

Table 3. Distribution of cephalometric and growth measures among different genotypes of studied polymorphisms.

Cephalometric	Genotype			P value
	AA	AG	GG	
SNA, °	82.23±1.26	82.28±1.22	82.28±1.42	0.657
SNB, °	78.49±1.58	75.26±1.69	78.36±1.39	0.046
ANB, °	4.16±1.85	7.02±1.64	4.46±1.74	0.024
FH-MP, °	31.52±3.85	33.58±4.25	32.42±4.15	0.457
(Co-Go), mm	45.36±4.18	44.38±5.22	44.68±5.29	0.369
(Go-Gn), mm	75.36±4.29	74.28±4.22	74.42±4.72	0.367
(Co-Gn),mm	120.25±6.23	118.53±4.95	118.62±4.85	0.624

Go – gonion; Gn – gnathion; SN – sella-nasion line; FH – Frankfort horizontal; MP – mandibular plane.

gene and mandibular retrognathism in a Chinese population. In cases of mandibular retrognathism, a minor "A" allele of rs67920064 was expressed far less than that of the controls. Moreover, the heterozygous form (GA) has potential coordinate relations with mandibular retrognathism. Our study suggests that rs67920064 involved in *ADAMTS9* gene is related to mandibular retrognathism.

The *ADAMTS9* gene is located in 3p14.2-14.3, contains about 400 exons, and has a gene size of 172 kilobase pairs. *ADAMTS9* is the largest *ADAMTS* as well as the most highly conserved family member [17]. It is essentially important for ECM remodeling by metalloproteinases during development. Two ECM proteoglycans, versican and aggrecan, were found to be substrates of *ADAMTS9*; aggrecan is a specialized product of cartilage [18].What's more, Enomoto et al. [19] found that the embryos of *Adamts9* $^{LacZ/1}$ produced completely cleft palates in stillbirths. These results suggest that *ADAMTS9* plays an important role in maxillofacial development.

In our research. we found that there was no statistical differences in the length of the mandible between the two groups. There was no significant difference in SNA, but the SNB of subject group was significantly smaller and the ANB was significantly larger in subject group, suggesting that the subjects had a clockwise rotation of the jaw during development. This change may be related to the expression of *ADAMTS9*. Because of the expression of *ADAMTS9* in the brain, the craniofacial structures are similar for mice and *Xenopus* [20]. Desanlis et al. studied the possible role of *ADAMTS9* in human development by studying the role of *ADAMTS9* in *Xenopus* development [21]. They found that *ADAMTS9* is highly expressed from early tailbud in various regions, especially in the midbrain-hindbrain boundary, and the migrating cells along the branchial arches and at tadpole stages. *ADAMTS9* expression was also seen in several tissues such as branchial arches and pronephros. Taken together, these findings suggest that *ADAMTS9*'s contribution is essential for craniofacial development. Further research shows that *ADAMTS9* is highly expressed throughout the development of the palate in mice, which also indicates that *ADAMTS9* plays an important role in skeletal development [22]. Therefore, we hypothesized a relationship between mandibular retrognathism and *ADAMTS9* gene.

A recently published research finding confirms this hypothesis, showing that *ADAMTS9* gene is related to mandibular retrognathism [15]. Our research indicates that the rs67920064 polymorphism in *ADAMTS9* gene plays an important role in

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mandibular retrognathism in a Chinese population. This research suggests that rs67920064 of *ADAMTS9* gene may affect mandibular development by adjusting the angle of development rather than the length of the mandible, but the mechanism is not clear.

However, there are some deficiencies in this study. The small sample size is the main limitation, which may affect the result of the association between mandibular retrognathism and *ADAMTS9* gene polymorphisms. Furthermore, since the study was performed in a Chinese population, our findings may not be generalized to other populations, so further research is needed.

Conclusions

Our research shows that polymorphism rs67920064 in *ADAMTS9* gene is associated with mandibular retrognathism in a Chinese population.

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