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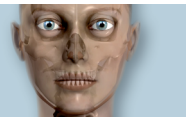

ORIGINAL ARTICLE

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Analysis of the association of *COL2A1* and *IGF-1* with mandibular prognathism in a Chinese population

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Structured Abstract

Objective **–** In this study, we performed a case–control association analysis to determine whether the candidate genes *COL2A1* and *IGF-1* are susceptibility genes for mandibular prognathism (MP).

Methods **–** Eleven and five single-nucleotide polymorphisms (SNPs) located in *COL2A1* and *IGF-1*, respectively, were selected and genotyped in 211 cases and 224 controls. The individual SNPs and the relevant haplotypes were analyzed and tested for an association with MP, to identify genes potentially associated with MP.

Results **–** In the analysis of individual SNPs, the SNP rs1793953 in the *COL2A1* gene showed a possible association with MP with regard to allelic frequency and genotypic distribution ($p = 0.031$; $p = 0.025$, respectively) in the 211 cases and 224 controls. The A allele of rs1793953 was associated with a significantly decreased risk of MP (OR: 0.74; 95% CI: 0.58–0.97). Linkage disequilibrium and haplotype analysis revealed that MP was not associated with haplotypes that included the rs1793953 alleles. *IGF-1* gene did not show the association with MP.

Conclusion **–** An association between polymorphism in the *COL2A1* gene and MP was observed. The results suggested that the *COL2A1* gene could be a new susceptibility gene for use in the study of genetic risk factors for MP.

Key words: association study; *COL2A1*; *IGF-1*; mandibular prognathism; SNPs

Introduction

Mandibular prognathism (MP) is a dentofacial anomaly, characterized by an anterior cross-bite and a protrusive mandible. The reported prevalence of MP is 5–10% (1–3) in Asian populations, which is relatively high in comparison with Caucasian populations

(0.5–4%) (4, 5). The etiology of MP is not understood completely, but it is well known that both genetic components and environmental factors contribute to the development of MP. In a number of previous reports, research from family and twin studies has revealed that MP can be accounted for mainly by a polygenic model of inheritance (6–8), and recent studies in molecular genetics have identified the susceptibility loci and genes that underlie the development of MP.

The results of genome-wide linkage analyses have suggested that several chromosomal loci might be linked to MP. Yamaguchi et al. (9) were the first to map susceptibility loci for MP to chromosomes 1p36, 6q25, and 19p13.2. In our previous study, we chose to investigate 1p36 and found that the *EPB41* gene on 1p36 was associated significantly with MP (10). Recently, another genome-wide study revealed that five suggestive loci, namely 1p22.1, 3q26.2, 11q22, 12q13.13, and 12q23, were associated with MP (11). In studies of craniofacial growth in mice, loci on murine chromosome 10 were determined to be responsible for mandibular length, and these corresponded to regions on human chromosome 12 (12). This comparative result supports previous findings that regions on chromosome 12 are biologically relevant to craniofacial development, and it suggests that the loci 12q23 and 12q13 might be linked to MP. Candidate genes of interest, *IGF-1* and *COL2A1*, are located within the regions 12q23 and 12q13, respectively. *IGF-1* has been proven to be involved in the proliferative activity of condylar cartilage (13), and *COL2A1* is expressed in cartilage and is important for craniofacial growth (14, 15).

In this study, we systematically genotyped 16 single-nucleotide polymorphisms (SNPs) in the *IGF-1* and *COL2A1* genes among 211 MP cases and 224 controls. The genetic SNPs and their haplotypes were analyzed and tested for association with MP to identify potential susceptibility genes for MP.

Material and methods

Participants

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster, and all participants gave written informed consent. The participants comprised 211 unrelated cases (mean age: 17.3 years; 108 males and 103 females) and 224 controls (mean age: 18.1 years, 115 males and 109 females), who were recruited from the registered patients of the Prince Philip Dental Hospital. The MP group was selected on the basis of assessment of their clinical features, which included visual inspection of whether the middle of the face was flat and the presence of anterior cross-bite (10, 16). The final diagnosis of MP was made on the basis of the critical cephalometric parameter of an ANB angle $< -2.0°$ (17, 18). Clinical examinations were carried out to exclude participants who had cleft lip or palate, or any generalized medical condition. The controls were patients who had been diagnosed with Class I malocclusion in our department. All the participants were of Chinese Han ethnicity and were resident in Hong Kong.

Selection and genotyping of SNPs

The SNPs were selected using a HapMap genotyping panel and NCBI dbSNP databases; only SNPs with minor allele frequencies (MAF) of at least 10% in the Chinese Han population were considered. Sets of five and 12 tag SNPs (tSNPs) that were located in *IGF-1* and *COL2A1*, respectively, were selected for genotyping. However, only 16 tSNPs within the two candidate genes were genotyped, because one SNP (rs6703976), located in *COL2A1*, could not be genotyped successfully in the assay.

Genomic DNA was extracted from whole peripheral blood using an Omega Bio-Tek DNA Isolation Kit (Doraville, GA, USA). All the samples of genomic DNA were diluted and arrayed on 96-well plates in accordance with the specifications of the service provider (Genome Research Centre, HKU) (10). The SNPs were mapped to the human genome sequences in the NCBI database, and the flanking sequences (250–300 bp) on either side of each SNP were used to design the PCR primers. The SNP genotypes for each sample were determined using the Sequenom MassArray genotyping platform (San Diego, CA, USA). The

genotyping call rate for each SNP was more than 98%, and the success rate of the duplicate check was >99.5%.

Statistical analysis

Each SNP was evaluated independently in both the cases and controls for conformity to Hardy–Weinberg equilibrium (HWE) (http://www.oege.org/software/hardy-weinberg.html). The genotype distributions and allele frequencies of the SNPs were compared between the cases and controls using a chi-square test, and $p < 0.05$ (two-tailed) was considered statistically significant. The strength of the allelic association was expressed by the odds ratio (OR). Linkage disequilibrium (LD) was assessed for each pair of SNPs, and haplotype analysis was performed using the Haploview 4.1 software (Broad Institute, Cambridge, MA, USA).

Results

Analysis of association of single SNPs

All 16 SNPs conformed to HWE, both in the cases and in the controls. The allelic MAF for each SNP was consistent with the information on the NCBI dbSNP database. Table 1 lists the results of the genotyping of the 16 SNPs in genes *COL2A1* and *IGF1*. The SNP rs1793953, which is located in *COL2A1*, showed statistically significant differences with regard to its genotype distribution and allele frequency between the 211 cases and 224 controls ($p = 0.025$; $p = 0.031$, respectively).

Table 2 shows the results of the analysis of the association of the SNP rs1793953 with MP. We found that the A allele of rs1793953 was less frequent in cases than in controls and was associated with a significantly decreased risk of MP (OR: 0.74; 95% CI: 0.58–0.97).

Linkage disequilibrium and haplotype analysis

The 16 SNPs within the two candidate genes formed six blocks of linkage disequilibrium (LD), and a total of 24 haplotypes were constructed. The haplotype GA of block 2 in *COL2A1* (Fig. 1, left) showed a significant association with MP in the 211 cases as compared with the 224 controls ($p = 0.014$). None of the other haplotypes constructed in any block were observed to be statistically significant.

Table 1. **Selection and genotyping of 16 SNPs in the genes *COL2A1* and *IGF1***

SNP No.	Genes	SNP ID	Location	Allele (1/2)	MAF*
1	*IGF1*	rs972936	101349051 (intron)	C/T	0.46
2	*IGF1*	rs2162679	101395389 (intron)	T/C	0.37
3	*IGF1*	rs6214	101317699 (intron)	T/C	0.49
4	*IGF1*	rs6217	101317916 (intron)	A/G	0.49
5	*IGF1*	rs7296464	101358140 (intron)	A/G	0.16
6	*COL2A1*	rs1793949	46657862 (intron)	G/A	0.48
7	*COL2A1*	rs1793958	46678700 (intron)	A/G	0.23
8	*COL2A1*	rs1793953	46679793 (intron)	G/A	0.44
9	*COL2A1*	rs2071437	46673186 (intron)	A/G	0.31
10	*COL2A1*	rs954326	46681090 (intron)	G/T	0.16
11	*COL2A1*	rs1793923	46670389 (intron)	C/T	0.43
12	*COL2A1*	rs12228854	46683187 (intron)	G/T	0.35
13	*COL2A1*	rs1034762	46675910 (intron)	C/A	0.47
14	*COL2A1*	rs4760608	46655256 (intron)	A/C	0.35
15	*COL2A1*	rs2070739	46654243 (intron)	C/T	0.38
16	*COL2A1*	rs11168337	46662797 (intron)	A/C	0.40

*Minor allele frequency.

Table 2. **Analysis of the association of the *COL2A1* SNP rs1793953 with MP among the 211 cases and 224 controls**

		Genotype frequencies (N)				Allele frequencies (N)			
Population	No. of Participants	Major homozygote GG	Heterozygote AG	Minor homozygote AA	p*	Major allele G	Minor allele A	p†	OR‡ (95% CI)§
Cases	211	72	109	30	0.025	253	169	0.031	0.74 (0.58–0.97)
Controls	224	67	102	55		236	212		

*Statistically significant differences in genotype distributions between cases and controls.
†Statistically significant differences in allelic frequencies between cases and controls.
‡Odds ratio of minor allele vs. major allele.
§Confidence interval.

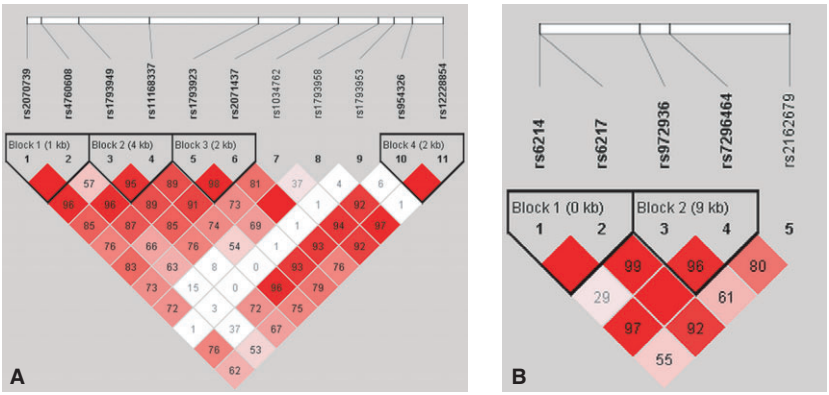

Fig. 1. Linkage disequilibrium and haplotype blocks in *COL2A1* and *IGF-1* genes. Eleven tSNPs formed four haplotype blocks in the *COL2A1* gene (A) and five tSNPs formed two haplotype blocks in the *IGF-1* gene (B). The pairwise D'value is displayed in each square (its value is not shown when D' = 1), and the cells in red indicate strong LD. The SNP rs1793953, which showed a significant association with MP, was not in strong LD with any of the other SNPs. The bar at the top of the figure represents the candidate genes and the location of each SNP; kb indicates kilobase.

Discussion

In the present study, we found that there was a significant association between the SNP rs1793953 and MP in a case–control population of Chinese origin. The results of single-SNP analysis indicated that the SNP rs1793953 was associated significantly with MP with regard to its genotype distribution ($p = 0.025$) and allele frequency ($p = 0.031$) in 214 cases and 221 controls. The SNP rs1793953 is located within the candidate gene *COL2A1*, and the association between rs1793953 and MP suggests that it is likely the *COL2A1* gene confers susceptibility to this phenotype. The *COL2A1* gene, which is located on chromosome 12q13 and has six exons that span almost 41 kb of genomic DNA, encodes the alpha-1 chain of type II collagen and plays an important role in cartilage formation (14, 15).

Type II collagen occurs exclusively in cartilage, and it is the marker for cartilage formation in the mandibular condyle (19). Disturbances in craniofacial growth, including undergrowth of mandibular body length, have been found in mice in which type II collagen has been knocked out (20). Mature chondrocytes express type II collagen after they have differentiated from mesenchymal cells (21). Some studies have reported that mandibular advancement accelerates the differentiation of chondrocytes and the formation of cartilage in the condyle through enhancing the expression of type II collagen (19, 22). In experiments on rat mandibular advancement, Rabie et al. (23) showed that temporal expression of the transcription factor Sox9 coincided with the expression of type II collagen, and they proved that Sox9 regulates the synthesis of type II collagen by chondrocytes in condylar cartilage

(24). This implies that the *COL2A1* gene is involved in chondrocyte differentiation and the formation of cartilage, and that polymorphisms in the *COL2A1* gene might lead to abnormal growth of the mandibular condyle.

The SNP rs1793953 was associated significantly with MP when individual SNPs were analyzed. The results of association identified by single-SNP analysis showed a direct association with MP compared with haplotype analysis ($p = 0.014$). rs1793953 did not form haplotypes with the other genotyped SNPs and was not in the LD block of the *COL2A1* gene that included a haplotype that was associated significantly with MP (block 2). Although haplotype analysis could not provide additional information that further verified that rs1793953 was associated with MP, it suggested that other functional polymorphisms located in *COL2A1* might be in LD with this SNP. Given that only a selective group of tSNPs were studied in each gene, further genotyping is required to investigate all the polymorphisms in each gene to determine those that are associated with the risk of MP.

To our knowledge, this is the first report of an association study with mandibular prognathism that is based on an assessment of the biological functions of the candidate genes. We present evidence that the *COL2A1* gene is associated with MP in the Chinese Han population. The *COL2A1* gene could be a new susceptibility gene for use in the study of genetic risk factors for MP. If the genetic association reported here is replicated in independent groups, the mechanism and signaling pathway of *COL2A1* in the regulation of MP will need to be elucidated further.

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