

Targeted sequencing in *FGF/FGFR* genes and association analysis of variants for mandibular prognathism

Xueyan Xiong, MD^a, Shuyuan Li, MD^{b,c}, Ying Cai, MD^a, Fengshan Chen, MD^{a,*}

Abstract

To identify variants of the genes in fibroblast growth factors/fibroblast growth factor receptors (FGF/FGFR) signal pathway that predispose to mandibular prognathism (MP) in the general Chinese population systematically.

Targeted sequencing of the FGF/FGFR genes was conducted in 176 MP individuals and 155 class I malocclusion controls. The associations of common and rare variants with MP as a categorical phenotype and also continuous malocclusion phenotypes generated by principal component (PC) analysis were analyzed.

One common variant, rs372127537, located in the 3'-untranslated region of *FGF7* gene, was significantly related to PC1 ($P = 4.22 \times 10^{-4}$), which explained 23.23% of the overall phenotypic variation observed and corresponded to vertical discrepancies ranging from short anterior face height to long anterior face height, after Bonferroni correction. Also, 15 other variants were associated with PC1–4, although not significant after multiple corrections ($P < .05$). We also identified 3 variants: rs13317 in *FGFR1*, rs149242678 in *FGF20*, and rs79176051 *FGF12* associated with MP ($P < .05$). With respect to rare variant analysis, variants within the *FGF12* gene showed significant association with MP ($P = .001$).

Association between FGF/FGFR signaling pathway and MP has been identified. We found a previously unreported SNP in *FGF7* significantly related to increased facial height. Also, rare variants within the *FGF12* were associated with MP. Our results provide new clues for genetic mechanisms of MP and shed light on strategies for evaluating rare variants that underlie complex traits. Future studies with larger sample sizes and more comprehensive genome coverage, and also in other population are required to replicate these findings.

Abbreviations: MAF = minor allele frequency, MP = mandibular prognathism, OR = odds ratio, PC = principal component, SNPs = single-nucleotide polymorphisms, UTR = untranslated region.

Keywords: association study, FGF/FGFR signaling pathway, mandibular prognathism, targeted sequencing

1. Introduction

Mandibular prognathism (MP) is a complex maxillofacial disorder, which imposes significant aesthetic and functional burden on affected individuals worldwide. The prevalence of MP

varies greatly relative to the population examined. Epidemiology studies suggested that the prevalence of MP is highest in individuals of Asian descent (approximately up to 15%) and lowest in individuals of Caucasian descent (about 1%).^[1] Genetic components play an important role in the pathogenesis of MP. However, the inheritance pattern of MP is still controversial. Both monogenic (dominant or recessive) and polygenic inheritance manners have been suggested by different studies.^[2–4] With the evidence accumulated, it is now accepted by most researchers that MP is a polygenic disorder with both environmental and genetic risk factors attributed to its etiology.^[1,5,6] Although genetic linkage analysis and association studies have identified many genes and loci associated with MP,^[1,7–17] the genes underlying the risk of MP in the general population remain elusive, leaving some impetus to search for new candidate genes.

As characterized by overgrowth of the lower jaw with or without undergrowth of the upper jaw,^[18] MP is deemed to be caused by abnormal craniofacial morphogenesis. The genetic mechanisms of craniofacial development has been elucidated, with FGF, bone morphogenetic protein, sonic hedgehog, and many other signal pathways playing critical roles.^[19] In the past decade, the role of FGF signaling in craniofacial development has been extensively investigated. It has been shown that FGF signaling exerts an inductive impact on facial primordia formation. *Fgfr1* and *Fgfr2* spread widely in facial primordia, whereas the FGF ligands are present in restricted region: *Fgf8*, *Fgf9*, and *Fgf10* are highly expressed at nasal pits, whereas expression of *Fgf3*, *Fgf15*, and *Fgf17* is confined to the medial

Editor: Jian Liu.

X.X. and S.L. are equal contributors.

Funding: This work was supported by National Natural Science Foundation of China (No. 81170942 and 81371129).

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Supplemental Digital Content is available for this article.

^a Department of Orthodontics, School & Hospital of Stomatology, Tongji University, Shanghai Engineering Research Center of Tooth Restoration and Regeneration, ^b Institute of Embryo-Fetal Original Adult Disease, ^c International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

* Correspondence: Fengshan Chen, Department of Orthodontics, School & Hospital of Stomatology, Middle Yanchang Road 399, Shanghai, China (e-mail: chenfengshan@163.com).

Copyright © 2017 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the Creative Commons Attribution-NoDerivatives License 4.0, which allows for redistribution, commercial and non-commercial, as long as it is passed along unchanged and in whole, with credit to the author.

Medicine (2017) 96:25(e7240)

Received: 31 December 2016 / Received in final form: 26 April 2017 / Accepted: 26 May 2017

<http://dx.doi.org/10.1097/MD.0000000000007240>

Table 1
Demographical characteristics of the cases and controls.

	Male/Female	Mean age (SD)	Mean ANB (°, SD)	Mean Wits appraisal (mm, SD)
Cases	79/97	23.36 ± 9.83	-2.62 ± 2.25	3.01 ± 1.16
Controls	60/95	23.01 ± 6.30	5.88 ± 3.48	-1.20 ± 3.31

ANB angle = point A-Nasion-point B, SD = standard deviation.

side of the nasal pits.^[20,21] Exogenous *Fgf2* and *Fgf4* could give rise to increased length of the cartilage rod formed in the frontonasal and mandibular mesenchyme.^[22] Inactivation of *Fgf8* in the ectoderm of the first branchial in zebrafish results in almost complete loss of first-arch derived structures, including the mandible.^[23] In addition, FGF signaling also has a vital function in craniofacial skeletogeny. It is expressed in both endochondral and intramembranous bones and involved in modulating their development and growth.^[24,25]

Given the crucial roles of FGF signaling in craniofacial development, it is not surprising that mutations in the FGF pathways are involved in various congenital bone diseases. It has been reported that gain-of-function mutations in *Fgfr1* and *Fgfr2* lead to craniosynostosis syndromes, such as Apert, Crouzon, and Pfeiffer syndrome, all of which often manifest MP phenotype.^[26–28] Recently, we have identified a novel mutation in *FGF23*, c.35C>A, strongly associated with MP.^[11] Moreover, 2 single-nucleotide polymorphisms (SNPs), rs2162540 and rs11200014, of *FGFR2* are suggested to increase the risk for classes II and III skeletal malocclusion.^[7] According to these clues, it is reasonable to hypothesize that the variants of genes in FGF/FGFR signal pathway play a significant role in MP pathogenesis. The purpose of this study was to identify variants of the genes in FGF/FGFR signal pathway that predispose to MP in the general Chinese population systematically.

2. Materials and methods

2.1. Study subjects

In all, 176 subjects with MP (mean age 23.36 ± 9.83 years; 79 males) and 155 subjects with normal skeletal class I (mean age 23.01 ± 6.30 years; 60 males), who were seeking orthodontic treatment at the affiliated Stomatology Hospital of Tongji University, were recruited from January 2013 to September 2014 (Table 1). All participants were unrelated and were of Han Chinese ancestry. Written informed consent (including the release for dental records) was acquired from each participant or the parental guardians in case of the minors. This study was approved by the Human Ethics Committee of Tongji University and was conducted according to Declaration of Helsinki principles.

All individuals were first diagnosed by lateral cephalograms, in conjunction with orthodontic study models or visual inspection. Digital lateral cephalograms were taken by a dental X-ray equipment (Veraviewepocs X550, Kyoto, Japan), using a standardized technique with the patients' jaws in centric occlusion. Individuals were diagnosed as MP if they had an ANB angle (Point A-Nasion-Point B) of the centric jaw relationship less than 0.0 degrees^[2,17] and a negative Wits appraisal greater than -2.0 mm.^[18] And the criteria of normal skeletal class I was defined as follows: an ANB angle range from 0.3 to 4.8 degree along with a Wits appraisal between -1.3 and 2.4 mm. Individuals who had previous orthodontic treatment,

severe facial trauma, congenital abnormalities (eg, cleft lip and palate), or general physical disease (eg, endocrine diseases) were excluded from this study.

Approximately 5 mL of ethylenediaminetetraacetic acid-anti-coagulated peripheral blood were obtained from each individual, and genomic DNA was extracted using a QIAamp DNA Blood Kit (QIAGEN GmbH, Hilden, Germany). All the samples were stored at <-80°C until analysis.

2.2. Cephalometric analysis

Pretreatment cephalometric tracing was performed using NemoCeph NX software (version 6.0, Nemotec, Madrid, Spain) by 2 independent orthodontists. All the lateral cephalograms were traced twice by each rater at least 2 weeks apart. Sixty-one cephalometric parameters digitized with 27 skeletal landmarks and 9 soft landmarks were used for phenotyping. Inter-rater and intrarater reliability was tested by intraclass correlation method as described previously.^[29] A Procrustes routine was implemented to wipe out variants irrelevant to shape.^[30] After that, the Procrustes residuals was employed for principal component (PC) analysis to determine the most significant aspects in the data.

2.3. Targeted region sequencing and data analysis

The coding and flanking regions of 26 genes in the FGF/FGFR signaling pathway, approximately 91.3 kb, were selected and sequenced in this study. The targeted regions (shown in Supplemental Table S1, <http://links.lww.com/MD/B757>) were captured according to the standard procedures using a customized NimbleGen capture array (Roche-NimbleGen Inc. Madison, WI) and then sequenced on the Illumina HiSeq2000 platform (Illumina Inc, San Diego, CA). The raw reads were aligned to the human reference genome (hg19) using Burrows-Wheeler Alignment tool v0.7.1 (<http://maq.sourceforge.net>), producing binary sequence alignment/map files containing various mapping information. The duplicate reads were then removed using Picard v1.137 (<https://github.com/broadinstitute/picard/releases>) and realigned using Genome Analysis Toolkit v3.4–46. The coverage, mean quality, and global depth of aligned reads were calculated by perl scripts based on the pileup files generated by SAMtools v1.2. Only the bases with ≥20 base quality were taken into account. Variants were called using SNPTools and annotated using the ANNOVAR software package (<http://www.openbioinformatics.org/annovar/>). Indels (insertion/deletion) were verified manually. To access reproducibility, 6 random selected samples (3 cases and 3 controls) were analyzed in duplicate and Sanger sequencing of the positive SNPs in this study was also conducted.

2.4. Statistical analysis

For common variants (minor allele frequency [MAF] ≥1%), the allelic and genotypic distributions of the case and control groups were compared using the Pearson chi-square test. Fisher exact test was used when the expected count was <5. Logistic regression analysis was used to identify the effects of the variants on MP with odds ratio (OR) and 95% confidence interval (CI). Linear regression analysis adjusting for age and sex was performed to identify the associations between the variants and PCs, explaining more than 5% of the total variance in the cephalometric data. For rare variants (MAF <1%), the cumulative variants within each gene region in cases and controls were compared by Fisher

Table 2**The associations between SNPs identified in FGF/FGFR signaling pathway and MP.**

SNP-gene-function	Genotype/allele	Genotype/allele		P	Logistic regression	
		Cases	Controls		OR (95% CI)	P
rs13317-FGFR1-UTR3	TT	86 (48.6)	63 (40.6)	.028	0.666 (0.470–0.944)	.022
	TC	82 (46.9)	73 (47.1)			
	CC	8 (4.5)	19 (12.3)			
	T:C	72.2:27.8	64.2:35.8			
rs149242678-FGF20-UTR5	GG	162 (92.0)	152 (98.1)	.022	4.379 (1.234–15.536)	.022
	GC	14 (8.0)	3 (1.9)			
	CC	0 (0)	0 (0)			
	G:C	96.0:4.0	99.0:1.0			
rs79176051-FGF12-intronic	AA	165 (94.3)	128 (85.9)	.013	0.369 (0.168–0.812)	.013
	AG	10 (5.7)	21 (14.1)			
	GG	0 (0)	0 (0)			
	A:G	97.1:2.9	93.0:7.0			

CI = confidence interval, FGF/FGFR = fibroblast growth factors/fibroblast growth factor receptors, MP = mandibular prognathism, OR = odds ratio, SNP = single-nucleotide polymorphism.

exact test. All statistical analyses were performed using SAS, version 9.2 (SAS institute Inc., Cary, NC), and 2-tailed *P* values <0.05 were considered statistically significant.

3. Results

3.1. Targeted sequencing data

The average sequencing coverage was $67\times$ (interquartile range $43\text{--}87\times$). The concordance of the calling variants in duplicate samples were more than 99%. The variants with calling rates less than 95% or inconsistent with Hardy–Weinberg equilibrium ($P < .01$) in the control group were removed. Based on the MAF of the SNPs tested in the control group, the retained variants were then classified into 2 groups: common variants (MAF ≥ 0.01) and rare variants (MAF < 0.01). Overall, 460 variants were identified across all sequenced individuals in the targeted regions, including 139 common variants and 321 rare variants. Among the 139 common variants identified, 43 of the variants conducted 15 haplotype blocks with the fact that the variants in the same block were in almost complete linkage disequilibrium (LD, $r^2 > 0.90$, $D' = 1$). In this case, only 1 variant from each block was chosen for further analysis randomly and the final number of common variants to be analyzed was 111.

3.2. Association analysis of common variants

Among the 111 common variants, we only presented those significantly associated with MP and PCs which explaining more than 5% of the facial variation. The results of Sanger sequencing of the positive SNPs in this study were in accordance with the raw results. No common variants within the 26 genes were significantly associated with MP after Bonferroni correction for multiple testing (cut-off *P* value = $.05/111 = 4.50 \times 10^{-4}$). Only 3 SNPs reached nominal significance ($P < .05$), including rs13317 in the 3'-untranslated region (UTR) of *FGFR1*, rs149242678 in the 5'-UTR of *FGF20*, and rs79176051 in the intron of *FGF12*. As shown in Table 2, the genotypic and allelic frequencies at rs13317, rs149242678, and rs79176051 were significantly different between case and control groups. The C allele of rs149242678 decreased the risk of MP, whereas the C allele of rs13317 and the G allele of rs79176051 increased the

risk of MP, with ORs of 4.379 (1.234–15.536), 0.666 (0.470–0.944), and 0.369 (0.168–0.812), respectively.

The results of the principal component analysis revealed 5 PCs accounted for 73.4% of the total variance, and each of them represented 23.2%, 19.8%, 13.6%, 10.0%, and 6.7% of the total variance, respectively (Fig. 1). As shown in Table 3, 1 common variant (rs372127537; $P = 4.22 \times 10^{-4}$) in the 3'-UTR of *FGF7* gene was significantly associated with PC1, which depicted vertical discrepancies ranging from short anterior face height to long anterior face height, after Bonferroni correction. This variant was also associated with PC2 ($P = 4.96 \times 10^{-3}$), which captured mandibular shapes ranging from a larger mandibular body, a large ramus height to a small mandibular body, a small ramus height, and PC4 ($P = 1.35 \times 10^{-2}$), which captured inclination of mandibular incisor, although the associations were not significant after multiple corrections. We also detected 3 other SNPs associated with PC1, 2 other SNPs associated with PC2, and 2 other SNPs associated with PC4 with nominal significance. In addition, 6 SNPs associated with PC3, which captured the inclination of maxillary incisor, and 3 SNPs associated with PC5, which captured horizontal discrepancies of the jaws with respect to anterior skull base, were also identified at the $P < .05$ significance level.

3.3. Association analysis of rare variants

Table 4 displays the association results of rare variants with MP for the 26 targeted genes. Compared with the controls, the MP group had more rare variants in *FGF12* ($P = .001$). However, when restricted to nonsynonymous variants, none of the targeted genes showed association with MP.

4. Discussion

It is widely held that genetic components play an important role in MP. So far, numerous chromosomal loci implicated in MP pathogenesis has been reported, and also a host of genes that predispose to MP, such as *EPB41*, *MATN1*, *COL2A1*, *MYO1H*, *TGFB3*, *LTBP2*, *ADAMTS1*, *DUSP6*, *FGFR2*, and *FGF23*.^[1,7–11,13–17,31] Most of these studies were based on family linkage study. However, the polygenic nature of MP makes it possible to study its genetic mechanism by case-control design.

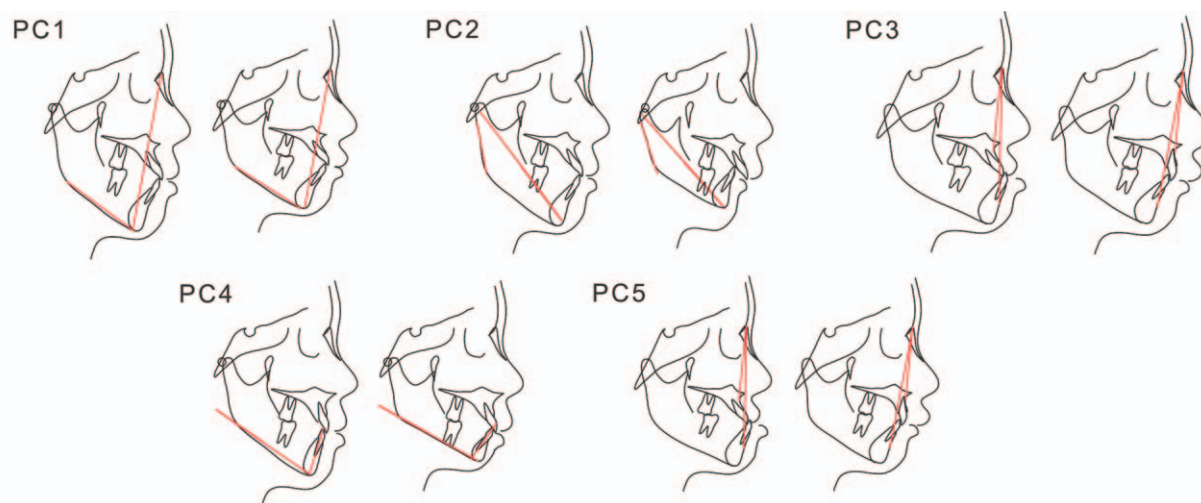


Figure 1. Examples of individuals at opposite extremes of the distributions for each of the 5 principal components. PC1 explained 23.2% of the observed facial variation and corresponded to variations within the anterior facial height. PC2 explained 19.8% of the observed facial variation and corresponded to differences in the ramus height (Co-Gn in mm) and mandibular body length (Ar-Go in mm). PC3 corresponded to characteristics of incisor inclination and explained 13.6% of the observed facial variation. PC4 corresponded to the mandibular incisor angulation and explained 10% of the observed facial variation. PC5 corresponded to the position of the mandibular and accounted for 6.7% of the variation.

With the evidence accumulated, it is now accepted by most researchers that MP is a polygenic disorder with both environmental and genetic risk factors attributed to its etiology.^[1,5,6] Although genetic linkage analysis and association studies have identified many genes and loci associated with MP, the genes underlying the risk of MP in the general population remain elusive, leaving some impetus to search for new candidate genes. In the current study, we aimed to identify the association between variants in the FGF/FGFR signaling pathway and MP in MP cases and controls using target sequencing strategy and have found some novel variants in these genes associated with MP.

By analyzing common variants in the coding and flanking regions of 26 selected FGF/FGFR genes in 176 MP cases and 155 controls, we found that 1 SNP, rs372127537, was significantly

associated with PC1 after Bonferroni correction. This SNP was also correlated with PC2 and PC4 with nominal significant difference. This SNP was located in the 3'-UTR of *FGF7* gene and may influence the gene expression.^[32] *FGF7* is a member of FGF family, which is known as a mediator of epithelial-mesenchymal tissue interactions in several organs.^[33] It may act directly to induce the formation of an additional apical ectodermal ridge in the ectoderm of the dorsal midline,^[34] and the apical ectodermal ridge is indispensable for limb outgrowth proceeds.^[35] Furthermore, *FGF7* is also expressed in perichondrium of growth plate during bone formation.^[36] Perlecan, a prominent component of human cartilage, is a receptor for FGF7. Their interaction initiates cell signaling and subsequent down-line effects on cell proliferation and differentiation, thus coordinates chondro-

Table 3

The associations of common SNPs with continuous malocclusion phenotypes.

Trait	SNP	A1/A2	Gene	Fun	Beta	SE	P
PC1	rs372127537	T/-	FGF7	UTR3	5.458	1.522	4.22×10^{-4}
	rs118040588	C/T	FGF1	UTR5	3.109	1.059	3.68×10^{-3}
	rs34347344	G/A	FGF18	Exon/syn	1.53	0.5785	8.79×10^{-3}
	rs3109189	G/T	FGF12	UTR5	0.8829	0.411	3.28×10^{-2}
PC2	rs372127537	T/-	FGF7	UTR3	4.03	1.418	4.96×10^{-3}
	rs60771113	A/T	FGF7	UTR3	0.782	0.3784	4.01×10^{-2}
	rs3740639	C/T	FGF4	UTR3	1.02	0.4943	4.02×10^{-2}
PC3	rs2305182	C/A	FGFR3	Intronic	-4.886	1.647	3.36×10^{-3}
	rs35420992	C/T	FGF3	Exon/syn	-2.215	0.9727	2.38×10^{-2}
	rs34003	A/C	FGF1	Intronic	0.6643	0.3056	3.08×10^{-2}
	rs17224024	-/G	FGF1	UTR3	0.6855	0.3163	3.13×10^{-2}
	rs2278202	G/A	FGFR2	Intronic	-0.5736	0.2791	4.11×10^{-2}
	rs2936871	A/T	FGFR2	Intronic	0.6238	0.3098	4.53×10^{-2}
PC4	rs115452181	C/T	FGF3	Exon/nonsyn	2.165	0.7531	4.46×10^{-3}
	rs372127537	T/-	FGF7	UTR3	-2.485	0.9973	1.35×10^{-2}
	rs1721100	C/G	FGF20	UTR3	0.5048	0.2384	3.54×10^{-2}
PC5	rs2936871	A/T	FGFR2	Intronic	0.4558	0.2171	3.70×10^{-2}
	rs45504296	T/C	FGF2	UTR3	-1.826	0.9081	4.57×10^{-2}
	rs2290070	C/G	FGF10	Intronic	-0.5385	0.2717	4.88×10^{-2}

PC=principal component, SE=standard error, SNP=single-nucleotide polymorphism, UTR=untranslated region.

Table 4
Burden test of rare variants.

Gene	All			Nonsyn		
	Cases	Controls	P	Cases	Controls	P
FGF1	9/176	7/155	1.0	0/176	0/155	1.0
FGF2	33/176	17/155	.047	3/176	1/155	.626
FGF3	2/176	0/155	.501	1/176	0/155	.468
FGF4	2/176	2/155	1.0	0/176	0/155	1.0
FGF5	10/176	6/155	.609	1/176	0/155	.468
FGF6	10/176	9/155	1.0	4/176	4/155	1.0
FGF7	2/176	2/155	1.0	1/176	0/155	.468
FGF8	1/176	3/155	.344	0/176	0/155	1.0
FGF9	18/176	11/155	.337	2/176	0/155	.501
FGF10	2/176	3/155	.668	1/176	2/155	.601
FGF11	3/176	5/155	.481	1/176	2/155	.601
FGF12	34/176	11/155	.001	0/176	0/155	1.0
FGF13	2/176	3/155	.668	0/176	0/155	1.0
FGF14	6/176	8/155	.586	0/176	0/155	1.0
FGF16	0/176	1/155	.468	0/176	1/155	.468
FGF17	5/176	4/155	1.0	1/176	1/155	1.0
FGF18	4/176	7/155	.359	0/176	1/155	.468
FGF19	8/176	3/155	.229	0/176	0/155	1.0
FGF20	7/176	2/155	.182	2/176	1/155	1.0
FGF21	4/176	5/155	.739	4/176	4/155	1.0
FGF22	1/176	3/155	.344	1/176	3/155	.344
FGF23	7/176	10/155	.330	4/176	3/155	1.0
FGFR1	15/176	15/155	.848	9/176	3/155	.148
FGFR2	13/176	13/155	.839	0/176	3/155	.102
FGFR3	37/176	25/155	.263	7/176	5/155	.776
FGFR4	11/176	17/155	.165	4/176	9/155	.154

Compared with the controls, the MP group had more rare variants in FGF12 ($P=.001$).

genesis and angiogenesis during skeletal development.^[37] And condylar cartilage are key to the regulation of mandibular growth.^[38] Our results indicated that rs372127537 was significantly associated with an increased anterior and posterior height of mandible. This SNP may play an important role in the increase of facial height by affecting growth of condyle cartilage, hence mandible. The potential function of this SNP and the *FGF7* gene in MP development needs to be studied further.

We also detected 3 SNPs in *FGFR1*, *FGF12*, and *FGF20*, respectively, associated with MP, with nominal significant difference. *FGFR1* has an extensive function during craniofacial morphogenesis, and is almost involved in all the structures, including craniomaxillofacial skeleton, muscle, palate, tooth, and submandibular salivary gland.^[19] It modulates osteoblast differentiation as a positive regulator for skeleton formation.^[39] Mutation in *FGFR1* leads to craniosynostosis syndrome (Pfeiffer syndrome), in which relative MP due to maxillary hypoplasia is a common finding.^[27] Loss-of-function variants in *FGFR1* are also determined in patients with combined pituitary hormone deficiencies, which can be associated with complex phenotypes such as cranial/facial midline defects.^[40] Moreover, ColIII-FGFR31^{ach} transgenic mice exhibit shortened long bones and a domed-shaped skull, probably owing to craniofacial hypoplasia.^[41] And according to HaploReg v.4.1, rs13317 is on protein-binding region of CCAAT enhancer-binding protein β (CEBPB), which is demonstrated as an important determinant of osteoblast function and bone mass.^[42] p20C/EBP β (a dominant negative inhibitor of *Cebpb*) transgenic mouse exhibits significant bone volume reduction of mandible.^[43] Therefore, the SNP rs13317 in *FGFR1* is presumed to interfere normal craniofacial shaping and result in MP. On the contrary, FGF20 is shown to be a major

downstream effector of *Eda*, and affects *Eda*-regulated characteristics of tooth morphogenesis, including the number, size, and shape of teeth.^[44] And *fgf20a* is demonstrated to directly affect suture and skull development in zebrafish, and *fgf20a* deficiency also causes craniofacial defects similar to Albertson syndrome.^[45] The identified SNP rs149242678 was located in the 5'-UTR of *FGF20* gene, and related to CCCTC-binding factor (CTCF) protein binding. CTCF is a heritable component of an epigenetic system regulating the interplay between DNA methylation, higher-order chromatin structure, and lineage-specific gene expression.^[46] Future work is needed to gain an insight into the role of these variants in MP pathogenesis.

With respect to *FGF12*, which has high sequence identity with the FGF family, but does not activate FGFRs, except for detecting common variant associated with MP, we also identified more rare variants in *FGF12* in MP individuals compared with controls. Emerging researches have figured out that the common variants with given complex diseases are unable to explain fully of their genetic etiology. Thus, the rare variants are suggested to give rise to explaining a slice of these genetic diseases. Multiple rare variants have been examined affect complex traits strongly, especially the extremes of a disease.^[47,48] A recent study reveals significant association of cleft lip with variants in *FGF12*. Cleft lip can cause other dentofacial malformations in humans such as MP. Also, dysfunction of *FGF12* is found to contribute to skeletal growth and development failure of grade II and III Kashin-Beck disease.^[49] There are no previous association studies with *FGF12* and MP, and it is also the first time that we identified rare variants associated with MP in *FGF12*. However, as none of these effects survived multiple testing correction, further independent replication of these findings will be required.

In a previous research, we have identified a novel heterozygous mutation in *FGF23*, c.35C>A, associated with MP, in a Chinese pedigree by family linkage analysis.^[4] However, no significant association was found between variants of *FGF23* and MP in this case-control association study. These suggests us that the genetic mechanisms of MP are complex,^[37] the main effects of the identified variants are considered to confer relatively small increments in risk, and explain only a small proportion of the heritability.^[50] The mutation in *FGF23*, c.35C>A, may only explain the development of MP in a small number of family samples. The contribution of *FGF23*C35A to MP may be small, and not the susceptibility gene of the group of MP individuals studied in this study.

Identification of the variants to MP in FGF/FGFR signal pathway is the first step to reveal the genes contributing to this disease. What necessary to be done is to detect the genetic architecture across more candidate genes, and to test the variants in large-scale individuals and verify the function of the genes pertinent to MP. Knowledge of these important genes will be helpful in elucidating the mechanisms of MP, and in improving diagnosis or even treatment by simple intervention strategies.

In this study, we have identified 1 common variant in *FGF7* significantly associated with PC1, which demonstrated vertical discrepancies ranging from short anterior face height to long anterior face height. We also identified 3 other variants associated with MP and 15 other variants associated with PC1–4, although not significant after multiple corrections. Moreover, the rare variant within *FGF12* showed significant association with MP. Future studies with larger sample sizes and more comprehensive genome coverage, and also in other population are required to replicate these findings, and further functional studies are also warranted.

Acknowledgments

We are indebted to participants for providing an excellence study sample, and we appreciate the dentists who took clinical examination.

References

- Chen F, Li Q, Gu M, et al. Identification of a mutation in *FGF23* involved in mandibular prognathism. *Sci Rep* 2015;5:11250.
- Cruz RM, Krieger H, Ferreira R, et al. Major gene and multifactorial inheritance of mandibular prognathism. *Am J Med Genet A* 2008;146a:71–7.
- El-Gheriani AA, Maher BS, El-Gheriani AS, et al. Segregation analysis of mandibular prognathism in Libya. *J Dent Res* 2003;82:523–7.
- Ko JM, Suh YJ, Hong J, et al. Segregation analysis of mandibular prognathism in Korean orthognathic surgery patients and their families. *Angle Orthod* 2013;83:1027–35.
- Jena AK, Duggal R, Mathur VP, et al. Class-III malocclusion: genetics or environment? A twins study. *J Indian Soc Pedod Prev Dent* 2005;23:27–30.
- Kawala B, Antoszewska J, Necka A. Genetics or environment? A twin-method study of malocclusions. *World J Orthod* 2007;8:405–10.
- da Fontoura CS, Miller SF, Wehby GL, et al. Candidate gene analyses of skeletal variation in malocclusion. *J Dent Res* 2015;94:913–20.
- Frazier-Bowers S, Rincon-Rodriguez R, Zhou J, et al. Evidence of linkage in a Hispanic cohort with a class III dentofacial phenotype. *J Dent Res* 2009;88:56–60.
- Guan X, Song Y, Ott J, et al. The *ADAMTS1* gene is associated with familial mandibular prognathism. *J Dent Res* 2015;94:1196–201.
- Ikuno K, Kajii TS, Oka A, et al. Microsatellite genome-wide association study for mandibular prognathism. *Am J Orthod Dentofacial Orthop* 2014;145:757–62.
- Jang JY, Park EK, Ryoo HM, et al. Polymorphisms in the *Matrilin-1* gene and risk of mandibular prognathism in Koreans. *J Dent Res* 2010;89:1203–7.
- Li Q, Li X, Zhang F, et al. The identification of a novel locus for mandibular prognathism in the Han Chinese population. *J Dent Res* 2011;90:53–7.
- Li Q, Zhang F, Li X, et al. Genome scan for locus involved in mandibular prognathism in pedigrees from China. *PLoS One* 2010;5: doi: 10.1371/journal.pone.0012678.
- Nikopensus T, Saag M, Jagomagi T, et al. A missense mutation in *DUSP6* is associated with class III malocclusion. *J Dent Res* 2013;92:893–8.
- Xue F, Rabie AB, Luo G. Analysis of the association of *COL2A1* and *IGF-1* with mandibular prognathism in a Chinese population. *Orthod Craniofac Res* 2014;17:144–9.
- Xue F, Wong R, Rabie AB. Identification of SNP markers on 1p36 and association analysis of *EPB41* with mandibular prognathism in a Chinese population. *Arch Oral Biol* 2010;55:867–72.
- Yamaguchi T, Park SB, Narita A, et al. Genome-wide linkage analysis of mandibular prognathism in Korean and Japanese patients. *J Dent Res* 2005;84:255–9.
- van Vuuren C. A review of the literature on the prevalence of class III malocclusion and the mandibular prognathic growth hypotheses. *Aust Orthod J* 1991;12:23–8.
- Nie X, Luukko K, Kettunen P. FGF signalling in craniofacial development and developmental disorders. *Oral Dis* 2006;12:102–11.
- Bachler M, Neubuser A. Expression of members of the *Fgf* family and their receptors during midfacial development. *Mech Dev* 2001;100:313–6.
- Wilke TA, Gubbels S, Schwartz J, et al. Expression of fibroblast growth factor receptor genes (*FGFR1*, *FGFR2*, *FGFR3*) in the developing head and face. *Dev Dyn* 1997;210:41–52.
- Richman JM, Herbert M, Matovinovic E, et al. Effect of fibroblast growth factors on outgrowth of facial mesenchyme. *Dev Biol* 1997;189:135–47.
- Trumpp A, Depew MJ, Rubenstein JL, et al. Cre-mediated gene inactivation demonstrates that *FGF8* is required for cell survival and patterning of the first branchial arch. *Genes Dev* 1999;13:3136–48.
- Eswarakumar VP, Monsonego-Ornan E, Pines M, et al. The *IIIc* alternative of *Fgfr2* is a positive regulator of bone formation. *Development* 2002;129:3783–93.
- Yu K, Xu J, Liu Z, et al. Conditional inactivation of *FGF receptor 2* reveals an essential role for *FGF* signaling in the regulation of osteoblast function and bone growth. *Development* 2003;130:3063–74.
- Piccione M, Antona V, Niceta M, et al. Q289P mutation in the *FGFR2* gene: first report in a patient with type 1 Pfeiffer syndrome. *Eur J Pediatr* 2009;168:1135–9.
- Roscioli T, Flanagan S, Kumar P, et al. Clinical findings in a patient with *FGFR1* P252R mutation and comparison with the literature. *Am J Med Genet* 2000;93:22–8.
- Wang Y, Xiao R, Yang F, et al. Abnormalities in cartilage and bone development in the Apert syndrome *FGFR2(+S252W)* mouse. *Development* 2005;132:3537–48.
- Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull* 1979;86:420–8.
- Chang HP, Liu PH, Chang HF, et al. Thin-plate spline (TPS) graphical analysis of the mandible on cephalometric radiographs. *Dentomaxillofac Radiol* 2002;31:137–41.
- Tassopoulou-Fishell M, Deeley K, Harvey EM, et al. Genetic variation in *myosin 1H* contributes to mandibular prognathism. *Am J Orthod Dentofacial Orthop* 2012;141:51–9.
- Wilkie GS, Dickson KS, Gray NK. Regulation of mRNA translation by 5'- and 3'-UTR-binding factors. *Trends Biochem Sci* 2003;28:182–8.
- Post M, Souza P, Liu J, et al. Keratinocyte growth factor and its receptor are involved in regulating early lung branching. *Development* 1996;122:3107–15.
- Yonei-Tamura S, Endo T, Yajima H, et al. *FGF7* and *FGF10* directly induce the apical ectodermal ridge in chick embryos. *Dev Biol* 1999;211:133–43.
- Saunders JW Jr, Gasseling MT, Errick JE. Inductive activity and enduring cellular constitution of a supernumerary apical ectodermal ridge grafted to the limb bud of the chick embryo. *Dev Biol* 1976;50:16–25.
- Veistinen L, Aberg T, Rice DP. Convergent signalling through *Fgfr2* regulates divergent craniofacial morphogenesis. *J Exp Zool B Mol Dev Evol* 2009;312b:351–60.
- Melrose J, Roughley P, Knox S, et al. The structure, location, and function of perlecan, a prominent pericellular proteoglycan of fetal,

- postnatal, and mature hyaline cartilages. *J Biol Chem* 2006;281:36905–14.
- [38] Xue F, Wong RW, Rabie AB. Genes, genetics, and class III malocclusion. *Orthod Craniofac Res* 2010;13:69–74.
- [39] Du X, Xie Y, Xian CJ, et al. Role of FGFs/FGFRs in skeletal development and bone regeneration. *J Cell Physiol* 2012;227:3731–43.
- [40] Correa FA, Trarbach EB, Tusset C, et al. FGFR1 and PROKR2 rare variants found in patients with combined pituitary hormone deficiencies. *Endocr Connect* 2015;4:100–7.
- [41] Wang Q, Green RP, Zhao G, et al. Differential regulation of endochondral bone growth and joint development by FGFR1 and FGFR3 tyrosine kinase domains. *Development* 2001;128:3867–76.
- [42] Harrison JR, Huang YF, Wilson KA, et al. Col1a1 promoter-targeted expression of p20 CCAAT enhancer-binding protein beta (C/EBPbeta), a truncated C/EBPbeta isoform, causes osteopenia in transgenic mice. *J Biol Chem* 2005;280:8117–24.
- [43] Savage T, Bennett T, Huang YF, et al. Mandibular phenotype of p20C/EBPbeta transgenic mice: reduced alveolar bone mass and site-specific dentin dysplasia. *Bone* 2006;39:552–64.
- [44] Haara O, Harjunmaa E, Lindfors PH, et al. Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. *Development* 2012;139:3189–99.
- [45] Whitehead GG, Makino S, Lien CL, et al. fgf20 is essential for initiating zebrafish fin regeneration. *Science* 2005;310:1957–60.
- [46] Phillips JE, Corces VG. CTCF: master weaver of the genome. *Cell* 2009;137:1194–211.
- [47] Cohen JC, Pertsemlidis A, Fahmi S, et al. Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. *Proc Natl Acad Sci U S A* 2006;103:1810–5.
- [48] Rivas MA, Beaudoin M, Gardet A, et al. Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. *Nat Genet* 2011;43:1066–73.
- [49] Zhang F, Dai L, Lin W, et al. Exome sequencing identified FGF12 as a novel candidate gene for Kashin-Beck disease. *Funct Integr Genomics* 2016;16:13–7.
- [50] Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature* 2009;461:747–53.