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Masseter Myosin Heavy Chain Composition Varies With Mandibular Asymmetry

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Abstract

Human jaw dysmorphologies are frequent and often affect young patients, resulting in malocclusion of teeth and inappropriate jaw relationships. Treatment is performed by means of orthodontics with orthognathic surgery as required. Mandibular asymmetry is one of the most frequent dysmorphologies, but in many cases, the specific cause is unknown.

In healthy patients who were undergoing orthognathic surgery for correction of malocclusion, we tested the hypothesis that masseter muscle phenotype composition, which determines contractile properties, was different between sides in patients with mandibular asymmetry but not in those without mandibular asymmetry.

After cephalometric analysis, 50 patients from whom we obtained samples of both right and left masseter muscles were separated into 2 groups: with or without mandibular lateral deviation. Samples were immunostained with myosin-isoform-specific antibodies to identify 4 skeletal

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muscle fiber types, and their fiber areas and proportions were measured. Two-tailed Wilcoxon test for paired samples was used to compare the 4 fiber-type compositions by means of percent occupancy and mean fiber area on both sides.

Patients with mandibular asymmetry were associated with a significant increase of type II fiber occupancy ($P = 0.0035$) on the same side as the deviation.

This finding that masseter muscle phenotype is significantly linked to mandibular asymmetry is of relevance to physiotherapeutic and surgical managements of jaw discrepancies and merits further investigation in the light of its possible role in the etiology of this condition.

Keywords

Myosin heavy chains; masseter muscle; human; malocclusion; plasticity; immunohistochemistry; asymmetry

Jaw Dymorphologies

Human jaw dymorphologies are frequent in the general healthy population and often affect young patients. The clinical presentation is expressed by malposition of teeth and sometimes inappropriate jaw relationships. Treatment of severe cases of jaw dymorphologies is performed by means of orthodontics in combination with orthognathic surgery.

Mandibular Asymmetry

Mandibular asymmetry is one of the most frequent jaw dymorphologies^{1,2} and may result from congenital malformations of genetic origin, irregular variations in growth and development, fractures, tumors, or soft tissue atrophy or hypertrophy. Symmetry is defined by the correspondence in size, shape, and location of facial landmarks on both sides of the median sagittal plane. The origin of mandibular asymmetry may involve either bone growth (overgrowth and undergrowth³) or muscle growth and function.^{4,5} Mandibular asymmetry is important because of its effect on facial appearance and, to some extent, function (different motion patterns between 2 sides). Mandibular asymmetry is also reported to be commonly associated with internal derangements of the temporomandibular joint, although the causal relationship between them remains unclear.^{6,7}

Muscle Phenotypes

Muscles express different sarcomeric proteins that determine different contractile properties. These may change with age, hormonal status, some pharmacologic treatments, training status, innervations, and activity patterns. Muscle plasticity is widely illustrated in the literature with implications for pathology and physiology.^{8–10}

Human masticatory muscles express a large variation in phenotype, especially in their myosin heavy chain (MHC) composition.^{11,12} Variations are quite large, both between individuals and in different areas of the same masseter.^{13,14} Muscle fiber phenotypes may be adequately assessed by ATPase staining in limb muscle, which contains only the 3

commonest MHC isoforms (I, IIA, IIX); however, in masseter that contains additional isoforms, immunostaining is needed to characterize the additional fiber types.^{15–17}

In a previous study, we found significant relationships between anterior facial height and fiber type II myosin isoform in the masseter of orthognathic surgery patients,^{16,18} indicating an association between masseter composition and craniofacial morphology (especially the vertical dimension).

Statement of the Problem

In a significant number of patients with craniofacial dysmorphologies, the cause of the condition is unknown. Bone is readily remodeled during growth in response to a number of factors, which can include muscle action, as encapsulated in the Wolfe law.¹⁹ Skeletal muscle is also able to adapt its phenotypic properties in relation to conditions such as unloading.^{20,21} It is possible that both contribute to the development of craniofacial dysmorphologies, possibly at different stages of their development. A better understanding of the relationship between craniofacial dysmorphologies and muscle phenotype should help us improve the clinical management of jaw discrepancies and malocclusion, especially with regard to postsurgical therapies to avoid relapse.

In this study, we looked for evidence of any clear association of masseter muscle phenotype with mandibular asymmetry by comparing left and right side samples of masseter from orthognathic surgery patients, 24 with mandibular asymmetry and 26 without mandibular asymmetry.

MATERIALS AND METHODS

Patient Selection

In our study, we analyzed left and right masseter muscle samples from 50 patients treated for orthognathic surgery after presurgical orthodontic treatment was completed. None of these patients had a history of injury, tumors, overgrowth/undergrowth in the condyle, internal derangement of the temporomandibular joint, or known congenital disease. Only patients with mandibular bilateral sagittal split osteotomy performed to correct sagittal, midline, or vertical discrepancies of the mandible were included. During the surgical osteotomy, the periosteum is separated from the bone; in addition, we partially cut the masseter and pterygoidian aponeurosis.^{22,23} To stop bleeding and before closing, we removed some fragments of the deep portion of the masseter muscle in this specific area (the same on both sides, as determined by the surgical procedure). These tissue pieces were usually evacuated using suction drain or vacuum, and in that situation, we collected them for analysis. This is considered waste tissue, and permission to use it for analysis was obtained from the local research ethics committee (reference no. CP 03/36). The patients' consent was also obtained for this, and patient list was declared to the board that enforces the law on data protection.

Muscle Samples

The masseter samples were then sent to the pathology department, which gave a reference number to the biopsy to ensure patient anonymity during its subsequent processing. Masseter samples were snap frozen and kept at -80°C until they could be cryosectioned for fiber-type composition characterization.

Immunostaining

Immunostaining analysis was carried out as described previously.¹⁶ Immunostaining used 5 antibodies (Table 1). Eight fiber types were identified for each sample: types I, IM, IIC, IIA, and IIX, neonatal, atrial, and other. Those 8 fiber types were merged into 4 major phenotype categories (and shown in Table 2):

- Type I
(fiber type I): slow fibers
- Type hybrid
(fiber types IM and IIC): intermediate fibers
- Type II
(fibers types IIA, IIX and IIAx): fast fibers
- Type neoatrial
(hybrid fibers containing neonatal and/or atrial MHC in addition to the other MHC isoforms).

For each of these types, the relative number of fibers and fiber areas were measured, and mean fiber area and percent occupancy were calculated as described previously.¹⁶ Percent occupancy of a fiber type was calculated from the mean fiber area and relative number of fibers of a specific fiber type within each biopsy. This represents the fractional area of the sample occupied by that fiber type.

To ensure the same technical conditions throughout the study, immunostaining was performed by the same team in the same laboratory, always using internal reference samples (human limb muscle) to verify antibody specificity.

Diagnosis of Malocclusion and Mandibular Asymmetry

Diagnosis was performed by clinical examination, dental study models, and radiographic analysis of lateral, axial, and Sub-Mento-Vertex cephalograms and panoramic radiographs. Surgical treatment planning was done using a computer-assisted cephalometric analysis from Delaire (Tridim and Orqual ceph, Orthalis). An orthodontic classification of these patients using a WITS analysis for class³¹ and maxillomandibular plane angle for vertical bite³² is summarized in Table 3.

Mandibular asymmetry was identified by measuring the deviation of the midpoint of the mandibular central incisors, using as the vertical reference a line through the crista galli and the superior aspect of the nasal septum, drawn perpendicular to the line between the

intersections of the greater wing of the sphenoid bone and the lateral margin of the orbits (Fig. 1). Changes in tooth position achieved purely as part of presurgical orthodontic treatment were ignored.

Patients were included in the asymmetric group if the horizontal deviation in the midpoint of the mandibular central incisors was 2 mm or greater relative to the vertical reference line. These patients all had a larger skeletal asymmetry of the mandible, in which a shorter and a longer side could be identified, when examining the position of the gonial angles relative to the horizontal reference line (Fig. 1). The mandibular dental asymmetry deviated to the shorter side of the mandible, and these patients would therefore be classified as being in group A subtype of facial asymmetry as defined by Hwang et al.³³

Using this criterion, the patients were separated in 2 groups: with or without mandibular asymmetry. During mandibular surgery, repositioning of the incisor midline was performed for all the patients belonging to the deviated group.

Statistical Treatment

Two-tailed Wilcoxon tests for paired samples were used to compare the 4 fiber-type compositions by mean fiber area and percent occupancy in the asymmetric ($n = 24$) and symmetric ($n = 26$) patient groups. $P < 0.01$ was taken as the threshold for statistical significance of differences between groups.

RESULTS

Of the 50 patients, 26 had no asymmetry and 24 had mandibular asymmetry. Table 3 shows the distribution of dysmorphologies in these patients.

The asymmetric group had a median age of 27.5 years at the time of the surgery and included 13 women and 11 men. The symmetric group had a median age of 24.7 years at the time of the surgery and included 17 women and 9 men.

The fiber type data from analysis of masseter samples are shown in Table 4. In the asymmetric patient group, fiber type data were pooled so that samples from the “short” mandibular side (toward the deviation) could be compared against the “long” side (opposite to the deviation). In this group, there was a significant increase in type II fiber occupancy ($P = 0.0035$) on the same side as the deviation, that is, on the short side. Other fiber types showed no differences.

No significant differences were seen between left and right side samples in the symmetric group (Table 4). Only a trend was present concerning type II fibers mean area of the asymmetric group, with an increase of the mean area correlated to an increase of the percent occupancy.

DISCUSSION

In the current study, we found for the first time a relationship between mandibular asymmetry and the occupancy of type II fibers in masseter muscle in patients undergoing

surgical correction of various malocclusions. This finding is potentially clinically significant because the mandibular asymmetry seems to be associated with a significant modification in masseter fiber-type composition, independent of the other coexisting discrepancies (vertical, anteroposterior, and mixed). If the association is causal, with increased type II fiber reflecting a difference in the muscle activity of the affected side, this might offer an opportunity for functional treatment of jaw discrepancies for instance by means of botulinum toxin therapy.^{4,34}

An important contributor to the significant increase in percent occupancy of type II fibers on the short side of the asymmetric group was the increase in mean fiber area of the type II fibers, as shown in Table 4 (although the *P* value for difference between mean fiber area was 0.079, ie, less significant than that for the occupancy). Thus, the increase of the percent occupancy of the fibers type II on the short side of the asymmetric group is associated mainly with an increase of the mean area of the type II fibers in the asymmetric group.

Origin of Asymmetry

In the craniofacial context, we consider symmetry to be the reference (normal) condition, and one of the aims of orthodontic treatment is to restore it. However, there is often a preference for one side during mastication; in addition, emotional expression is also described as being asymmetric.^{35–37}

If there is no discrepancy in the cranial base and maxilla, craniofacial asymmetry indicates an isolated lateral deviation of the mandible. In that situation, different causes are possible: mandibular condyle overgrowth/undergrowth, mandibular tumors, trauma, and malformations.³⁶ If these causes can be excluded, variations in tooth positions, bone or muscle growth, or functional preferences for one side may contribute, individually or in combination, to the development of mandibular asymmetry. Our results clearly demonstrate that there is a muscle phenotype difference associated with mandibular asymmetry.

Study Population and Classification of Asymmetry

The patients described here are from a larger study population of 180 subjects undergoing surgical treatment of malocclusion. This larger study population has a median patient age of 24 years (range, 15–65 y), consisting of 112 women (62%) and 68 men (38%). Nearly half of the patients (*n* = 84) in this population had mandibular asymmetry (46.6%).

In the 50 patients from whom we were able to analyze both left and right masseter samples, the incidence of lateral deviation was nearly the same: 24 patients with asymmetry (48%).

In our study, we considered mandibular asymmetry was present only if the incisor midline deviation represented approximately half a mandibular incisor width (2 mm). We choose this value because with such a deviation, traditional orthodontic treatment alone cannot easily correct the misalignment, and this is one of the criteria justifying mandibular repositioning by means of surgery.

There are various kinds of craniofacial asymmetry,^{33,38} but our study describes patients with asymmetry limited only to the mandible. In addition, our patients were characterized as

having the same type of mandibular asymmetry in which the midline structures were deviated toward the short side of the mandible. Patients with condyle hypertrophy or osteoarthritis or internal derangement of the temporomandibular joint were not included. We assume, therefore, that the asymmetry in mandibular form in these patients occurred as a result of essentially normal remodeling processes during growth. This study allowed us to examine the relation of mandibular form to masseter muscle composition, but it does not a priori show if the change in muscle was a causal factor of the altered skeletal growth or was an adaptation to different bone growth and shape.

Muscle Phenotypes

It is known that masseter volume does not correlate with asymmetry,^{35,39,40} but there are a few indications in the literature that the composition of the muscle in the fiber phenotypes does show some association with alterations in craniofacial form. Human masseter shows large variations in myosin isoform composition, both between individuals and between different portions of the muscle.^{14,41} This means that, for cross-sectional studies (such as this one), examining the relation of muscle composition with craniofacial form, a large sample population is needed to obtain significant results, and the sample must be taken from the same position within the muscle (which was the case in our study).

Conventional myosin ATPase activity does not allow definition of all the masseter muscle fiber phenotypes with sufficient accuracy, so immunostaining is preferred for the investigation of masseter muscle phenotype variations in craniofacial discrepancies. The advantage of using a myosin-based method is that the myosin isoforms are good markers of contractile performance of muscle fibers.⁴²

Our study shows that mandibular asymmetry is significantly linked to type II (fast) fiber percentage occupancy and effect was mainly due to an increase in the relative mean area of fast fibers. This finding has to be considered in the light of surgical management of jaw discrepancies (especially during postsurgical rehabilitation) and a possible contribution to the incidence of relapse. On another hand, one may advocate the possibility to modulate muscle phenotype during growth of asymmetric patient and avoid discrepancy such asymmetry.

Both in this study and in our previous study,¹⁶ it was the type II (fast) fiber population that was significantly altered in relation to the craniofacial form. In the previous study, type II fiber occupancy was increased in patients with deep bite (reduced anterior facial height), and in the patients with mandibular asymmetry described here, it was increased on the side of the deviation (as considered as the short side). This is consistent with the idea that the muscle in such cases was developing more force because type II fibers (in larger motor units) are normally recruited principally at higher contraction strengths^{41,43} and that this may have influenced mandibular form. The opposite condition, jaw muscle weakness, is known to predispose toward open bites (increased vertical dimension). We cannot, however, exclude the possibility that the change in the muscle was an adaptation to the skeletal discrepancy. In this context, it would also be interesting to determine whether the difference in muscle phenotype is corrected after surgical correction of the mandibular asymmetry.

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Figure 1.

Measurement of mandibular asymmetry. The horizontal and vertical reference lines are shown on the main image (as seen in a plane angle cephalogram), and the insert shows how the lateral discrepancy was obtained. This situation is typical of our patient group, in which the mandible is shorter on the side of the deviation (see text). The drawing was obtained by contouring the patient's postero-anterior cephalogram.

TABLE 1

Origin and Specificity of Antibodies Used for Immunostaining

Antibody (Company)	Host Species	Identified Myosin Isoform(s)	Identified Fiber(s)	References
MY-32 (Sigma)	Mice	IIA, IIB, IIX	IIA, IIB, IIX, IIAX	Naumann and Pette ²⁴ and Sciote et al ¹¹
BA-F8 (DSM Germany)	Mice	Slow	I	Borrione et al ²⁵
SC-71 (DSM Germany)	Mice	IIA	IIA	Schiaffino et al ²⁶ and Gorza ²⁷
Antiatrial MAS 366 (Sera Lab, UK)	Mice	Atrial (α -cardiac)	Atrial	Sciote et al ¹¹ , Sciote and Kentish, ²⁸ and Leger et al ²⁹
Antineonatal (polyclonal, gift)	Rabbit	Neonatal	Developmental	Scapolo et al ³⁰

TABLE 2

Fiber Type Classification by Means of Immunostaining Intensity Reaction

Antibody Name	Myosin HC Isoform(s) Recognized	Reference for Source and Supplier	Immunoreaction With Fiber Phenotypes in Masseter						
BAF8	I (beta)	Borrione et al ²⁵ ; DSM	✓	✓	(✓)	–	–	*	*
SC71	IIA	Schiaffino et al ²⁶ ; DSM	–	*	*	✓	–	*	*
MY32	IIA, IIX, and neonatal	Naumann and Pette ²⁴ ; Sigma	–	(✓)	✓	✓	✓	*	✓
Anti-neo	Neonatal	Scapolo et al ³⁰ ; gift	–	–	–	–	✓	–	✓
Anti-alpha	Atrial	Leger et al ²⁹ ; SeraLab	–	–	–	–	–	–	✓
Individual fiber types identified:			I	IM	IIC	IIA and IIX	IIX	Neo	Atrial [†] Neoatrial [†]
Type groups for statistical analysis:			I	I/II hybrid	II				

Intensity reaction ranges from strong to absent. Every fiber type is defined associating different levels of staining intensity for each antibody. *May be positive or negative. †These types usually contain at least one other of the isoforms I, IIA, IIX. ✓ indicates strong reaction; (✓), weak reaction; –, negative.

TABLE 3

Distribution of Dysmorphologies in the Patient Group Defined by Using WITS Analysis for Class (I, II, or III) and Maxillomandibular Plane Angle for Vertical Bite (Open, Normal, or Deep)

	Class I A/S	Class II A/S	Class III A/S	All A/S
Open	3/0	5/6	3/8	11/14
Normal	0/1	3/2	5/1	8/4
Deep	0/1	4/5	1/2	5/8
All A/S	3/2	12/13	9/11	24/26

A indicates asymmetric; S, symmetric.

Values for Mean Fiber Area and for Percent Occupancy for the Main Muscle Fiber Types in Masseter on the 2 Sides in Symmetric and Asymmetric Cases

TABLE 4

Parameter	Symmetric (n = 26)				Asymmetric (n = 24)			
	Right		Left		Long Side		Short Side	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mean fiber area (μm^2)								
Type I	2256	745.1	2203	626.4	2277	930.4	2333.6	780.2
Hybrid (I/II) fibers	1601.7	559.4	1527.7	401.8	1524.6	708.6	1619.3	637.6
Type II	889.5	704	912.6	513	791.2	613.6	1030.1	663.1
Neotrial	899.8	579.2	962.6	583.9	996.3	558.6	962.7	346
Percent occupancy								
Type I	60	17.8	61	16.3	60.9	16.8	58	16.8
Hybrid (I/II) fibers	20	18.3	16.8	14.9	16.3	14.5	12.5	12.1
Type II	13.3	17.3	12.6	17.5	10.6*	13.3	18.4*	15.9
Neotrial	6.7	9	9.6	10.5	12.2	13.1	11.1	10.1

* A significant difference ($P = 0.0035$) was found between sides for the percentage occupancy of type II fibers (short side has larger occupancy).