Association between Mandibular Prognathism and MATRILIN-1 Gene

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Abstract

Background and Aim: Mandibular prognathism (MP) is a craniofacial deformity resulting from the combined effects of environmental and genetic factors.Secondary condylar cartilage is the postnatal growth site of the mandible. Chromosome location 1p36 contains MATRILIN-1, which is related to cartilage matrix formation. Present study aims to find out the correlation of mutations in MATRILIN-1 gene with MP in the central Indian population residing in the same city to exclude regional and geographical bias. Gene encoding MATRILIN-1, that is, 1p36, was studied at the restriction site rs20566 and region corresponding to rs1065755.

Material and Methods: This case control study included 50 MP patients and 50 control individuals with normal occlusion. The cephalometric parameters studied for the inclusion in MP cases and control group were ANB (Point A-Nasion-Point B), SNA (Sella-Nasion-Point A) and SNB (Sella-Nasion-Point B) angles and mandibular length in Legan–Burstone analysis.DNA was extracted from venous blood and genotyped. Two loci on chromosome 1p36 (rs20566 and rs1065755) encoding MATRILIN-1 were studied for mutation.

Results: A total of 59% MP cases and 14% of control individuals showed SNP at rs20566. On alignment of sequences of the region corresponding rs1065755, we found frame shift mutation in 80.1% of MP subjects and 36% of control subjects. Out of 50 MP cases, 14 were first-generation relatives.Single-nucleotide polymorphism (SNP) at rs20566 and frameshift mutation at rs1065755 had a significantly greater frequency in MP cases than in control.

Conclusion: The results of this study show association of mutation in MATRILIN-1 gene with MP. A greater potential for frameshift mutation was found with resultant alteration of the protein. The identification of genetic influences in malocclusions helps in the prevention and improves treatment modalities of maxillomandibular discrepancies.

Key Words: Chromosome,Mandibular prognathism, MATRILIN-1, Single-nucleotide polymorphism.

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Introduction

Malocclusion is a term used to represent the lack of growth harmony of dental and facial skeletal structures, especially the growth harmony of the maxilla and mandibular. Skeletal malocclusion is classified into three groups, i.e., class I, class II, and class III malocclusions. Class I skeletal malocclusion describes a normal maxilla and mandible relation with a clinically flat facial profile. Class II skeletal malocclusion is declared when the maxilla is relatively more protruded than the mandible with convex facial profile while the class III facial malocclusion represents a condition when the mandible is relatively more protruded than the maxilla with a relatively concave facial profile. When the mandible overgrows in the sagittal direction resulting in a clinically observed protruded chin, the term mandibular prognathism is used .[1-4]

Genetic predisposition in the causation of mandibular prognathism (MP) is an established fact.[5,6] It is an autosomaldominant trait with incomplete penetrance.[7,8] Prevalence of MP is found to be high in Asians, particularly in eastern Asian races. In India, the prevalence of MP is found to be at 3.4%.[9]Generally, MP presents itself as a concave profile, reverse overjet, and Angle's class III malocclusion. This is not only aesthetically disfiguring but may also lead to functional impairment. MP may be associated with maxillary retrognathism that aggravates the severity of disfigurement. Early diagnosis of class III malocclusion and its components are necessary in deciding treatment modality, time of intervention, and visualizing prognosis.

In orthodontics, one of the most challenging aspects of treating patients is predicting mandibular growth, especially in patients who show more pronounced characteristics of mandibular development. The etiology of mandibular prognathism is not understood completely, but it is well known that both genetic components and environmental factors contribute to its development. In some previous studies conducted on family members and twin siblings, it is well documented that there is a strong link between mandibular prognathism and genetics, accounting mainly for the polygenic model of inheritance.[10] Since genetic factors could contribute to the etiology of skeletal class-III malocclusion, the identification of predisposing gene variants would help in predicting the condition and helps in early prevention or intervention. Although various genetic linkage analysis and genome-wide association studies have identified many genes and loci associated with mandibular prognathism, the genes underlying the risk of mandibular prognathism in the general population remain ambiguous, leaving some impetus to search for new candidate genes.

Postnatal growth of the mandible takes place by endochondral bone formation at the condylar cartilage that is a secondary cartilage. MATRILIN-1 is a non-collagenous protein secreted by chondrocytes and expressed during cartilage matrix formation. It is an adhesion protein for chondrocytes and fibroblasts that stabilizes cartilage matrix by altering its tensile strength and elasticity.[11] MMp is found to be a polygenic trait affected by multiple genes, each making a small contribution to the overall outcome. Various candidate chromosomal regions have been found to be associated with it.[12]However, statistical significance of linkage to MP was noted at chromosomes 1p35, 1p36, 6q25, and 19q13.2. Among these sites, chromosome 1p36 area contains many genes related to the skeletal system, such as alkaline phosphate, heparansulphate proteoglycan 2, and cartilage matrix protein (MATRILIN-1).[13]

Jang et al[14] investigated the association of MP with single nucleotide polymorphisms (SNPs) in MATRILIN-1 gene in the Korean

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population. Three restriction sites studied on the chromosomal location 1p36, in MP cases and control, were rs20566, rs1149045, and rs1065755. Loci rs20566 and rs1065755 showed statistically significant difference in the nucleotide sequence in MP cases than in control.[14] On the basis of these facts, the present study aims to find out the correlation of mutations in MATRILIN-1 gene with MP in the central Indian population residing in the same city to exclude regional and geographical bias. Gene encoding MATRILIN-1, that is, 1p36, was studied at the restriction site rs20566 and region corresponding to rs1065755.

Material and Methods

This case control study included 50 MP patients and 50 control individuals with normal occlusion. All subjects were recruited from the Department of Orthodontics and DentofacialOrthopaedics of tertiary care institute of India. Ethical approval was taken from the institutional ethical committee and written informed consent was taken from all the participants. Lateral cephalograms of all subjects were taken using a single machine. All lateral cephalograms were traced by one of the authors. The cephalometric parameters studied for the inclusion in MP cases and control group were ANB (Point A-Nasion-Point B), SNA (Sella-Nasion-Point A) and SNB (Sella-Nasion-Point B) angles and mandibular length in Legan–Burstone analysis.[15] MP cases had smaller than 0 degree ANB values with normal SNA (82 ± 2 degrees) and increased SNB (>80 degrees), with increased mandibular length according to Legan and Burstone analysis. Control group individuals had normal ANB (2 ± 2 degrees) with normal SNA (82 ± 2 degrees) and normal SNB (80 ± 2 degrees), with normal mandibular length according to Legan and Burstone analysis. Patients with craniofacial syndromes including cleft lip and palate; endocrinal disturbances; anomalies of tooth, number, morphology, and eruption; facial asymmetries; and cases with retrognathic maxilla—were excluded from the study.

For genotyping of samples, 2 mL of venous blood was collected from antecubital area of the arm, and DNA was extracted from each sample. Amplification of DNA fragment was performed through polymerase chain reaction. The restriction sites rs20566 and rs1065755 were in the coding region of 1p36 MATRILIN-1 gene. The primer sets used for amplification and sequencing analysis were designed based on the Gene Bank reference sequence. Primers were obtained from Integrated DNA Technologies (IDT) and marketed by Allied Scientific Products. Restriction fragment length polymorphism (RFLP) was performed to genotype rs20566 with restriction enzyme Bsr1. DNA sequencing was performed on the region corresponding to rs1065755 to check for the mutation. Amplicons were sent to the first base laboratories for sequencing. The obtained sequences were aligned with the help of Clustal X2 software along with the reference sequence deposited in NCBI. Allele presence or absence in the given sample was mentioned as YES or NO, respectively, and then the data were collected in terms of percentage. The allele frequencies in MP cases and controls were analyzed and compared for the distribution.

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.

Results

In the present study there were 26 males and 24 females in the age range from 7 years to 64 years; mean age was 21.80 ± 12.70 years) and 50 control individuals with normal occlusion (29 males, 21females in the age range from 18 years to 26 years; mean age was 21.45 ± 2.48 years)Repeatability

examination of the lateral cephalograms showed good agreement (0.79) as assessed by the kappa coefficient. The genotype distribution of the MATRILIN-1 at rs20566 and rs1065755 was analyzed in MP cases and controls. RFLP was performed on genotype rs20566. Gel electrophoresis of the fragments showed double- or single banding pattern, depending on the digestion of fragment, which was indicative of either normal or altered sequence (SNP). A total of 59% MP cases and 14% of control individuals showed SNP at rs20566. On alignment of sequences of the region corresponding rs1065755, we found frame shift mutation in 80.1% of MP subjects and 36% of control subjects. Out of 50 MP cases, 14 were first-generation relatives. The mutation pattern was found to be similar in the relatives. Mutation at both sites was strongly associated with greater degree of disfigurement with ANB below −3 degrees and strong familial tendency. A total of 52% of MP subjects showed mutation at both the sites. The association between both site mutation and MP was found to be highly significant ($P \leq 0.05$). (Table 2) The results suggest that the mutation in MATRILIN-1 at rs20566 and region corresponding rs1065755 can be attributed to MP.

Table 1: Demographic details of the study Population

Variable	MP Cases (n=50)	Control (n=50)
Age (years) Mean±SD	21.80 ± 12.70	21.45 ± 2.48
Male	52 (%)	58 (%)
Female	48 (%)	42 (%)

Table 2: Genotype Frequencies of Polymorphism in MP Cases and Control

Restriction Site	Type of Mutation	MP Cases (n = 50)	Control (n = 50)	P value
rs20566	SNP	59%	14%	0.002*
rs1065755	Frameshift	80.1%	36%	0.05*
Presence of mutation in both sites		52%	9.25%	0.001*

* indicates statistically significance at p≤0.05

Discussion

Mandibular prognathism is a common craniofacial deformity characterized by either mandibular protrusion or maxillary retrusion or a combination of both. It is mainly due to the overgrowth of the mandible in the sagittal direction.[16] Mandibular growth is regarded as a result of the combined effect of environmental and genetically predetermined intrinsic factors. Research on growth and development has shown that heredity and the mechanical modulation of growth and development share a common pathway via genes.[17] Singh, in his review article, stated that comorphologies of craniomaxillary and mandibular complexes are likely dependent on candidate genes that undergo gene-environmental interactions to bring about mandibular prognathism.[1] Thus, mandibular prognathism is multifactorial and has a complex trait. Since there is consensus that the development of mandibular prognathism is genetically predetermined, various researchers (Wolf et al and EI-Gheriani et al) have investigated the candidate genes governing mandibular development.[18,19]

Out of all class III malocclusion cases, 19% are due to MP, while 45.6% are due to a combination of MP with maxillary retrognathism.[20,21] Identification of the jaw responsible for class III is important in

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making a decision regarding early or late treatment, as well as visualizing prognosis of the case. Inability to do so may lead to either loss of growth period, prolonged treatment, or relapse. Yamaguchi, in 2005, mapped 3 chromosomal loci 1p36, 6q25, and 19p13.2, out of which 1p36 is the loci of interest for the study of skeletal system as it harbors candidate genes related to it.[13] Genome-wide association study in the Japanese population showed association of 2 loci with the susceptibility for MP—1p32.2 and 1p22.3. The locus Ip22.3 mentioned in the study was found to be near 1p36.

MATRILIN-1 is a cartilage-specific homotrimer localized in the growth plate of long bones. It is transcribed in the late proliferative and hypertrophic chondrocytes of the growth plate. During endochondral bone formation, the sequence of expression is collagen type II, aggrecan, and MATRILIN-1.[22] It is an adhesion factor for fibroblasts and chondrocytes, which is mediated by Integrin a1b1 and thus may play an important role in the development and repair of skeletal tissues.[23] Mutation in gene encoding MATRILIN-1 may lead to altered polypeptide synthesis, resulting in altered chemical and physical properties of protein that is either itself responsible for altered phenotype or makes it more vulnerable to environmental influences.

Several gene candidates have been reported in previous studies as the genetic factors in skeletal malocclusion class III and mandibular prognathism. Referring to studies on various ethnicities, the genes that are suggested to correlate with mandibular prognathism are located in different loci. One of the loci commonly found in Asian is the MATN1 gene locus 1p35.[24-27] No study on genetic factors in the etiology of skeletal malocclusion class III mandibular prognathism has been done in Indonesian subjects; hence, the involvement of heredity factors in this disorder remains unknown.

MATRILIN-1 gene variants in Equusasinus have been found to be an effective genetic marker for MP in the species. According to a study by Rodrigues et al,[28] MATRILIN-1 might have a role in protein regulation, by affecting splicing, maturation, or elongation of RNA. It can also change enhancer or silencer, affecting transcription rate. Ultimately, the genetic variation seems to reduce the expression of resultant protein.Jang et al[14] investigated the association of MATRILIN-1 with MP in the Korean population. Three restriction sites on the 1p36 locus were studied—rs20566, rs1149045, and rs1065755. At rs20566, SNP was found as a substitution of C > T. The significance of its association was found to be high in MP cases than in control ($P < .05$) Study results showed a significant difference in the distribution of SNP at rs20566 in MP cases and control. Allele frequency in MP cases was 60%, which was much higher than that in control (14.3%). So the attributed risk of developing MP in presence of mutation at rs20566 was calculated to be 9%. These findings for rs20566 are in concordance with the study performed by Jang et al.[14]To genotype the region encoding rs1065755, DNA sequencing was performed. We found significantly higher frequency of frameshift mutation in MP cases as compared to controls. A total of 80.6% of MP cases showed frame shift when compared to control samples (36.3%).Tassopoulou-Fishellet al[29] conducted a study wherein they used PCR along with TaqMan chemistry to amplify the genome and to trace polymorphisms. A correlation was found between the rs10850110 polymorphism of the MYO1H gene and mandibular prognathism in their study population. Though he analyzed the role of other polymorphisms rs2503243, rs972054, rs1413533, rs1490055, rs2101560, rs1601948, rs1387168, rs2940913, rs7718944, rs3016534, and rs9458378 in mandibular prognathism, they could not find any correlation. This shows that rs10850110 MYO1H is an important marker in analyzing mandibular prognathism. Sun et al[30] used the whole-mount in situ hybridization (WISH) technique to analyze

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the pattern of expression of MYO1H and they concluded that MYO1H plays a vital role in mandibular growth because of the involvement of this gene in the proliferation and morphology of the mandibular condyle chondrocytes.

The association of SNP at rs20566 and frameshift mutation at rs1065755 together were significantly greater in MP cases than in control ($P \leq 0.05$). The frequency of occurrence of MP in the presence of mutation at both the sites was 51.6%. Samples which showed mutation at both the sites had strong MP component with average ANB lesser than −3 degrees. Therefore, to study the predisposition to MP in an individual, the mutational screening of both sites—rs20566 and rs1065755—is reliable.

Conclusion

The results of this study show association of mutation in MATRILIN-1 gene with MP. A greater potential for frameshift mutation was found with resultant alteration of the protein.The identification of genetic influences in malocclusions helps in the prevention and improves treatment modalities of maxillomandibular discrepancies.The data generated in the present study can offer the foundation for mutational screening analysis to predict the susceptibility of developing an MP.

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