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Epigenetic influence of *KAT6B* and *HDAC4* in the development of skeletal malocclusion

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Abstract

Introduction—Genetic influences on the development of malocclusion include heritable effects on both masticatory muscles and jaw skeletal morphology. Beyond genetic variations, however, the characteristics of muscle and bone are also influenced by epigenetic mechanisms that produce differences in gene expression. We studied 2 enzymes known to change gene expressions through histone modifications, chromatin-modifying histone acetyltransferase *KAT6B* and deacetylase *HDAC4*, to determine their associations with musculoskeletal variations in jaw deformation malocclusions.

Methods—Samples of masseter muscle were obtained from subjects undergoing orthognathic surgery from 6 malocclusion classes based on skeletal sagittal and vertical dysplasia. The muscles were characterized for fiber type properties by immunohistochemistry, and their total RNA was isolated for gene expression studies by microarray analysis and quantitative real-time polymerase chain reaction.

Results—Gene expressions for fast isoforms of myosins and contractile regulatory proteins and for *KAT6B* and *HDAC4* were severalfold greater in masseter muscles from a patient with a deepbite compared with one with an open bite, and genes related to exercise and activity did not differ substantially. In the total population, expressions of *HDAC4* ($P = 0.03$) and *KAT6B* ($P = 0.004$) were significantly greater in subjects with sagittal Class III than in Class II malocclusion, whereas *HDAC4* tended to correlate negatively with slow myosin type I and positively with fast myosin gene, especially type IIX.

Conclusions—These data support other published reports of epigenetic regulation in the determination of skeletal muscle fiber phenotypes and bone growth. Further investigations are needed to elucidate how this regulatory model might apply to musculoskeletal development and malocclusion.

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The etiology of dentofacial deformities and malocclusion is multifactorial and includes genetic, behavioral, and environmental components. The soft-tissue envelope, including the muscles of mastication, contributes to the development of jaw deformation by exerting variable loads during stretching and contractile activities.[1] An example of how changes in masticatory muscle activity influence skeletal structures is the development of open-bite malocclusion in children with Duchenne muscular dystrophy.[2–4] Open bite in these patients is often progressive because of decreasing muscle function as the condition exacerbates with age. Previous studies have also demonstrated an association between masticatory muscle fiber type, size, and numbers with the development of open-bite and deep-bite malocclusions.[5–7]

Skeletal muscles are composed of mixtures of fiber types, and each type is associated with specific functional characteristics. What causes variations in fiber type properties that are so closely associated with differences in facial skeletal morphology during growth remains an interesting question in the clinical treatment of dentofacial deformities. The importance of genetic variations (gene polymorphisms) in the growth of muscle and bone is currently being explored and highlighted by genome-wide association studies.[8,9] Epigenetic factors, the mechanisms that change gene expression, must also be explored to determine how genetic influences, beyond primary DNA structure, contribute to growth and facial phenotype. Epigenetic mechanisms can often include acetylation of lysine residues in histone chromatin proteins by acetyltransferase (*KAT*) and deacetylase (*HDAC*) enzymes.[10] In eukaryotic cell nuclei, genomic DNA is folded around octamers of the core histone proteins H2A, H2B, H3, and H4 to form nucleosomes. Nucleosomes are further compacted into chromatin fibers. Histone modification through acetylation can regulate transcription by promoting conformational changes in regions of chromatin, making them more accessible to transcriptional complexes.

Our earlier findings demonstrated that increases in the size and proportion of fast-contracting type II fibers in masticatory muscle are a key environmental influence on the development of skeletal deepbite malocclusion.[7,11] Among the several molecular mechanisms that might influence fiber type variations is a model of epigenetic regulation in which increased acetylation promotes an open conformation of chromatin domains containing the genes for fast-type myosin heavy chain (MHC) gene expression in a rat model.[12,13] Conversely, increased deacetylation promotes a closed chromatin confirmation in chromatin areas to limit accessibility of transcriptional complexes to the slow-contracting (type I) MHC gene. Because of the large variations in fiber types identified in human masseter muscles, it is of interest to determine what role chromatin acetylation/deacetylation plays in fiber composition and MHC gene expression during the development of malocclusion. To address this question, we have begun to evaluate expression of the genes for K (lysine) acetyltransferase 6B (*KATB4*) and histone deacetylase 4 (*HDAC4*) and contractile proteins in masseter muscle samples from orthognathic surgery patients to assess whether there are associations with malocclusion.

MATERIAL AND METHODS

Recruitment was from patients undergoing orthognathic surgery for treatment of malocclusion in the Hopital Roger Salengro, Service de Chirurgie Maxillo-Faciale et Stomatologie, Centre Hospitalier Universitaire, in Lille, France. During the mandibular bilateral sagittal split osteotomy surgical procedure, a small sample of masseter muscle, usually discarded as clinical waste, was collected for study in accordance with the approvals of the French Committee for Personal Protection and the Temple University institutional review board. Either unilateral or bilateral masseter samples about 0.5 cm^3 were snap frozen and stored until shipped to Dr Sciote's laboratory. Muscle tissue was consistently taken from

a point on the superficial area of the deep portion of the masseter muscle about 1.5 cm from the lowest point of the mandible's angle during bilateral sagittal split mandibular osteotomy procedures. Fibers in this area are oriented at almost right angles to the Frankfort horizontal plane[14] and are activated for maintenance of jaw posture.[15] This area's ability to control jaw position in tonus is evidenced by exceptionally large muscle spindles, with the highest muscle spindle density.[16] Consistency of sampling is evidenced when samples from both left and right masseter muscle were compared because there were no significant differences for either mean fiber type area or fiber type percentage of occupancy in the muscle sample between sides.[11]

Malocclusion classification was based on the surgical treatment plan and the presurgical orthodontic diagnosis, which was confirmed in an independent review of the cephalograms at Temple University. The surgical team in Lille uses the analysis of Delaire et al[17] for malocclusion classification during treatment planning for surgical procedures. In addition to being the most popular cephalometric analysis among orthodontists in France, this classification is particularly useful for surgical treatment planning[18] since it clearly shows maxillofacial deformities that need correction to balance the facial skeleton.[19] Diagnostic groupings were confirmed by the orthodontists in Philadelphia using the metric method first proposed by Bjork.[20] Rather than geometric measurements traditionally used in cephalometry, the metric method confirms the presence of most of the 7 structural signs of growth rotation to characterize skeletal dysplasia in the vertical and sagittal dimensions. More recently, the metric method has been validated as perhaps a better classification approach when compared with cephalometric measurements for diagnosis of open-bite malocclusions.[21] Overall, our subjects had jaw deformities that were morphogenetic and not caused by functional behaviors such as thumb-sucking or other oral habits. Representative cephalograms of our subjects have been presented previously in articles by Rowlerson et al[5] (Fig 5) and Sciote et al[22] (Fig 1). After confirmation of the diagnoses, the subjects were placed into 1 of 6 malocclusion classifications based on sagittal and vertical dysplasia: either skeletal sagittal Class II or Class III, or vertical open, normal, or deepbite (Table I). All clinical information and tissue samples were deidentified, and the masseter muscles were stored at −800°C before processing and analysis.

The samples were placed on wet sterile gauze surrounded by ice and taken from the operating room for processing. Each specimen weighed 60 to 120 mg and was about 0.5 cm^2 in size. To prevent molecular degradation of mRNA, the tissues were snap frozen in isopentane cooled by liquid nitrogen (−196°C) within minutes of excision. Before freezing, each masseter sample was examined for fiber orientation and placed perpendicular on a cork board and mounted with Tissue-Tek OCT (optimum cutting temperature; Sakura Finetek, Torrance, Calif). The frozen muscle was sectioned at 10 μm on a cryostat, and the sections picked up on polylysine-coated slides for immunohistochemical staining of myosin isoforms and morphometric analysis. Serial muscle sections were immunostained with anti-MHC antibodies to classify skeletal fibers into 1 of 4 fiber types as detailed previously: type I (slow-contracting), type II (fast-contracting), type I/II hybrid (mixture of fast and slow isoforms), and type neonatal/atrial (containing atypical myosins for adult skeletal muscle).[5,23] For fiber type classification, only tissue sections with good antibody reactions for all myosin isoforms and acceptable morphology were used.

After sectioning, the rest of the muscle samples were processed for gene expression analysis. Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, Calif), treated with DNAse I, and reisolated with RNAqueous (Invitrogen) as described previously.[24] Two muscle samples, from an open-bite subject and a deepbite subject, were selected for microarray analysis based on their diagnoses as prototypical representatives of malocclusion types and fiber type compositions. cDNA libraries of the muscle transcriptome from these

specimens were prepared by reverse transcription of RNA and amplification using a WTA2 TransPlex RNA kit (Sigma-Aldrich, St Louis Mo).

Microarray preparation and analysis were done at the Core Facility of the Fox Chase Cancer Center in Philadelphia, Pa. Aliquots (2 μg) of amplified cDNA were labeled twice with cyanine 3-CTP (cy-3) and cyanine 5-CTP (cy-5) (Genomic DNA Labeling Kit PLUS; Agilent, Santa Clara, Calif) to minimize dye bias. Amounts of 1 μg of labeled cDNA were hybridized on a Whole Human Genome Oligo 4 × 44 K microarray slide (Agilent) for 17 hours at 65°C and washed according to Agilent procedures. One slide contains 4 arrays imprinted with oligonucleotides representing over 41 K genes and transcripts. The hybridized slides were scanned at 5-μm resolution on an Agilent scanner, and images were extracted using Agilent feature extraction software. The data were subjected to background subtraction, log transformation, and normalization using the locally weighted scatterplot smoothing procedure implemented in the feature extraction software before ratios between experimental conditions were calculated. Data from 2 arrays were combined, and an average ratio for every gene was calculated and reported as the processed signal for each labeled sample and the $\log_2$ values of ratios between samples. When thresholds greater than 100 for the processed signal and ±2.0-fold ratio values were set for the normalized data, approximately 7.7 K transcripts remained; this represented about 18% of the array total.

For gene expression studies, masseter samples from 38 subjects were assayed. mRNA for *HDAC4*, *KAT6B*, and the endogenous control gene hypoxanthine phosphoribosyltransferase-1 (*HPRT1*) were quantified in triplicate assays by TaqMan (Applied Biosystems, Foster City, Calif) real-time polymerase chain reaction (RT-PCR) using specific primer-probe sets and RNA-to-C_T 1-step reagent (Applied Biosystems). Message for MHC genes was quantified using custom-designed primer sets.[15] Nomenclature for the MHC genes is different from the protein isoforms, so that the names used here are type I MHC isoform, MYH7 gene; type IIA, MYH2; type IIX, MYH1; type neonatal, MYH8; and type atrial, MYH6. All primer sets spanned exon junctions to prevent erroneous amplification of any residual genomic DNA. An authentic preparation of human skeletal muscle RNA (Ambion; Austin, Tex) was used as a positive control and internal reference standard in the assays. Typically, 15 to 30 ng of masseter muscle RNA was used per assay sample. Amplification efficiencies for all were over 90%, and standard curves were approximately parallel. A 25-ng amount of skeletal muscle standard was used as a reference calibrator. Relative expression quantities of the target genes were calculated using the ΔΔCT method.[25]

Statistical analysis

Malocclusion classification, either skeletal Class II or Class III and skeletal normal, open, or deepbite, were compared for differences in *HDAC4* and *KAT6B* expression. Values for the sagittal groups were compared with a 2-tailed *t* test, and values for the vertical dimension groups were compared with 1-way analysis of variance. A second set of statistical comparisons with the Kendall tau-b test were done for RT-PCR experiments to determine correlations between *HDAC4*, *KAT6B*, and MHC gene expressions. Data for all masseter muscle samples were first pooled to determine general relationships. Samples were then subdivided into the different malocclusion classes to investigate the strength of the epigenetic relationships between subgroups.

RESULTS

Samples from 2 subjects were selected for microarray analysis because their masseter muscle fiber type properties best represented the morphologies of a skeletal open bite and a skeletal deepbite (Table II). The masseter muscle in the subject with a skeletal open bite had

a small proportion of type II fibers, and the muscle in the skeletal deepbite subject had a large proportion of type II fibers (0.6% and 53% tissue occupancy in the samples, respectively). These values conform to the reported type II fiber distributions in masseter muscles from a large population of open-bite and deepbite subjects.[5] Another equally important criterion for this pilot data was that there be sufficient tissue remaining after sectioning for an immunohistochemical analysis to obtain adequate mRNA for microarray experiments. Among the differences in gene expression between subjects, we first compared the contractile protein genes (Table III). Contractile protein categories were organized into 4 functional groups: MHC isoforms, myosin light chain isoforms, myosin binding proteins, and troponins. When the expression values for the deepbite subject were compared relative to the open-bite subject, fast isoforms were expressed at severalfold higher levels than slow types, which were unchanged or down-regulated.

Because fiber type composition has the potential to change with increased muscle activity, we examined whether expression differences for exercise-related genes in the masseter muscles from deepbite and open-bite subjects might accompany the pattern seen for the contractile protein genes.[26] Genes with various functions, whose expressions are reported to change with activity levels, were surveyed from a review of the literature and identified on the microarray. Data from genes with processed fluorescence signals less than 100 were excluded from the analysis to prevent exaggerated expression ratio values. The remaining 24 genes from this survey are shown in Table IV, along with alternate names and values for their expression ratios between deepbite and open bite. Only 7 genes had expression ratios less than 2.0 or greater than 2.0. The gene for solute carrier family-2 type-4 (*SLC2A4*), commonly known as *GLUT-4*, that is an insulin and contraction-regulated facilitated glucose transporter had the lowest expression ratio. The gene for nicotinamide phosphoribosyltransferase (*NAMPT*) that catalyzes synthesis of nicotinamide mononucleotide had the highest expression ratio. Among the other 3 genes with low deep-to-open ratios, variant 7 of peroxisome proliferator-activated receptor alpha (*PPARA*) is a regulator of lipid metabolism, whereas forkhead box O3 (*FOXO3*) and forkhead box O1 (*FOXO1*) regulate transcription in response to oxidative stress. The last 2 genes with high expression ratios have mitochondrial functions that include proton transport for uncoupling protein 3 (*UCP3*) and transcription termination for mitochondrial transcription termination factor (*MTERF*).

We ascertained differences in genes that affect chromatin acetylation/deacetylation. Among the 4 histone acetyltransferase and 11 deacetylase genes on the microarray, deep-to-open expression ratios were greatest for *KAT6B* (2.25) and *HDAC4* (6.96). The other genes had ratio values that either were not ±2.0-fold or were detected at much lower signals than *KAT6B* and *HDAC4*. Therefore, TaqMan RT-PCR assays were done to quantify *HDAC4* and *KAT6B* expression in all masseter muscle samples. Average relative quantity (RQ) values for each gene among all subjects were compared between vertical normal, open, and deep (Fig, *A*), and sagittal Class II and Class III (Fig, *B*) malocclusion groups. RQ values were not significantly different between the vertical groups. However, Class III had significantly greater expression than Class II malocclusion for both *HDAC4* ($P = 0.03$) and *KAT6B* ($P = 0.004$), as determined by a 2-tailed *t* test.

Histomorphologic analyses confirmed that the masseter muscle samples used here had the same characteristic fiber type properties as described in previous studies.[7,11] The number and size of type I fibers were always greatest; on average, the number and size of type II fibers were the smallest and the most variable. RQ values of RNA for individual MHC genes, obtained after the histomorphologic analyses, were summed, and the total values were used to calculate the relative percentage of each gene expressed in extracts of the whole muscle. Correlation analyses between *HDAC4* and *KAT6B* and the percentages of

total expression for each MHC gene showed that *HDAC4* was negatively correlated to *MYH7* expression (P <0.03) and positively correlated to *MYH1* expression (P <0.05) (Table V). There were no significant correlations between the myosin genes and *KAT6B* expression. Analyses were then done to determine whether gene expression values might correlate differently with malocclusion classification. As shown in Table VI, *HDAC4* tended to be negatively correlated with *MYH7* expression and positively correlated with *MYH2*, *MYH1*, and *MYH8* expressions across malocclusion groups. Specifically, *HDAC4* had a significant negative correlation with *MYH7* in Class II malocclusion and significant positive correlations with *MYH1* in Class II malocclusion, and with both *MYH1* and *MYH8* in vertical normal bite malocclusion (P <0.05). In Table VII, the highest correlations for *KAT6B* tended to be negative with *MYH7* and *MYH1* expression and positive with *MYH8* expression. Two high correlations with *MYH2* were negative with open bite and positive with deepbite. Correlation values with *MYH6* expression were all low. Overall, the negative correlation with *MYH1* in sagittal Class III was significant (P <0.05), and the positive correlations with *MYH2* in deepbite and *MYH8* in open bite approached significance, with P values between 0.09 and 0.05.

DISCUSSION

Human masticatory muscle fibers have uncharacteristic phenotypes when compared with other skeletal muscles. This differential fiber composition in masticatory muscles is of continuing interest to both basic and clinical investigators. In masseter muscles, type I fibers have approximately the same fiber size as limb type I fibers,[23] but the type II fibers have unusually small and highly variable fiber areas that constitute small type II motor units.[27] Hybrid fibers that contain variable amounts of type I and type IIA or type IIX MHCs are another unusual fiber type in masticatory muscles. This fiber type is infrequently identified in limb muscle but can constitute up to 25% of fibers in masseter muscles.[23] In studies with many subjects, there are robust statistical associations between variations in type II and hybrid fibers with the development of skeletal open and deepbite malocclusions.[7,11] Functional differences in the fibers provide a mechanism by which the compressive forces of muscle function can influence mechanotransduction and modeling of bone to produce jaw deformation phenotypes.[28] Animal models with electromyography and bone-strain measurements demonstrate that continuous masseter loading of the mandible is almost nonstop and represents the source of low-amplitude, high-frequency forces that influence the morphology and composition of bone.[29] With these relationships well established, we have begun to investigate how differences in gene expression influence facial phenotype and jaw growth.

To simultaneously investigate expressions of muscle gene groups, we did a pilot microarray experiment with 1 open-bite subject and 1 deepbite subject who had characteristic masseter muscle fiber morphology for these malocclusion groups. A hallmark of phenotypic differences is MHC expression levels, since as the main motor enzyme they constitute approximately 25% of total protein content in skeletal muscles.[13] The deepbite subject had type IIA and type IIX MHC gene expressions on the order of 6- to 13-fold greater than type I MHC. Type I had similar expression levels between subjects because it almost always represents the majority of MHCs in the masseter. Other contractile apparatus regulatory proteins that have slow or fast fiber type specific isoforms were also differentially expressed between open-bite and deepbite subjects. Notable among these differences was the fast isoform of myosin binding protein C (a thick filament protein playing an essential role in muscle contraction[30]) that was 13.6-fold greater in the deepbite sample. This overall trend between fast and slow isoform expressions in the pilot data presented here suggests that at the molecular level, differences in muscle contractile protein gene expressions characterize jaw deformation malocclusion in the vertical dimension. To determine whether contractile

protein levels might be influenced by differences in muscle activity, we compared expression differences for 34 genes whose response to exercise induces fiber type changes, mitochondrial biogenesis, and angiogenesis in muscles.[11] Among the genes that we considered, only 7 had gene expression differences ±2-fold, which is the threshold considered to have potential relevance. These included *FOXO1* and *FOXO3*,[31,32] *GLUT 4*,[33] *MTERF*,[34] *NAMPT*,[35] and PPARA.[36] The greatestfold differences for these genes was 3.34, which is much less than the expression differences seen for the contractile and structural protein genes, indicating that differences in exercise levels probably are not the primary epigenetic determinant for contractile protein differences seen in open-bite vs deepbite malocclusion. Not reported here are a larger number of differences at or below ±2-fold, which are not directly related to contractile protein expression or muscle function. Since the microarray comparison was conducted on only 2 subjects, it is valid to report results that were substantiated in conjunction with contractile protein gene expression data. Further microarray experiments are underway with a larger subject population that will validate greater gene expression differences between malocclusion classifications.

An epigenetic mechanism in skeletal muscle has been identified in which enzymatic activities of acetyltransferases and deacetylases affect the acetylation of histones to elicit dynamic changes in chromatin at loci of the MHC genes that alter their expression.[13] Our microarray data from masticatory muscles conforms to this regulatory model because we found *HDAC4* gene expression levels that were 7-fold increased and *KAT6B* levels that were 2.6-fold increased in the deepbite subject compared with the open-bite subject. In the animal model, where muscle unloading experiments were conducted, increases in *HDAC* were associated with transcriptional activation of type II MHC genes.[12] Therefore, epigenetic modification at MHC gene loci in humans might be pivotal in the regulation of masticatory muscle fiber phenotypes. The general trend was for negative correlations for both *HDAC4* and *KAT6B* with type I myosin gene expression and positive correlations for type IIA and neonatal myosin gene expression (Table V). For type IIX myosin gene expression, *HDAC4* was positively correlated; this was similar to the results obtained in the rat limb muscle unloading experiments conducted by Pandorf et al.[12] Correlation values differed somewhat between malocclusion classifications; this suggests that differences in epigenetic regulation might be important in the development of specific types of malocclusion.

Finally, in comparisons of *HDAC4* and *KAT6B* expressions between vertical and sagittal malocclusions, there were statistically significant differences between the Class II and Class III groups but not for the skeletal open, normal, and deepbite groups. In particular, expressions of both *HDAC4* ($P < 0.05$) and *KAT6B* ($P < 0.005$) were significantly greater in Class III than in Class II patients. These differences, especially for *KAT6B*, will require further exploration. Although there is limited information about *KAT6B* effects in humans, its disruption by haploinsufficiency is the underlying cause of Noonan syndrome-like phenotype.[37] This phenotype has numerous skeletal dysplasias, but 1 hallmark is skeletal Class II open-bite malocclusion with increased dental overjet. Recently, new dominant mutations in *KAT6B* have been identified in people with Say-Barber-Biesecker variant of Ohdo syndrome.[38] Although the overall appearance is different from Noonan syndrome-like phenotype, subjects with Say-Barber-Biesecker variant of Ohdo syndrome share the skeletal Class II open-bite malocclusion phenotype. These syndromes emphasize the importance that histone acetylase activities can have on skeletal-based malocclusions in normal subjects. One possible explanation of how *KAT6B* influences mandibular shape is its molecular interaction with the transcription factor *RUNX2*. *KAT6B* has a highly conserved C-terminal domain that is known to bind to *RUNX2*.[39] Endogenous *KAT6B* is required for *RUNX2*-dependent transcriptional activation.[38] *RUNX2*-deficient mice lack mandibular condylar cartilage[40]; after distraction osteogenesis,[41] *RUNX2* expression in the periosteum is essential for bone formation and regeneration.[42,43] Furthermore, it has recently been found that

biomechanical stimulation of osteoblast gene expression requires activation of *RUNX2*.[34] Because *HDAC4* and *KAT6B* can be linked to both myosin gene expression differences and condylar and periosteal growth modifications, they appear to be important epigenetic factors in craniofacial growth.

CONCLUSIONS

1. Expression of the fast isoforms of contractile protein genes were severalfold greater in masseter muscles from a skeletal deepbite orthognathic surgery patient when directly compared with another subject with an open bite. Expression levels of exercise and activity-related genes are relatively consistent between those with open bite and deepbite in this same set of comparisons, suggesting that the differences in muscle fiber properties during the development of a vertical bite malocclusion are probably due to genetic factors other than those that respond to strenuous masticatory function. Also, genes for *HDAC4* and *KAT6B* that regulate histone acetylation to modify chromatin accessibility and transcription were both expressed at levels severalfold greater in the deepbite muscle than in the open-bite muscle, further suggesting that an epigenetic mechanism might participate in a vertical skeletal malocclusion. Independent sets of assays on a patient population show that expressions of both *HDAC4* and *KAT6B* are significantly greater in those with skeletal Class III malocclusion than in Class II malocclusion of the sagittal dimension.
2. The expression of *HDAC4* generally associates negatively with slow type I and positively with fast myosin type IIX gene expression. *KAT6B* associates negatively with type IIX but shows no correlation with slow type I myosin gene expression. The pattern of associations seen here is consistent with an epigenetic model for regulation of MHC expression. Histone deacetylase activity promotes closed chromatin conformations that diminish type I MHC RNA, and histone acetylation drives the opening of chromatin to enhance type IIX RNA.
3. Recent findings in craniofacial syndromes highlight the important influence of *KAT6B* in epigenetic regulation of skeletal growth. It is a potent activator of *RUNX2*, which in turn activates osteoblasts and chondroblasts especially at the condylar level during normal growth and after distraction osteogenesis during bone regeneration. We find that epigenetic regulation through coordinate activities of both *KAT6B* and *HDAC4* might be important in the entire masticatory musculoskeletal complex during the development of a malocclusion.

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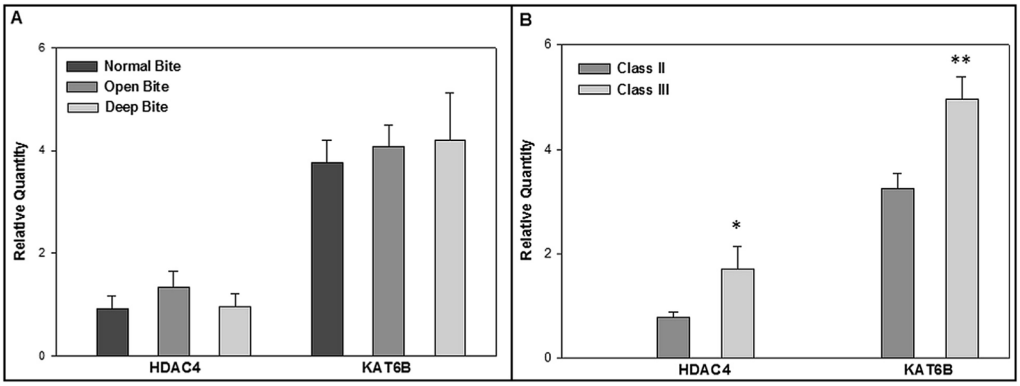

Fig.

Expression of *HDAC4* and *KAT6B* in masseter muscles of subjects with malocclusion: **A**, vertical dimension malocclusion (normal, $n = 12$; open, $n = 18$; deep, $n = 11$); **B**, sagittal dimension malocclusion (Class II, $n = 24$; Class III, $n = 17$). *$P < 0.05$. **$P < 0.005$.

Table I

Distribution of malocclusion types among subjects

				Sagittal class	
	Average age (y)	**Vertical class**	**Total (*n*)**	**Class II**	**Class III**
Female (*n* = 22)	20.2	Normal bite	7	4	3
		Open bite	12	8	4
		Deepbite	3	2	1
Male (*n* = 19)	20.1	Normal bite	5	3	2
		Open bite	6	3	3
		Deepbite	8	4	4

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Table II

Masseter muscle fiber type properties for open-bite and deepbite subjects selected for microarray analysis

	Fiber type							
	I		I/II hybrid		II		Neo/atrial	
Vertical malocclusion	**Open**	**Deep**	**Open**	**Deep**	**Open**	**Deep**	**Open**	**Deep**
Fiber number	136	22	95	7	10	40	29	10
Mean fiber area (μm^2)	2181	3592	828	1017	272	3184	654	1179
Muscle occupancy (%)	61	33	34	9	0.6	53	4	5

Table III

Expression differences in contractile protein genes from masseter muscles of deepbite and open-bite subjects

Description	Symbol	Gene name	Type	Deep:open
Myosin heavy chain	*MYH7* (I)	Heavy polypeptide 7, cardiac, beta	Slow	−1.37
	MYH2 (IIA)	Heavy polypeptide 2, skeletal muscle	Fast	6.54
	MYH1 (IIX)	Heavy polypeptide 1, skeletal muscle	Fast	13.74
Myosin light chain	*MYL2*	Light polypeptide 2, regulatory, cardiac	Slow	−2.07
	MYL1	Light polypeptide 1, alkal, skeletal	Fast	1.84
	MYLPF	Fast skeletal light chain 2	Fast	8.63
Myosin binding	*MYBPC1*	Binding protein C, slow type	Slow	1.29
	MYBPC2	Binding protein C, fast type	Fast	13.64
Troponins	*TNNC1*	Troponin C type 1	Slow	1.36
	TNNI1	Troponin I type 1 (skeletal)	Slow	−1.06
	TNNC2	Troponin C type 2	Fast	2.3
	TNNI2	Troponin I type 2 (skeletal)	Fast	5.39
	TNNT3	Troponin T type 3 (skeletal)	Fast	9.29

Table IV

Detection of -fold change for exercise-related genes in a microarray of masseter muscles from deepbite and open-bite subjects

Gene	Deep:open
SLC2A4 (*GLUT-4*)	−2.79
FOXO3 (*FOXO3A*)	−2.47
PPARA, var 7	−2.19
FOXO1 (*FOXO1A*)	−2.01
PPARGC1A (*PGC-1α*)	−1.91
PPARA var 6	−1.89
ANKRD2	−1.74
TRIM63 (*MURF*)	−1.37
FOXO4 (*MLLT7*)	−1.22
TFAM	−1.14
FBXO32 (*Atrogin-1*)	−1.13
ESRRA	−1.09
ESR1	−1.09
EPAS1 (*HIF2A*)	−1.07
PSMA1	1.07
UBR1	1.17
UCP3 (short var)	1.19
TFB1M	1.2
TFB2M	1.25
SOX6	1.39
NAMPT (*PBEF1*), var 1	1.71
UCP3 (long variant)	2.03
MTERF	2.28
NAMPT (*PBEF1*), var 2	3.34

Table V

Correlation of HDAC4 and KAT6B to MHC gene expression

	Myosin heavy chain gene				
	MYH7	**MYH2**	**MYH1**	**MYH8**	**MYH6**
HDAC4	−0.24*	0.18	0.22*	0.05	−0.05
KAT6B	−0.06	0.01	−0.08	0.14651	−0.0669

*Significant correlation: $P \leq 0.05$.

Table VI

Correlation of HDAC4 to MHC gene expression in malocclusion groups

	Myosin heavy chain gene				
Malocclusion	**MYH7**	**MYH2**	**MYH1**	**MYH8**	**MYH6**
Sagittal					
Class II (*n* = 23)	−0.30*	0.13	0.38*	0.10	0.11
Class III (*n* = 15)	−0.1	0.18	−0.01	0.03	−0.24
Vertical					
Normal (*n* = 11)	−0.45	0.16	0.49*	0.53*	0.09
Open (*n* = 17)	−0.29	0.12	0.25	−0.09	0.07
Deep (*n* = 10)	−0.02	0.24	0.02	−0.02	0.29

*Significant correlation: $P < 0.05$.

Table VII

Correlation of KAT6B to MHC gene expression in malocclusion groups

	Myosin heavy chain gene				
Malocclusion	**MYH7**	**MYH2**	**MYH1**	**MYH8**	**MYH6**
Sagittal					
Class II (*n* = 23)	−0.01	−0.13	0.01	0.22	0.03
Class III (*n* = 15)	−0.07	0.14	−0.39*	0.30	−0.09
Vertical					
Normal (*n* = 11)	0.02	0.13	−0.05	0.13	−0.02
Open (*n* = 17)	0.06	−0.29	−0.13	0.15	−0.07
Deep (*n* = 10)	−0.29	0.33	−0.11	0.07	−0.11

*Significant correlation: $P < 0.05$.