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Molecular motor *MYO1C*, acetyltransferase *KAT6B* and osteogenetic transcription factor *RUNX2* expression in human masseter muscle contributes to development of malocclusion

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ABSTRACT

Objective: Type I myosins are molecular motors necessary for glucose transport in the cytoplasm and initiation of transcription in the nucleus. Two of these, MYO1H and *MYO1C*, are paralogs which may be important in the development of malocclusion. The objective of this study was to investigate their gene expression in the masseter muscle of malocclusion subjects. Two functionally related proteins known to contribute to malocclusion were also investigated: *KAT6B* (a chromatin remodelling epigenetic enzyme which is activated by *MYO1C*) and *RUNX2* (a transcription factor regulating osteogenesis which is activated by *KAT6B*).

Design: Masseter muscle samples and malocclusion classifications were obtained from orthognathic surgery subjects. Muscle was sectioned and immunostained to determine fibre type properties. RNA was isolated from the remaining sample to determine expression levels for the four genes by TaqMan® RT-PCR. Fibre type properties, gene expression quantities and malocclusion classification were compared.

Results: There were very significant associations ($P < 0.0000001$) between *MYO1C* and *KAT6B* expressions. There were also significant associations ($P < 0.005$) between *RUNX2* expression and masseter muscle type II fibre properties. Very few significant associations were identified between *MYO1C* and masseter muscle fibre type properties.

Conclusions: The relationship between MYO1C and KAT6B suggests that the two are interacting in chromatin remodelling for gene expression. This is the nuclear myosin1 (NM1) function of MYO1C. A surprising finding is the relationship between RUNX2 and type II masseter muscle fibres, since RUNX2 expression in mature muscle was previously unknown. Further investigations are necessary to elucidate the role of *RUNX2* in adult masseter muscle.

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1. Introduction

Jaw growth imbalances often require interdisciplinary orthodontic treatment and orthognathic surgery to restore function and aesthetics to the entire masticatory unit. While much is known about the behavioural and environmental contributions to these dysplasias, the genetic aetiology of skeletal growth and adaptation of facial growth patterns remains substantially unknown.[1] A key environmental influence on jaw deformation during growth is masticatory muscle strength, which is determined by the size and proportion of muscle fibre types that associate with vertical growth.[2,3] Sagittal jaw bone length deformations are more closely influenced by genetic variations associated with skeletal tissue growth. This is especially true for mandibular prognathism, which has an autosomal dominant inheritance with incomplete penetrance.[4] Since general heritability estimates for muscle strength and bone length traits in adolescents are at >80%,[5,6] genetic contributions to growth of both jaw bones and muscles are important determining factors and the mechanisms by which deviations in craniofacial morphology develop are complex and require further investigation.

In a cross sectional study, we identified a genetic variation in Myosin 1H (*MYO1H*), which contributes to class III malocclusion due to mandibular prognathism.[7] Unlike Class II myosin heavy chains (MHCs), which are responsible for muscle contraction and are the basis for classification of skeletal muscle fibre types, Class I is an unconventional myosin group of single-headed monomers involved in cellular signalling mechanisms that regulate membrane dynamics, intracellular vesicle transport and auditory mechanotransduction.[8] Eight Class I myosins, designated *MYO1A* to *MYO1H*, are found in humans; some function as tension-sensors that respond to load changes by altering their ATPase activity and mechanical properties, but others have as of yet no known function.[9] Among the Class I myosins, *MYO1C* and *MYO1H* are vertebrate-specific sister paralogs which evolved from a gene duplication event.[10] The molecular functions of *MYO1H* are not known, but there is extensive information for *MYO1C* since it codes for the first single-headed myosin identified in mammals.[11]

The *MYO1C* gene produces three protein isoforms through alternative splicing. Isoforms 1 and 2 can be found in both nuclear and cytoplasmic locations, but isoform 3 is restricted to nuclear functioning only. Isoforms 1 and 2 have been given the name cytoplasmic *MYO1C* protein and isoform 3 the name nuclear myosin 1 (NM1). The proteins have redundancy since *MYO1C* can replace NM1 functioning in the nucleus when NM1 is knocked out in animal experiments.[12] Isoforms 1 and 2 of *MYO1C* regulate glucose uptake via facilitated glucose transporter 4 (*GLUT* 4) in skeletal muscle by acting as a motor for movement of *GLUT4*-stored vesicles to plasma membranes after stimulation with insulin and contraction.[13–15] In microarray experiments we recently found that expression of *GLUT4* is nearly 3× higher in masseter from open-bite compared to deep bite patients.[16] GLUT4 is expressed at higher levels in Type I, slow contracting fibres in human vastus lateralis muscle, but not in soleus or triceps brachii muscles.[17,18] It is possible that both *MYO1C* and *GLUT4* expression in masseter muscle may be increased in skeletal open bite due to elevated levels of Type I fibres, or hybrid fibres which express some Type I myosin in addition to other myosin heavy chain isoforms.

NM1 performs a separate and critical role in activation of transcription in the nucleus.[19] NM1 participates in the formation of the multi-protein assembly B-WICH, which is comprised of the William syndrome transcription factor complex (WSTF), Cockayne syndrome group B protein (CSB) and NM1, that is necessary for chromatin remodelling.[20] In this process nucleosomes are repositioned which leads to their binding with histone acetyltransfeases (HATs). The HATs confer transcriptional specificity since they function as active gene promoters.[21] Using RT-PCR we recently found that expression of a HAT, K(lysine) acetyltransferase 6B (*KAT6B*), positively correlates with mandibular prognathism, and with Class II myosin heavy chain (MHCs type IIA and IIX) expression in masseter muscle.[16] Increased expression of these fast-twitch type II MHC isoforms and type II skeletal muscle fibres enhances masticatory strength, which contributes to the development of deep bite malocclusion by decreasing vertical growth of the face.[2,3] The association of *KAT6B* with mandibular prognathism could be related to its activation of the osteogenic transcription factor *RUNX2*[22] which is required for mandibular condylar cartilage growth.[23]

Given the importance of these genetic and epigenetic influences on sagittal and vertical jaw growth, we compared gene expression of *MYO1H*, *MYO1C*, *KAT6B* and *RUNX2* in masseter muscle to malocclusion classification and muscle fibre type distribution.

2. Materials and methods

2.1. Patient population and surgical procedure

Recruitment was from orthodontic patients undergoing orthognathic surgery at the *Hôpital Roger Salengro, Service de Chirurgie Maxillo-Faciale et Stomatologie at the Centre Hospitalier Universitaire de Lille* in Northern France. Subject participation was in accordance with the research ethics committee's approval at Temple University and at the University of Lille. Masseter muscle samples were obtained from 28 females and 21 males (average age 22 yrs) undergoing the sagittal split procedure. The surgeries were performed by two surgeons, the Department Head and the Graduate Program Director for Maxillofacial Surgery. Surgical procedures for all subjects in this study included at least a mandibular bilateral sagittal split osteotomy using Epker's technique. This osteotomy separates the ascending branch of the mandible from the dental arch and mandibular body. The Epker technique uses structural elasticity to split the bone through the bony channel of the inferior alveolar nerve. The technique is advantageous since during the split, the inferior alveolar nerve and blood vessels are visualized and protected to avoid damage, which would affect chin and lip sensation. The bony separation is performed with a Tessier distractor in order to drive the split by using bone flexibility, which assures a more accurate and consistent sagittal split. At separation the deep portion of the masseter muscle is exposed, and some muscle fibres are