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REVIEW ARTICLE

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Genes, genetics, and Class III malocclusion

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Abstract

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To present current views that are pertinent to the investigation of the genetic etiology of Class III malocclusion. Class III malocclusion is thought to be a polygenic disorder that results from an interaction between susceptibility genes and environmental factors. However, research on family pedigrees has indicated that Class III malocclusion might also be a monogenic dominant phenotype. Recent studies have reported that genes that encode specific growth factors or other signaling molecules are involved in condylar growth under mechanical strain. These genes, which include Indian hedgehog homolog (*IHH*), parathyroid-hormone like hormone (*PTHLH*), insulin-like growth factor-1 (*IGF-1*), and vascular endothelial growth factor (*VEGF*), and variations in their levels of expression play an important role in the etiology of Class III malocclusion. In addition, genome-wide scans have revealed chromosomal loci that are associated with Class III malocclusion. It is likely that chromosomal loci 1p36, 12q23, and 12q13 harbor genes that confer susceptibility to Class III malocclusion. In a case–control association study, we identified erythrocyte membrane protein band 4.1 (*EPB41*) to be a new positional candidate gene that might be involved in susceptibility to mandibular prognathism. Most of the earlier studies on the genetic etiology of Class III malocclusion have focused on the patterns of inheritance of this phenotype. Recent investigations have focused on understanding the genetic variables that affect Class III malocclusion and might provide new approaches to uncovering the genetic etiology of this phenotype.

Key words: association study; Class III malocclusion; condylar growth; linkage analysis; single nucleotide polymorphisms

Introduction

According to Angle's classification, Class III malocclusion is defined in cases that mandibular first molar is positioned mesially relative to the first molar of maxilla (1). Class III malocclusion is clinically heterogeneous and can be associated with many combinations of skeletal and dental morphological variants. It has been reported that approximately 75% of Class III cases in male Caucasians have a skeletal origin and are a result of mandibular prognathism or macrognathia (2). The prevalence of Class III malocclusion among Caucasian people ranges from 0.48% to 4% (3), but it rises to as high as 10% in the Japanese population (4). In a population of

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Chinese origin, the frequencies of anterior crossbites and edge-to-edge incisal relationships among permanent and deciduous teeth were 4.9% and 8.1%, respectively (5).

In a number of previous studies, the etiology of Class III malocclusion has been attributed to various patterns of genetic inheritance and to environmental factors (6–8). It has been known for many years that skeletal Class III malocclusion has a significant genetic component. To date, many investigations have focused on understanding the genetic factors that underlie Class III malocclusion and on determining how these genetic factors might influence the response of patients to orthodontic treatment.

Recent genetic and molecular data might provide new information that will enable the genetic etiology of Class III malocclusion to be clarified. In this review, we present different models of inheritance of Class III malocclusion, discuss the genes that affect condylar growth, and suggest chromosomal loci that might confer susceptibility to this phenotype.

Models of inheritance of Class III malocclusion

Evidence from population studies has demonstrated that Class III malocclusion is influenced strongly by genetic factors, and multiple environmental factors have been shown to affect mandibular growth. As a result, Class III malocclusion is assumed to show polygenic inheritance. Polygenic or multifactorial inheritance refers to the inheritance of a phenotypic trait that can be attributed to two or more susceptibility genes and their interaction with the environment. Genetic analysis of families with the Class III phenotype supports the hypothesis of polygenic inheritance. Litton et al. (9) examined the families of 51 probands with Angle Class III malocclusion and found that approximately 13% of the siblings of probands exhibited the trait. In addition, there is a high prevalence of Class III malocclusion in families of patients with skeletal Class III malocclusion who have been treated with orthognathic surgery (10). Further evidence was obtained from a study of twins, which indicated that the concordance rate of mandibular prognathism in monozygotic twins is six times higher than that among dizygotic twins; again this suggests a polygenic mode of inheritance (11). Both family and twins studies suggest a polygenic mode of inheritance as the primary cause of Class III malocclusion.

However, a monogenic model has been reported that fits the data from several unrelated European noble families who are known to possess this phenotype. Unlike polygenic inheritance, monogenic inheritance is the result of a single mutated gene and follows the Mendelian pattern of inheritance. Wolff et al. (12) analyzed the pedigrees of 13 European noble families that showed mandibular prognathism and found that this phenotype was determined by a single autosomal dominant gene. El-Gheriani et al. (13) came to a similar conclusion after analyzing the families of 37 Libyan patients with mandibular prognathism. Recent research on 2562 individuals from 55 families has indicated that one major gene influences the expression of mandibular prognathism and shows Mendelian inheritance, but that environmental factors also influence the penetrance of the trait (14).

How do genetic components affect Class III malocclusion?

Condylar cartilage is an important site of growth in the mandible and it forms part of the temporomandibular joint as well (15). The condylar cartilage is categorized as secondary cartilage, which has distinct biological characteristics, and is considered to support the growth of the mandibular bone. Mechanical loading or functional stimuli might influence the responses of the condylar cartilage and the subsequent growth of the mandible (16). Experimental generation of Class III malocclusion in monkeys has been attributed to an increased rate of condylar growth (17). Therefore, McNamara and Carlson (18) hypothesized that the cartilage of the mandibular condyle is responsive to biophysical environmental changes, and it is highly likely that Class III malocclusion might be precipitated under these biomechanical conditions by the inheritance of genes that predispose to a Class III phenotype (Fig. 1).

A number of reports have documented the influence of various genes that are involved in the regulation of mandibular morphogenesis. Recent research has focused on the expression of specific growth factors or other signaling molecules that are involved in condylar

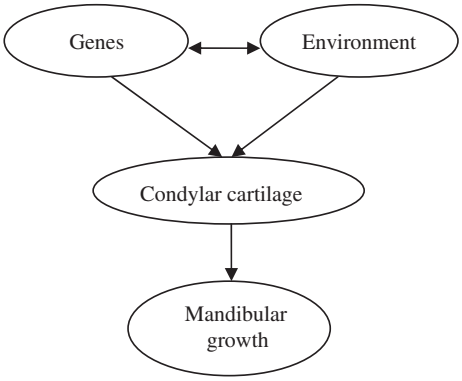

Fig. 1. Effects of gene–environment interactions on mandibular growth. Susceptibility genes, environmental factors, and condylar cartilage are key to the regulation of mandibular growth, which results from the activity of the condylar cartilage. Fundamentally, mandibular growth is induced by both genetic and environmental mechanisms, which interact with each other to produce the Class III phenotype.

growth. Growth factors and cytokines are local mediators and can be secreted in response to mechanical strain. These mediators regulate cell proliferation and the expression of differentiation products by activating signal transduction pathways in the target cells (19). In an experimental model of enhanced condylar growth, Rabie et al. (20, 21) indicated that forward positioning of the mandible triggered the expression of Ihh and Pthlh, which promote mesenchymal cell differentiation and proliferation, respectively, and that these proteins acted as mediators of mechanotransduction to promote increased growth of the cartilage. In another rat model, the expression of *IGF-1* increased significantly when the mandible was repositioned by means of a propulsive appliance (22). In addition, growth factors such as Vegf and transcription factors such as the sex-determining region Y (SRY)-box 9 (Sox9) and runt-related transcription factor 2 (Runx2) play important roles in the differentiation of chondrocytes in the growth plate under conditions of mechanical loading or exposure to other stimuli (23–25).

The genes that have been implicated in condylar growth by studies in the mouse might serve as potential candidates to increase our understanding of Class III malocclusion in humans. The discovery of candidate genes provides the possibility to identify genes that confer susceptibility to this phenotype. In the search for susceptibility genes that are involved in Class III malocclusion, polymorphisms in the aforementioned genes and the genes for the molecules that they regulate will be prime targets.

Linkage analysis and association study of Class III malocclusion

Linkage analysis

Linkage analysis is performed to determine the chromosomal loci that might harbor genes associated with a particular disease or phonotype. The aim is to identify a genetic marker that is inherited by all family members that are affected by the disorder or trait, but is not inherited by any of the unaffected family members (26). During linkage analysis, the segregation of chromosomal regions that are marked by genetic variants is followed in affected families to identify regions that cosegregate with the disorder or trait (27). However, this approach can only provide an approximate location of the gene of interest relative to a genetic marker, and further association studies are needed to identify the susceptibility genes.

The results of genome-wide linkage analyses have suggested several chromosomal regions that might harbor susceptibility genes for Class III malocclusion. Yamaguchi et al. (28) were the first to map susceptibility loci to chromosomes 1p36, 6q25, and 19p13.2 in affected sibling pairs from Korean and Japanese families. Recently, another genome-wide study, which was carried out in four Hispanic families from a Colombian background, revealed five suggestive loci, namely 1p22.1, 3q26.2, 11q22, 12q13.13, and 12q23 (29). Therefore, there is support for the existence of susceptibility loci on chromosome 1. The region 1p36 harbors positional candidate genes of interest, which include heparan sulfate proteoglycan 2 (*HSPG2*), matrilin 1, cartilage matrix protein (*MATN1*), and alkaline phosphatase (*ALPL*). Recently, it has been reported that *HSPG2* is related to the formation of cartilage and to craniofacial abnormalities (30), and Matn1 and Alpl are considered to be markers for the formation of cartilage and bone, respectively (31, 32). Furthermore, in studies of craniofacial growth in mice, loci on murine chromosomes 10 and 11 were determined to be responsible for mandibular length, and these correspond to the human chromosomal regions 12q21 and 2p13, respectively (33). This comparative result supports the hypothesis that the regions 12q23 and 12q13 are relevant to craniofacial development and might be linked to the Class III phenotype. Candidate genes of interest are located within these regions, which include the homeobox region (*HOX3*), *IGF-1*, and the

collagen, type II, alpha 1 (*COL2A1*) gene. The *HOX* genes are believed to be pivotal in craniofacial development (34); *IGF-1* has been proven to be involved in the proliferative activity of condylar cartilage (22); and Col2a1 encodes type II collagen in cartilage and is important for craniofacial growth (35). Therefore, studies have suggested that the major gene(s) that are responsible for Class III malocclusion might be located at chromosomal loci 1p36, 12q23, and 12q13.

Association study

The aim of association study is to identify differences in the frequencies of genetic variants between ethnically matched cases and controls to find variants that are associated strongly with the disease (36). If a variant is more common in cases than in controls, an association can be inferred. Such studies require relatively large sample sizes and phenotypes that are defined accurately. Association study can be based on candidate genes or can be genome-wide and free of hypotheses (37). They can be used to resolve and refine the candidate interval further (38). Research on a Japanese population showed that polymorphisms of the gene growth hormone receptor (*GHR*) are associated with mandibular height (39). Zhou et al. (40) reported a similar conclusion from research on a Chinese population, but there is no direct evidence that shows an association with Class III malocclusion. In a case–control association study that included 158 people with mandibular prognathism, we genotyped 106 single nucleotide polymorphisms (SNPs) on 1p36 across an 8.6-Mb critical interval and four candidate genes. Significant genetic associations with mandibular prognathism were observed for rs2249138, rs2254241, rs2788890, and rs2788888, respectively ($p = 0.018$; $p = 0.015$; $p = 0.028$; $p = 0.023$); all four SNPs were located within the gene *EPB41*. The results of our study suggested that the *EPB41* gene might be a new positional candidate gene that is involved in susceptibility to mandibular prognathism (Table 1).

Genome-wide association study (GWAS) uses statistical tools that are similar to those used in association studies based on candidate genes. However, instead of relying on candidate genes that are selected on the basis of previous knowledge of the disorder or phenotype, GWAS involves an unbiased scan of the whole genome and therefore is more likely to reveal genetic and physiological connections. It is known that the human genome contains many millions of SNPs, which can either cause changes in the phenotype directly or might tag nearby mutations that influence variation among individuals and susceptibility to specific phenotypes. Such GWASs allow researchers to sample 100 000 or more SNPs from each subject to capture the variation across the genome (41). With the completion of the human genome project (HGP) in 2003, the pace of GWAS has been accelerated by several developments in science and technology: the availability of sequence data from the HGP; improved bioinformatic and

Table 1. **Linkage analysis and association study on Class III malocclusion**

Methodology	Candidate genes/loci	References
Linkage analysis	1p36, 6q25, 19p13.2	Yamaguchi et al. (28)
	12q23, 12q13, 1p22.1, 3q26.2, 11q22	Frazier-Bowers et al. (29)
Association studies	*GHR*	Yamaguchi et al. (39)
		Zhou et al. (40)
	EPB41, ALPL, HSPG2, MATN1 (1p36)	Yamaguchi et al. (28)
		Rabie et al.[†]
	HOX3, IGF1, COL2A1 (12q13-12q23)	Frazier-Bowers et al. (29)
	IHH, PTHLH	Rabie et al. (21)
	IGF-1	Hajjar et al. (22)
	VEGF, RUNX2, SOX9	Rabie et al. (23)
		Papadopoulou et al. (24)
		Tang et al. (25)

[†]The data for the *EPB41* gene are unpublished.

statistical tools for handling large volumes of data (42, 43); and high-throughput genotyping platforms (44).

Conclusion

The relative contributions of genetic and environmental factors to Class III malocclusion have been the subject of a number of previous studies. Typically, Class III malocclusion is inherited in a polygenic manner, but monogenic inheritance has also been reported. It is likely that the skeletal morphology of the dentofacial complex is dependent upon susceptibility genes that are involved in gene–environment interactions that result in the Class III phenotype. Progress in molecular biology has made it possible to recognize various genes that are involved in mandibular growth. A number of genes have been described as key regulatory factors that contribute to condylar growth under mechanical strain, and these genes could play a role in the development of Class III malocclusion. Each gene involved in this biological pathway might be a candidate gene that is related to Class III malocclusion. Recent genetic research using linkage analysis and association studies has identified genes that confer susceptibility to Class III malocclusion. GWAS is a new and very powerful tool that will be used to identify genetic contributors to the Class III phenotype in the future.

Clinical relevance

Class III malocclusion is the result of multiple factors that interact during the morphogenetic period of the mandible, and it might be possible to regulate some of these factors during infancy. For this reason, it has been suggested that a better understanding of the genetic variables that contribute to the Class III phenotype is necessary to develop new preventive strategies for the condition. These promising approaches could allow the clinician to select early courses of dentofacial and orthodontic treatments that are aimed at preventing the development of Class III malocclusion.

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