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Expression of myosin heavy chain isoforms mRNA transcripts in the temporalis muscle of common chimpanzees (*Pan troglodytes*)☆

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ABSTRACT

Purpose: The common chimpanzee (*Pan troglodytes*) is the primate that is phylogenetically most closely related to humans (*Homo sapiens*). In order to shed light on the anatomy and function of the temporalis muscle in the chimpanzee, we have analyzed the expression patterns of the mRNA transcripts of the myosin heavy chain (MyHC) isoforms in different parts of the muscle.
Basic procedures: We dissected the superficial, deep and sphenomandibularis portions of the temporalis muscle in five adult *P. troglodytes* and quantified the expression of the mRNA transcripts of the MyHC isoforms in each portion using real-time quantitative polymerase chain reaction.
Main findings: We observed significant differences in the patterns of expression of the mRNA transcripts of the MyHC-IIM isoform between the sphenomandibularis portion and the anterior superficial temporalis (33.6% vs 47.0%; P = 0.032) and between the sphenomandibularis portion and the anterior deep temporalis (33.6% vs 43.0; P = 0.016). We also observed non-significant differences between the patterns of expression in the anterior and posterior superficial temporalis.
Principal conclusions: The differential expression patterns of the mRNA transcripts of the MyHC isoforms in the temporalis muscle in *P. troglodytes* may be related to the functional differences that have been observed in electromyographic studies in other species of primates. Our findings can be applicable to the fields of comparative anatomy, evolutionary anatomy, and anthropology.

1. Introduction

The temporalis, masseter, and medial pterygoid muscles are the jaw-closing muscles functioning in occlusion of the mandible and other movements associated with chewing and biting (Taylor and Vinyard, 2013). The temporalis arises from the side of the skull and inserts onto the coronoid process of the mandible and the anterior edge of the mandibular ramus (Aiello and Dean, 1990). In the common chimpanzee (*Pan troglodytes*), the temporalis comprises a superficial and a deep portion that can be easily separated (Aiello and Dean, 1990; Oxnard and Franklin, 2008). In contrast, in humans (*Homo sapiens*), the temporalis has the equivalent of the deep temporalis in *P. troglodytes*, while the superficial temporalis is either completely lacking or only vestigially present (Oxnard and Franklin, 2008; Lee et al., 2012). This loss of the superficial temporalis in humans is a result of the general reduction in the masticatory apparatus that occurred in the evolution of the genus *Homo* (Aiello and Dean, 1990), which may in turn be due to a relatively soft diet (Oxnard and Franklin, 2008).

Some authors make a distinction between the sphenomandibularis and the temporalis muscles in humans and consider the sphenomandibularis to be a separate muscle arising from the maxillary surface of the sphenoid bone and inserting on the temporal

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crest of the mandible (Dunn et al., 1996). Others, however, consider the sphenomandibularis to be a part of the temporalis, without its own specific vascularization and innervation (Türp et al., 1997; Shimokawa et al., 1998; Schön-Ybarra and Bauer, 2001; Geers et al., 2005; Sedlmayr et al., 2009). In non-human primates, such as the *Macaca mulatta*, the sphenomandibularis has been described as part of the deep temporalis (Skinner and Aziz, 2003).

Electromyographic studies of the temporalis in *H. sapiens* and in the non-human primates *Macaca fuscata* and *Papio anubis* have found that the temporalis acts in the occlusal phase of the masticatory cycle, with different fibers acting at different points of the cycle (Blanksma et al., 1997; Hylander et al., 2005; Wall et al., 2008). The contraction of the anterior fibers, which run vertically, results in elevation of the mandible, closing the mouth. The anterior fibers reach their peak of maximum activity during the power stroke (Blanksma et al., 1997; Hylander et al., 2005; Vinyard et al., 2008; Vinyard and Taylor, 2010). The posterior fibers run horizontally and their contraction retracts the mandible, pulling the jaw posteriorly. The posterior fibers act during a later phase of the power stroke in which the mandible moves laterally to reposition the molars (Ahlgren et al., 1985; Blanksma et al., 1997; Hylander et al., 2005; Wall et al., 2008; Williams et al., 2011). The consistency of the food being chewed also influences which muscle fibers are called into play (Grünheid et al., 2009). In *M. fuscata*, *P. anubis*, and *H. sapiens*, more muscle fibers in the anterior than in the posterior temporalis are used when chewing tough food (Blanksma et al., 1997; Ross and Hylander, 2000; Hylander et al., 2005; Vinyard et al., 2008). In *P. anubis*, the increase in electromyographic activity in the anterior region of the temporalis occurred primarily in the superficial temporalis, which has a greater capacity to produce force (Wall et al., 2008). However, when chewing relatively softer food, there was greater electromyographic activity in the posterior temporalis in *M. fuscata* and *P. anubis* (Hylander et al., 2005; Vinyard et al., 2008). In electromyographic studies in *H. sapiens*, the sphenomandibularis portion of the deep temporalis worked together with the lateral pterygoid muscle to produce lateral movements of the mandible and also helped maintain the mandible in a stable position (Wood, 1986; Fuentes et al., 2012).

The functional characteristics of the temporalis can also be analyzed in terms of the expression of the myosin heavy chain (MyHC) isoforms (Bottinelli and Reggiani, 2000). Different types of muscle fibers have different contractile properties (force, contraction velocity, and resistance to fatigue) that are related to the expression patterns of MyHC isoforms (Pette and Staron, 2000; Bottinelli et al., 1996; Harridge et al., 1996). The main MyHC isoforms expressed in the skeletal muscles of mammals are MyHC-I, MyHC-IIA, and MyHC-IIX (Sciote and Morris, 2000). The MyHC-I isoform is mainly expressed in slow-twitch oxidative fibers and is the predominant isoform in slow-twitch (type I) muscles (Schiaffino and Reggiani, 2011), which are characterized by low force production and high resistance to fatigue. The MyHC-IIA and MyHC-IIX isoforms are primarily expressed in fast-twitch oxidative glycolytic fibers and fast-twitch glycolytic fibers, respectively, and are the predominant isoforms in fast-twitch (type II) muscles (Schiaffino and Reggiani, 2011). Type IIX fibers are characterized by very high force production and low resistance to fatigue, while type IIA fibers fall in between the type I and type IIX fibers. In addition to the MyHC-I, MyHC-IIA, and MyHC-IIX isoforms, the masticatory muscles of non-human primates also express the MyHC-IIM isoform in type IIM muscle fibers (Rowlerson et al., 1983), which have a moderate contraction velocity, similar to that of the type IIA fibers, and a high force production, greater than that of the type IIX fibers (Hoh, 2002; Toniolo et al., 2008).

The composition of muscle fibers and the expression patterns of the MyHC isoforms in the temporalis muscle of non-human primates have been studied by histochemistry in *M. mulatta* (Maxwell et al., 1979; Miller and Farias, 1988) and *Cebus apella* (Andreo et al., 2002), by immunohistochemistry in *Macaca irus* (Rowlerson et al., 1983), and by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in *P. anubis* (Wall et al., 2013). Using ATPase staining, Miller and Farias (1988) found that the temporalis muscle of *M. mulatta* had 47.9–58.5% of type IIX fibers, 27.3–42.6% of type IIA fibers, and 6.6–21.3% of type I fibers. When they compared the anterior and posterior superficial temporalis, they found no significant differences in the percentage of type IIA and IIX fibers, while the percentage of type I fibers was greater in the anterior than in the posterior superficial temporalis (13.1% vs 6.6%). They also found that the posterior superficial temporalis had a lower proportion of type I and IIA fibers (6.6% and 34.9%, respectively) than the posterior deep temporalis (9.5% and 42.6%, respectively). Maxwell et al. (1979) also used ATPase staining to study the temporalis muscle in *M. mulatta* and found a higher proportion of type I fibers in the anterior than in the posterior superficial temporalis (49.95% vs 20.65%). In contrast, Andreo et al. (2002), using ATPase staining to study the *C. apella*, found no significant differences between the anterior and posterior superficial temporalis. Importantly, none of these studies examined type IIM fibers, which are the most frequent type in the temporalis of non-human primates (Rowlerson et al., 1983). Using ATPase staining together with indirect immunoperoxidase staining, Rowlerson et al. (1983) found that the central temporalis in *M. irus* had 86% type IIM fibers and 14% type I fibers. A study using SDS-PAGE to analyze the temporalis in *P. anubis* found 87–88.7% expression of the MyHC-IIM isoform in the anterior and 85.2–90.8% expression in the posterior superficial temporalis, while the MyHC-I isoform was the only other isoform expressed (Wall et al., 2013). In the deep temporalis, the MyHC-IIM isoform was expressed at 60.7% in males and 14.4% in females, while the MyHC-IIA isoform was expressed at less than 5% and the MyHC-I isoform at 38.1% in males and 83.3% in females (Wall et al., 2013).

To the best of our knowledge, no electromyographic or molecular studies have examined the temporalis muscle in *P. troglodytes*, which, together with *Pan paniscus*, is the primate that is phylogenetically most closely related to *H. sapiens*. In order to shed further light on the anatomy and function of the temporalis muscle in the common chimpanzee, we have dissected the temporalis muscles of five adult chimpanzees and quantified the mRNA transcripts of the MyHC-I, MyHC-IIA, MyHC-IIX, and MyHC-IIM isoforms by real time quantitative polymerase chain reaction (RT-qPCR) in the anterior and posterior portions of the superficial and deep temporalis as well as in the sphenomandibularis portion of the temporalis. Our primary objective was to identify differences in the expression patterns of the MyHC isoforms among the different parts of the temporalis muscle, especially in the sphenomandibularis portion, which could be associated with the functional differences identified by electromyographic studies in humans and other primates.

2. Material and methods

2.1. Muscle samples

A total of five adult *P. troglodytes* were included in the study — two males and three females. All five chimpanzees came from Spanish zoos and had died of causes unrelated to the present study. All bodies had been cryopreserved without chemical fixation within 24–48 h after death. We dissected the right temporalis muscles of four of the chimpanzees and the left temporalis muscle of one female chimpanzee (specimen 03), since the right temporalis of this female had been damaged in a prior necroscopy. All dissections were performed by the same investigator at the Anatomy Museum of the University of Valladolid (Valladolid, Spain) (Table 1).

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