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Absence of Developmental and Unconventional Myosin Heavy Chain in Human Suprahyoid Muscles

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Abstract

Introduction—Contradictory reports of the myosin heavy chain (MHC) composition of adult human suprahyoid muscles leave unresolved the extent to which these muscles express developmental and unconventional MHC.

Methods—By immunohistochemistry, separation SDS-PAGE-Coomassie, separation SDS-PAGE-Western blot, and mRNA PCR, we tested for conventional MHCI, MHCIIA, MHCIIX, developmental MHC embryonic and MHC neonatal, and unconventional MHC alpha-cardiac, MHC extraocular, and MHC slow tonic in adult human anterior digastric (AD), geniohyoid (GH) and mylohyoid (MH) muscles.

Results—By separation SDS-PAGE-Coomassie and Western blot only conventional MHC are present. By immunohistochemistry all muscle fibers are positive for MHCI, MHCIIA, or MHCIIX, and fewer than 4 fibers/mm^2 are positive for developmental or unconventional MHC. By PCR, mRNA of MHCI and MHCIIA dominate, with sporadically detectable MHC alpha-cardiac and without detectable mRNA of other developmental and unconventional MHC.

Discussion—We conclude that human suprahyoid muscles AD, GH and MH are composed almost exclusively of conventional MHC isoforms.

Keywords

Suprahyoid; swallowing; myosin heavy chain; human; muscle

INTRODUCTION

Dysphagia is estimated to affect at least 6% of the aged population, although the incidence may be substantially greater.[1,2] Although the basis for dysphagia with aging is likely multifactorial, dysfunction of head and neck muscles is considered to be a contributing factor.[3] These muscles are similar to appendicular skeletal muscle, but some have suggested that their unique mechanical demands, innervation, and developmental origin may impose similarly unique functional and phenotypic patterns. Knowledge of adult human suprahyoid

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myosin heavy chain (MHC) composition can thus provide a baseline to establish pathological alterations of MHC with aging and disease.

The extrafusal fibers of adult human appendicular muscles are composed exclusively of conventional MHC isoforms MHCI, MHCIIA, and MHCIIX. Adult human head and neck muscles express conventional MHC (CON), but they may also express developmental MHC [MHC embryonic (MHCemb), MHC neonatal (MHCneo)] and unconventional MHC [MHC alpha-cardiac (MHCac), MHC extraocular (MHCeom), MHC slow tonic (MHCst), MYH15].[4,5,6] Expression of developmental and unconventional MHC in human head and neck muscles may be related to activation during behaviors with diverse kinematic requirements and to embryonic origin from branchiomeric somitomeres which differ from post-cranial muscles in muscle-specific transcription factors [4,7,8,9,10].

Expression of developmental MHC (DEV) and unconventional MHC (UNCON) is most clearly documented in human extraocular (EOM) and masticatory muscles. In addition to CON, human EOM contain MHCac, MHCemb, MHCeom, MHCneo, MHCst, and the recently identified MHC MYH15.[5,6] Human masticatory muscles, masseter and lateral pterygoid, contain MHCI, MHCIIA, MHCIIX, MHCneo, and MHCac with up to 52% hybrid fibers and as many as 5 MHC expressed in single fibers.[11,12]

The human suprahyoid muscles anterior digastric (AD), geniohyoid (GH), and mylohyoid (MH) are active during swallowing, oral transport, coughing, emesis, and speech. They develop from branchiomeric (AD, MH) or occipital somitic sources (GH)[10] and thus might be expected to express appreciable DEV and UNCON. Initial IHC and separation SDS-PAGE studies have identified predominantly CON in adult human suprahyoid muscles with no or limited MHCac and MHCneo[13,14,15]. More recent studies by IHC and immunoblot, however, have indicated widespread expression of MHCac, MHCneo, and MHCst in MH and MHCac and MHCst in AD.[16,17,18] In these studies, >50% of muscle fibers were reported to be composed of at least 1 conventional and 1 unconventional MHC, a pattern of hybridization markedly different from prior studies. To our knowledge, a re-evaluation of human suprahyoid MHC by separation-SDS-PAGE-Western blot and mRNA PCR in the light of these recent studies has not been published. We were not able previously to detect MHCst by IHC in suprahyoid muscles of 2 individuals.[19]

For these reasons we investigated MHC composition of AD, GH, and MH by immunohistochemistry, separation SDS-PAGE, and mRNA PCR to determine MHC composition of human suprahyoid muscles. We also tested the cross-reactivity of MHC antibodies used previously to document DEV and UNCON in human suprahyoid muscles.

MATERIALS AND METHODS

Subjects

Post-mortem muscle tissue was taken from the left or right anterior digastric, geniohyoid, and mylohyoid from 6 adult human subjects and the mylohyoid from 1 subject (Subjects 1–7; Table 1). Subjects were free of known neuromuscular disease. Muscle was sampled from the antero-lateral MH, but otherwise it was not identified with respect to muscle region.

Additional tissue for immunohistochemical and electrophoresis control was obtained from a human fetal tongue muscle of approximately 23 gestational weeks (FT1), a fetal tongue of approximately 40 gestational weeks (FT2), a neonatal monkey tongue body (*M. rhesus*, MT1), the medial gastrocnemius of an 80 year old man (MG), the biceps brachii of Subject 1 (BC), the inferior oblique (IO) and lateral rectus (LR) of subject 1, the medial rectus of an 80 year old man (MR), the stylohyoid (STY), and medial pharyngeal constrictor (MPC) of Subject 5, the atrium (HA) and ventricle of a 62 year old woman, the atrium and ventricle of an adult rat, and the anterior latissimus dorsus muscle of the chick (ALD). All samples were frozen in liquid nitrogen within 9 hours post-mortem with the exception of FT1 (15 hours post-mortem) and FT2 (24 hours post-mortem). Post-mortem degradation of myosin heavy chain is temperature-dependent with prolonged stability at 4 degrees but partial proteolysis by 24 hours at 37 degrees.[20,21] Myosin heavy chain ATPase activity is stable for >24 hours at room temperature,[22] and identification of MHC in autopsy tissue by IHC appears to be stable for at least 36 hours[11]. Tissue was obtained from the Emory University School of Medicine Body Donor Program (EUSMBDP), from the National Disease Research Interchange (NDRI), and from the California National Regional Primate Center (CNRPC). All tissue used in this study is IRB-exempt.

Sample Preparation

Muscle tissue was partly thawed, cut into <10mm^3 blocks with razor blades, mounted on tongue depressors with Tissue-Tek O.C.T. Compound (Sakura, Finetek) quick-frozen in melting isopentane cooled by liquid nitrogen, and stored at −80°C.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis of MHC Isoforms

Tissue Preparation—Samples of ALD, AD, BC, FT, GH, HA, IO, LR, MH, MG, MR, and MT were prepared for electrophoretic identification of MHC. Approximately 40–50 mg of muscle tissue was cut from the frozen tissue block, homogenized in 200μl of 0.1M potassium phosphate (PBS) buffer (pH 7.3) and 5% protease inhibitor cocktail (Sigma, Aldrich) following Kohn and Myburgh[23] with a tissue homogenizer (Fisher Scientific, PowerGen 500) in an ice bath, followed by centrifugation at 10,000g (4°C) for 10 minutes and re-suspended in 0.1M PBS buffer (pH 7.3) and 5% protease inhibitor cocktail for extraction of the myosin fraction. Total protein content was assayed by bicinchoninic acid assay according to manufacturer specifications (Synergy HT multimode microplate reader, Biotek Instruments, Inc., Pierce® BCA protein assay, Thermo Fisher Scientific Inc). Samples were stored at −80°C.

Gel Preparation—The separation gel electrophoresis protocol was modified from Talmadge and Roy[24], with stacking gels (0.75 mm thick) of 4% acrylamide (wt/vol; acrylamide:N,N′-methylene-bis-acrylamide in the ratio of 37.5:1), 30% glycerol, 70mM Tris, 4mM EDTA, 0.4% SDS, 0.1% APS, and 0.05% TEMED and separating gels of 8% acrylamide (wt/vol; acrylamide:N,N′-methylene-bis-acrylamide in the ratio of 50:1), 30% glycerol, 0.2M Tris, 0.1M glycine, 0.4% SDS, 0.1% APS and 0.05% TEMED. The lower electrode buffer consisted of 50mM Tris, 75mM glycine, and 0.05% SDS; the upper electrode buffer of 6X the lower electrode buffer plus 0.12% 2-mercaptoethanol. Protein

samples were mixed with Laemmli sample buffer (Bio-Rad Laboratories) at 1:1, and equivalent amounts of sample protein (approximately 1.0 μg/lane, except for chick ALD 0.50 μg/lane and 4.0–7.5 μg/lane EOM sample) were loaded. A 45–200 kDa molecular weight standard (Bio-Rad SDS-PAGE molecular weight standards, High range, Bio-Rad Laboratories, Hercules, CA) was loaded in the initial lane for reference. SDS-PAGE gels were run at 140 V for 22 hours at 4°C and on ice.

Coomassie Stain—SDS-PAGE gels were rinsed with water for 3 × 5 minutes, stained with Imperial™ Protein Stain (Thermo Fisher Scientific Inc.) at room temperature for 1 hour, de-stained with water for 1.5 hours and scanned at 2400 DPI resolution (Epson Perfection V33).

Western Blot—SDS-PAGE gels were transferred to Immuno-Blot™ PVDF membrane (Bio-Rad Laboratories) at 4°C, 200mA for 1 hour (Transblot SD semi-dry transfer cell, Bio-rad Laboratories). After incubation in 0.5X blocking buffer (USB Corporation) at room temperature for 40 minutes, membranes were incubated overnight with primary antibody in 0.5X blocking buffer with 0.1% Triton X-100 at 4°C (for Ab concentrations see Supplementary Information, Table 1, available online). Following PBS wash (4 × 5 minutes each), membranes were incubated with appropriate secondary IRDye 700DX goat anti-mouse IgG (Rockland, Gilbertsville, PA), IRDye 800CW goat anti-mouse IgM (Rockland, Gilbertsville, PA), or with Dylight 800 goat anti-rabbit IgG (Thermoscientific, Rockford, IL) and visualized by Odyssey Infrared Imaging System (LI-COR). On some membranes, different Abs were reacted sequentially to assist in identification of MHC bands. To optimize visualization of MHC, some Abs were reacted at very high concentrations (see Supplementary Information, Table 1 for specificity and concentration of Abs for Western blot, available online).

Immunohistochemical Methods

Serial 12-μm thick cross-sections of AD, GH, MH, and control tissue (BC, FT, HA, MR, LR, STY, MPC) were cut on a cryostat at −23°C and mounted on gelatin-subbed slides. The following antibody dilutions were used for IHC (see below for Ab specficities): Ab MY-32 at a dilution of 1:400, Abs MYH6, and SC-71 at dilution of 1:25, Ab S46 at dilutions of 1:10 and 1:25, NCL-MHCd and NCL-MHCn at a dilution of 1:10, and Abs A4.84, BA-G5, F1.652, N3.361, N2.261, and N1.551 at dilutions from supernatant (SN) to 1:5. Tissue was sometimes co-reacted with the anti-laminin Ab D18 (The Developmental Studies Hybridoma Bank; 1:50, 1:100) to facilitate identification of muscle fiber boundaries.

Tissue was reacted following the protocol of Eason et al.[25] and described in Daugherty et al.[26] Briefly, tissue sections were incubated in a blocking solution composed of 2% normal goat serum, 0.03% Triton-X, and 0.1M Tris-HCl (T-NGS) at room temperature for 1 hour, followed by incubation overnight with primary antibody in blocking solution in a humid chamber at 4 °C. Tissue was then washed in Tris-HCl buffer and incubated with the appropriate secondary antibody (peroxidase-conjugated goat IgM fraction to mouse immunoglobulins; peroxidase-conjugated goat IgM fraction to rabbit immunoglobulins, peroxidase-conjugated goat IgG fraction to mouse immunoglobulins, all at a dilution of

1:100) for 1 hour at room temperature. A standard DAB reaction was used to visualize label (0.5 mg DAB/mL, 0.1 M PBS, 0.03% H_2O_2). Slides were then washed with water for 5 minutes × 2, dehydrated, and coverslipped in Permount Mounting Medium (Fisher Scientific).

Tissue sections were viewed on an Olympus BX51 microscope at 100x, 200x, and 400x magnification. Images were collected with Neurolucida software (Microbrightfield, Burlington, VT) using a MicroFire digital microscope camera (Optronics, Goleta, CA), and stored onto computer (Dell Optiplex GX270, 1280 × 1024 pixel resolution).

To quantify expression of DEV and UNCON in suprahyoid muscles, the number of fibers positive for Abs NCL-MHCn, NCL-MHCd, and S46 were counted and expressed per mm^2 tissue.

Myosin Antibodies for Immunohistochemistry and Western blot

To enable comparison to previous studies we used 12 antibodies for IHC and/or Western blot study of control and experimental tissue: (1) Ab A4.84 [The Developmental Studies Hybridoma Bank (DSHB), developed under the auspices of the NICHD and maintained by The University of Iowa Department of Biology, Iowa City, IA 52242], which in humans is reported to be specific for MHCI (βcardiac)[27,28] but in our hands also labels MHCac[26]; (2) Ab BA-G5, reported to label MHac, MHCI, and MHCIIB in rat,[29] and MHCac in human[16,17]; (3) Ab F1.652 (DSHB), reported to be specific for MHCemb[30]; (4) Ab NCL-MHCd (Novocastra antibody, Leica), which in mammals is likely specific for MHCemb[31]; (5) Ab MY-32 (Ascites, Sigma-Aldrich, St Louis, MO), which in rats and humans reacts with MHCII isoforms and likely MHCneo and MHCeom[32,33]; (6) Ab MYH6 (Sigma-Aldrich), which in humans is reported to label MHCac by IHC[34] but in our hands labels MHCac, MHCI, MHCIIX, MHCemb, and putative MHCneo[26]; (7) Ab N1.551, reported to be specific in humans for neonatal MHCIIA[17] or adult MHCIIA[35]; (8) N2.261, which has been reported to be specific for MHCneo[16] or to react with MHCI, MHCac, and MHCIIA, but not MHCemb or MHCneo[26,27,36,5]; (9) Ab N3.36 reported to label MHCneo and MHCIIA in humans[37,30]; (10) Ab NCL-MHCn (Clone WB-MHCn, Vector Laboratories, Burlingame, CA), which in mammals is reported to be specific for MHCneo[38,12] but in our hands labels MHCemb and putative MHCneo in humans by Western blot[26]; (11) Ab S46 (DSHB), which reacts with putative MHCst in adult human muscle spindles and human extraocular muscles, does not cross-react with MHCac or MHCI in adult catarrhine primate muscle,[19] and labeled a single band (putative MHCst) in human extraocular muscle[26]; and (12) Ab SC-71 (ATCC) which in humans is reported to be specific for MHCIIA[28,39], to label MHCIIA>MHCIIX[40,12,26] or to label MHCIIA and MHCIIX.[41]

Control Tests: Identification of Myosin Heavy Chain by Separation/SDS-PAGE and Specificity of Myosin Heavy Chain Antibodies

Reactivity of Abs with multiple MHC isoforms can produce false-positive assignment of MHC by IHC if >1 MHC isoform is present in a fiber and by separation SDS-PAGE-Western blot if >1 isoform is present in a band. Because of differing reports of human suprahyoid muscle MHC composition and differing reports of MHC antibody specificities in

human muscle (see above), we sought to increase confidence in the assignment of MHC in our hands by: (1) testing for MHC migration by separation SDS-PAGE in a sample of control tissue containing DEV and UNCON; (2) testing for MHC migration by separation SDS-PAGE in control tissue with MHC Abs of putative specificity; (3) testing specificity of MHC Abs previously used to assign MHC identity by Western blot in human suprahyoid muscles; and (4) testing the specificity of the putative anti-MHCac antibody BA-G5 by IHC (see Supplementary Information, Figures 1–5, available online).

From these studies we assign MHC mobilities from fast to slow, MHCI, MHCac/MHCeom, MHCst, MHCIIA, MHCemb, and MHCIIX/putative MHCneo (Figure 1A; Supplementary Information, Figures 1–4, available online). In our hands, MHC migration was similar to other studies with the exception of DEV, 1 or both of which often migrate between MHCI and MHCIIA (e.g.,[42,43,37,44]). Specificities of Abs by Western blot in our hands are shown in Supplementary Information, Table 1 (available online). By IHC Ab BA-G5 supernatant strongly labeled fibers in the rat ventricle, but either did not label or weakly labeled fibers in human atrium, ventricle, biceps and suprahyoid muscles (Supplementary Information, Figure 5, available online). We attribute weak labeling to cross reaction with MHCI and conclude that supernatant Ab BA-G5 does not label human MHCac by IHC in our hands.

MHC Transcript Profiling by Quantitative PCR

MHC expression was evaluated by quantitative PCR in AD2, GH1, GH2, MH7, and MH2. Blocks of approximately 50 mg were homogenized in Trizol using a rotor-stator (TissueMizer), and RNA was separated according to the manufacturer's protocol. Purified RNA was quantified by ultaviolet spectroscopy, and 2 ug was subjected to reverse transcription (High Capacity RNA-to-cDNA master mix, ABI) using random hexamer primers. Amplification of MHCI, MHCIIA, MHCIIX, MHCIIB, MHCac, MHCemb, MHCeom, MHCneo, MYH15, and MHCmasticatory was performed using primer pairs described previously and validated[45] with β-actin as an internal standard. For each amplicon, a DNA standard was prepared by conventional PCR and quantified by Hoechst 33258 fluorescence. Standard curves were included in each PCR run to confirm amplification, provide absolute quantification, and validate detection threshold. Individual amplifications were performed in duplicate and validated by terminal melt-curve analysis. For each sample, expression of each mRNA was determined in moles and normalized to the total MHC mRNA content.

RESULTS

Separation SDS-PAGE-Coomassie of Suprahyoid Muscles

In our sample of AD, GH, and MH only MHCI, MHCIIA, and MHCIIX were visible by Coomassie stain (Figures 1A, 1B).

Separation SDS-PAGE-Western blot of Suprahyoid Muscles

Following Western blot of separation SDS-PAGE with Abs that exclusively label DEV and UNCON (Abs BA-G5, S46, F1.652, NCL-MHCd, NCL-MHCn) no protein was labeled in suprahyoid muscles (results for Subjects 2 and 3 shown in Figures 1–4). Following Western

blot of separation SDS-PAGE with Abs that label CON in addition to DEV and/or UNCON (Abs A4.84, SC-71, MY-32, MYH6, N2.261), MHCI and MHCIIA were labeled in all and MHCIIX in many suprahyoid muscles (results for Abs N2.261 and MYH6 are shown in Figures 1–3). No DEV or UNCON were labeled in any suprahyoid muscle with these Abs. Ab N1.551 weakly labeled all MHC in suprahyoid muscles (Figure 4). Ab ALD-58 did not label MHC in suprahyoid muscles (Figure 1).

Quantitative PCR

Expression of only MHCI, MHCIIA, MHCIIX, and MHCac were above the detection threshold of 4 fmole/g RNA in AD, GH, and MH. Total MHC amplified from sample MH7 was only 15–30% of that found in the other samples. We interpret this as RNA degradation and discount the results of MH7. Each of these muscles expresses primarily MHCI, with the balance being MHCIIA and MHCIIX and generally a trace of MHCac (Table 2).

Immunohistochemistry

Abs to MHCemb, MHCneo and MHCst labeled few or no fibers in human suprahyoid muscles (Figure 5; Table 3).

DISCUSSION

By IHC, separation SDS-PAGE-Western blot, and PCR we find appreciable expression of only conventional myosins in human AD, GH, and MH with the exception of limited MHCac in GH (<5%) by PCR.

Cross-reactivity of Abs with multiple MHC isoforms suggests that recent reports of abundant DEN and UNCON in human suprahyoid muscles represent misattribution of MHC due to Ab cross-reactivity with CON.

Prior Studies of DEV and UNCON in Human Suprahyoid Muscles

Initial studies using ATPase identified Type I and Type II fibers with minimal Type IIC/IM in the human AD[46] and 9% Type IM in the MH.[47] By IHC Bredman et al.[48] reported MHCac in human masseter and temporalis but not in the AD or in extrafusal fibers of post-cranial muscles. Also by IHC, Korfage and colleagues reported limited MHCneo (1% of fibers) and MHCac (5% of fibers) in suprahyoid muscles (AD, GH, MH, and suprahyoid). DEV and UNCON always hybridized with CON, with predominant fiber phenotypes MHCac/MHCIIA (2.0%) and MHCac/MHCI (1.5%).[15] MHCac was most notable in the GH (approximately 10% of fibers), and MHCac and MHCneo were absent in AD.[14] MHCneo was not identified with separation SDS-PAGE of human AD muscle fibers by either silver stain or Western blot.[49] With different antibodies, Monemi et al.[13, 50] did not identify MHCac, MHCemb and MHCneo in human AD by IHC or by separation SDS-PAGE. In a previous study of Subject 1 and Subject 2, the anti-MHCst Ab S46 was negative for AD, GH, and MH by immunoblot and labeled occasional extrafusal fibers by IHC.[19] The current study confirms and extends these earlier findings by demonstrating no or limited DEV and UNCON in human AD, GH, and MH by IHC, separation SDS-PAGE-Western blot, and mRNA PCR.

In contrast, Mu and colleagues reported robust expression of DEV and UNCON in human AD and MH. Approximately 80% of muscle fibers in MH and 50% of muscle fibers in AD were reported to be hybrids of CON/DEV or CON/UNCON.[16,17,18] Cross-reaction of putative anti-DEV and anti-UNCON Abs used by Mu and colleagues with CON may explain partly the discrepancy with our results. In our hands, by SDS-PAGE-Western blot Ab N2.261 (putative anti-MHCneo[16]) reacts with many MHC but does not react with MHCneo (see also[36,5,26]). Ab BA-G5 (putative anti-MHCac[16,17]) reacts with putative MHCac and MHCst in EOM but does not react with MHCac in atrium. At high concentrations, Abs BA-G5, N1.551 (putative anti-MHCneo[17,18]), and ALD-58 (putative anti-MHCst[16,17,18]) label MHC weakly and nonspecifically. In our hands, by IHC, Abs N1.551, and BA-G5 are either unreactive or react weakly with fibers positive for CON. Additionally, by IHC the putative anti-MHCst Ab ALD-58 reacts with MHCI and MHCac in mammals, including humans.[51,19] Label of MHCI by Ab ALD-58 is the most likely explanation for Ab ALD-58 reactivity in AD, MH, and masseter by Mu and colleagues. Previously, MHCst was not found in the human masseter with the anti-MHCst Ab ALD-19.[52]

In contrast, by both SDS-PAGE-Western blot and IHC, Ab F1.652 is considered specific for MHCemb (see also[37,30]). By IHC, Mu and colleagues reported 5% of fibers positive for Ab F1.652 in the anterior MH and 4% in the AD[16,17]. With the same Ab we found few fibers positive for MHCemb in the AD, GH, and MH, suggesting that expression of MHCemb in human suprahyoid muscles is individually variable but limited. Previously, MHCemb was not reported by IHC in the AD[50].

In the light of Ab cross-reactivities documented here, re-evaluation of studies of AD and MH by Mu and colleagues[16–18] indicates that only CON can be resolved in their studies with confidence, with the exception of occasional fibers positive for MHCemb. Antibody cross-reactivities may also explain their more recent findings of abundant DEV and UNCON in muscles of the human pharynx and upper esophageal sphincter.[53,54,55,56]

Limitations of Study

We recognize 3 primary limitations of this study. First, the absence of a specific Ab for MHCac precluded confident study of this isoform by immunohistochemistry (i.e., at the single fiber level). Thus we cannot rule out the translation of MHC below the sensitivity of Western blot suggested by MHCac mRNA. In human laryngeal muscles MHCac was detected by mRNA PCR[57] but not by SDS-PAGE[58,59]. In human extraocular muscles, MHCac was documented by IHC but was below the sensitivity of Western blot [5]. Second, although almost 5% of MHCac mRNA was detected by PCR in 1 GH sample, we did not observe MHCac in this or any other sample by separation SDS-PAGE/Western blot. The correlation between prevalence of MHC mRNA by PCR and prevalence of MHC protein by SDS-PAGE is variable by muscle,[60,61] and even in single muscle fibers this correlation is imprecise[62]. In the human ventricle, relative prevalence of MHCac was almost 5 times greater by mRNA PCR than SDS-PAGE,[63] and a similar relationship may be present in the GH. Because of the limited volume of muscle sampled by PCR, sampling bias may contribute to the discrepancy. Third, we conducted PCR and separation SDS-PAGE on a

subset of subjects. MHC expression is variable by individual and gender (e.g.,[59]), and DEV and UNCON may have been present in a larger sample. However, appreciable MHCemb, MHCneo, and MHCst were not identified by IHC in two men and three women. Fourth, we recognize uncertainty regarding the location of MHCneo by separation SDS-PAGE in our hands. We provisionally assign MHCneo to the slowest band (co-migratory with MHCIIX) based on reaction of this band with Ab NCL-MHCn and Ab N3.36 but not with the anti-MHCI-IIX Ab 8H2.[26] However, Abs NCL-MHCn and N3.36 also reacted with protein at the position of MHCemb (i.e., the band labeled with Abs F1.652 and NCL-MHCd) which can be explained by: (1) cross-reaction of Abs NCL-MHCn and N3.36 with MHCemb or by (2) co-migration of MHCneo and MHCemb at this position. This latter possibility would not change our evidence for no or limited DEV in human suprahyoid muscles, because this band was never observed in AD, GH, or MH by Coomassie stain or Western blot (i.e., with Abs 8H2, F1.652, MYH6, NCL-MHCd, NCL-MHCn and N3.36).

Significance of Limited DEV and UNCON in Human Suprahyoid Muscles

Appreciable DEV and UNCON are present in extrafusal fibers of some human head and neck muscles, in particular jaw closing muscles (e.g.,[13]) and extraocular muscles (e.g.,[5,6]). Expression of DEV and UNCON in these muscles may be related to complex functional demands (e.g.,[4,64]). At least for MHCst in extraocular muscles, UNCON expression is associated with peripheral and central neural specializations.[64,65]

Limited adult expression of MHCemb and MHCneo is a feature of many cranial muscles more generally (e.g.,[66,67]). In these muscles DEV expression may reflect fiber remodeling due to normative mechanical damage (e.g., stress at fiber terminations) or disease process (such as dennervation-reinnervation)[67]. Limited DEV in AD, GH and MH suggest that these muscles are adapted to normal use without persistent remodeling. Limited expression of MHCac has also been reported in some human cranial muscles including GH.[14] In our study, MHCac was not present by Western blot, but accounted for 5% of MHC mRNA in 1 GH sample. Whether MHCac mRNA is expressed routinely in human GH requires corroboration in a larger sample. Previously we documented MHCac mRNA in the human tongue.[45]

Implications for Human Swallowing Therapy

In concert with earlier studies we conclude that human AD, GH, and MH are composed almost exclusively of conventional MHC. Thus for these muscles neither developmental history nor putative complex patterns of muscle activation are associated with appreciable DEV and UNCON.

The suprahyoid muscles are a principal component of swallowing synergy,[68] and suprahyoid muscle activity is correlated with tongue-to-palate pressures.[69] Recently, atrophy of the geniohyoid muscle has been associated with aspiration in human aging,[70] which raises the possibility that sarcopenia of suprahyoid muscles contributes to age-related swallowing dysfunction. Age-related changes in hyoid and laryngeal movements during swallowing are variable by study [71,72]; however, enhancement of suprahyoid muscle function by exercise or stimulation is a target of current dysphagia interventions [73,74]. These interventions are based

on the expectation that branchiomeric muscles respond to strength training similar to limb muscles, but the effectiveness of exercise interventions in muscles with marked expression of DEV and UNCOV is unknown. This study, in demonstrating that human AD, GH and MH are not characterized by a unique MHC allotype [9], suggests that interventions based on appendicular muscle biology should enhance suprahyoid muscle performance. However, motoneurons in the hypoglossal nucleus and higher centers involved in their coordination may differ substantially from spinal motoneurons, and neural coordination is a significant factor in voluntary performance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

Ab	antibody
AD	anterior digastric muscle
ATPase	adenosine triphosphatase
CON	conventional myosin heavy chain
DEV	developmental myosin heavy chain
EOM	extraocular muscle
GH	geniohyoid muscle
IHC	immunohistochemistry
MG	medial gastrocnemius muscle
MH	mylohyoid muscle
MHC	myosin heavy chain
MHCac	myosin heavy chain alpha-cardiac
MHCemb	myosin heavy chain embryonic
MHCeom	myosin heavy chain extraocular
MHCneo	myosin heavy chain neonatal
MCHst	myosin heavy chain slow tonic
PCR	polymerase chain reaction

SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SN	supernatant
UNCON	unconventional myosin heavy chain

References

1. Cook IJ. Oropharyngeal dysphagia. Gastroenterol Clin North Am. 2009; 38(3):411–431. [PubMed: 19699405]
2. Roy N, Stemple J, Merrill RM, Thomas L. Dysphagia in the elderly: preliminary evidence of prevalence, risk factors, and socioemotional effects. Ann Otol Rhinol Laryngol. 2007; 116(11):858–865. [PubMed: 18074673]
3. Robbins J, Gangnon RE, Theis SM, Kays SA, Hewitt AL, Hind JA. The effects of lingual exercise on swallowing in older adults. J Am Geriatr Soc. 2005; 53(9):1483–1489. [PubMed: 16137276]
4. Butler-Browne GS, Eriksson PO, Laurent C, Thornell LE. Adult human masseter muscle fibers express myosin isozymes characteristic of development. Muscle Nerve. 1988; 11(6):610–620. [PubMed: 3386670]
5. Kjellgren D, Thornell LE, Andersen J, Pedrosa-Domellof F. Myosin heavy chain isoforms in human extraocular muscles. Invest Ophthalmol Vis Sci. 2003; 44(4):1419–1425. [PubMed: 12657575]
6. Rossi AC, Mammucari C, Argentini C, Reggiani C, Schiaffino S. Two novel/ancient myosins in mammalian skeletal muscles: MYH14/7b and MYH15 are expressed in extraocular muscles and muscle spindles. J Physiol. 2010; 588(Pt 2):353–364. [PubMed: 19948655]
7. Buckingham M, Bajard L, Chang T, Daubas P, Hadchouel J, Meilhac S, Montarras D, Rocancourt D, Relaix F. The formation of skeletal muscle: from somite to limb. J Anat. 2003; 202(1):59–68. [PubMed: 12587921]
8. Kelly RG, Jerome-Majewska LA, Papaioannou VE. The del22q11. 2 candidate gene Tbx1 regulates branchiomeric myogenesis. Hum Mol Genet. 2004; 13(22):2829–2840. [PubMed: 15385444]
9. Hoh JF. Laryngeal muscle fibre types. Acta Physiol Scand. 2005; 183(2):133–149. [PubMed: 15676055]
10. Noden DM, Francis-West P. The differentiation and morphogenesis of craniofacial muscles. Dev Dyn. 2006; 235(5):1194–1218. [PubMed: 16502415]
11. Korfage JA, Van Eijden TM. Myosin isoform composition of the human medial and lateral pterygoid muscles. J Dent Res. 2000; 79(8):1618–1625. [PubMed: 11023285]
12. Yu F, Stal P, Thornell LE, Larsson L. Human single masseter muscle fibers contain unique combinations of myosin and myosin binding protein C isoforms. J Muscle Res Cell Motil. 2002; 23(4):317–326. [PubMed: 12630706]
13. Monemi M, Kadi F, Liu JX, Thornell LE, Eriksson PO. Adverse changes in fibre type and myosin heavy chain compositions of human jaw muscle vs. limb muscle during ageing. Acta Physiol Scand. 1999; 167(4):339–345. [PubMed: 10632637]
14. Korfage JA, Brugman P, Van Eijden TM. Intermuscular and intramuscular differences in myosin heavy chain composition of the human masticatory muscles. J Neurol Sci. 2000; 178(2):95–106. [PubMed: 11018701]
15. Korfage JA, Schueler YT, Brugman P, Van Eijden TM. Differences in myosin heavy-chain composition between human jaw-closing muscles and supra- and infrahyoid muscles. Arch Oral Biol. 2001; 46(9):821–827. [PubMed: 11420054]
16. Mu L, Su H, Wang J, Han Y, Sanders I. Adult human mylohyoid muscle fibers express slow-tonic, alpha-cardiac, and developmental myosin heavy-chain isoforms. Anat Rec A Discov Mol Cell Evol Biol. 2004; 279(2):749–760. [PubMed: 15278946]
17. Wang J, Han Y, Su H, Mu L. Expression of unique and developmental myosin heavy chain isoforms in adult human digastric muscle. J Histochem Cytochem. 2004; 52(7):851–859. [PubMed: 15208352]

18. Ren M, Mu L. Intrinsic properties of the adult human mylohyoid muscle: neural organization, fiber-type distribution, and myosin heavy chain expression. Dysphagia. 2005; 20(3):182–194. [PubMed: 16362507]
19. Sokoloff AJ, Li H, Burkholder TJ. Limited expression of slow tonic myosin heavy chain in human cranial muscles. Muscle Nerve. 2007; 36(2):183–189. [PubMed: 17486578]
20. Bandman E, Zdanis D. An immunological method to assess protein degradation in postmortem muscle. Meat Sci. 1988; 22(1):1–19. [PubMed: 22055148]
21. Lametsch R, Roepstorff P, Bendixen E. Identification of protein degradation during postmortem storage of pig meat. J Agric Food Chem. 2002; 50(20):5508–5512. [PubMed: 12236671]
22. Eriksson O, Eriksson A, Ringqvist M, Thornell LE. The reliability of histochemical fibre typing of human necropsy muscles. Histochemistry. 1980; 65(3):193–205. [PubMed: 6445346]
23. Kohn TA, Myburgh KH. Electrophoretic separation of human skeletal muscle myosin heavy chain isoforms: the importance of reducing agents. J Physiol Sci. 2006; 56(5):355–360. [PubMed: 17014746]
24. Talmadge RJ, Roy RR. Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. J Appl Physiol. 1993; 75(5):2337–2340. [PubMed: 8307894]
25. Eason JM, Schwartz G, Shirley KA, English AW. Investigation of sexual dimorphism in the rabbit masseter muscle showing different effects of androgen deprivation in adult and young adult animals. Arch Oral Biol. 2000; 45(8):683–690. [PubMed: 10869480]
26. Daugherty M, Luo Q, Sokoloff AJ. Myosin heavy chain composition of the human genioglossus muscle. J Speech Lang Hear Res. 2012; 55(2):609–625. [PubMed: 22337492]
27. Hughes SM, Cho M, Karsch-Mizrachi I, Travis M, Silberstein L, Leinwand LA, Blau HM. Three slow myosin heavy chains sequentially expressed in developing mammalian skeletal muscle. Dev Biol. 1993; 158(1):183–199. [PubMed: 7687223]
28. Li ZB, Lehar M, Nakagawa H, Hoh JF, Flint PW. Differential expression of myosin heavy chain isoforms between abductor and adductor muscles in the human larynx. Otolaryngol Head Neck Surg. 2004; 130(2):217–222. [PubMed: 14990919]
29. Putman CT, Conjard A, Peuker H, Pette D. Alpha-cardiac-like myosin heavy chain MHCI alpha is not upregulated in transforming rat muscle. J Muscle Res Cell Motil. 1999; 20(2):155–162. [PubMed: 10412087]
30. Cho M, Hughes SM, Karsch-Mizrachi I, Travis M, Leinwand LA, Blau HM. Fast myosin heavy chains expressed in secondary mammalian muscle fibers at the time of their inception. J Cell Sci. 1994; 107 (Pt 9):2361–2371. [PubMed: 7531198]
31. Brueckner JK, Itkis O, Porter JD. Spatial and temporal patterns of myosin heavy chain expression in developing rat extraocular muscle. J Muscle Res Cell Motil. 1996; 17(3):297–312. [PubMed: 8814550]
32. Brandon CA, Rosen C, Georgelis G, Horton MJ, Mooney MP, Sciote JJ. Muscle fiber type composition and effects of vocal fold immobilization on the two compartments of the human posterior cricoarytenoid: a case study of four patients. J Voice. 2003; 17(1):63–75. [PubMed: 12705819]
33. Harris AJ, Fitzsimons RB, McEwan JC. Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles. Development. 1989; 107(4):751–769. [PubMed: 2534377]
34. Stirn Kranjc B, Smerdu V, Erzen I. Histochemical and immunohistochemical profile of human and rat ocular medial rectus muscles. Graefes Arch Clin Exp Ophthalmol. 2009; 247(11):1505–1515. [PubMed: 19609551]
35. Webster C, Silberstein L, Hays AP, Blau HM. Fast muscle fibers are preferentially affected in Duchenne muscular dystrophy. Cell. 1988; 52(4):503–513. [PubMed: 3342447]
36. Liu JX, Eriksson PO, Thornell LE, Pedrosa-Domellof F. Myosin heavy chain composition of muscle spindles in human biceps brachii. J Histochem Cytochem. 2002; 50(2):171–183. [PubMed: 11799136]
37. Cho M, Webster SG, Blau HM. Evidence for myoblast-extrinsic regulation of slow myosin heavy chain expression during muscle fiber formation in embryonic development. J Cell Biol. 1993; 121(4):795–810. [PubMed: 8491773]

38. Ecob-Prince M, Hill M, Brown W. Immunocytochemical demonstration of myosin heavy chain expression in human muscle. J Neurol Sci. 1989; 91(1–2):71–78. [PubMed: 2746293]
39. Serrano AL, Perez M, Lucia A, Chicharro JL, Quiroz-Rothe E, Rivero JL. Immunolabelling, histochemistry and in situ hybridisation in human skeletal muscle fibres to detect myosin heavy chain expression at the protein and mRNA level. J Anat. 2001; 199(Pt 3):329–337. [PubMed: 11554510]
40. Smerdu V, Soukup T. Demonstration of myosin heavy chain isoforms in rat and humans: the specificity of seven available monoclonal antibodies used in immunohistochemical and immunoblotting methods. Eur J Histochem. 2008; 52(3):179–190. [PubMed: 18840559]
41. Bamman MM, Clarke MS, Talmadge RJ, Feeback DL. Enhanced protein electrophoresis technique for separating human skeletal muscle myosin heavy chain isoforms. Electrophoresis. 1999; 20(3): 466–468. [PubMed: 10217154]
42. Pedrosa-Domellof F, Thornell LE. Expression of myosin heavy chain isoforms in developing human muscle spindles. J Histochem Cytochem. 1994; 42(1):77–88. [PubMed: 8263326]
43. Biral D, Damiani E, Margreth A, Scarpini E. Myosin subunit composition in human developing muscle. Biochem J. 1984; 224(3):923–931. [PubMed: 6395865]
44. Lloyd JS, Brozanski BS, Daood M, Watchko JF. Developmental transitions in the myosin heavy chain phenotype of human respiratory muscle. Biol Neonate. 1996; 69(2):67–75. [PubMed: 8713651]
45. Rahnert JA, Sokoloff AJ, Burkholder TJ. Sarcomeric myosin expression in the tongue body of humans, macaques and rats patent 1422–6421 (Electronic) 1422–6405 (Linking). 2010
46. Eriksson PO, Eriksson A, Ringqvist M, Thornell LE. Histochemical fibre composition of the human digastric muscle. Arch Oral Biol. 1982; 27(3):207–215. [PubMed: 6212040]
47. Vignon C, Pellissier JF, Serratrice G. Further histochemical studies on masticatory muscles. J Neurol Sci. 1980; 45(2–3):157–176. [PubMed: 6445000]
48. Bredman JJ, Wessels A, Weijs WA, Korfage JA, Soffers CA, Moorman AF. Demonstration of ‘cardiac-specific’ myosin heavy chain in masticatory muscles of human and rabbit. Histochem J. 1991; 23(4):160–170. [PubMed: 1836206]
49. Korfage JA, Van Eijden TM. Myosin heavy-chain isoform composition of human single jaw-muscle fibers. J Dent Res. 2003; 82(6):481–485. [PubMed: 12766203]
50. Monemi M, Liu JX, Thornell LE, Eriksson PO. Myosin heavy chain composition of the human lateral pterygoid and digastric muscles in young adults and elderly. J Muscle Res Cell Motil. 2000; 21(4):303–312. [PubMed: 11032341]
51. Shafiq SA, Shimizu T, Fischman DA. Heterogeneity of type 1 skeletal muscle fibers revealed by monoclonal antibody to slow myosin. Muscle Nerve. 1984; 7(5):380–387. [PubMed: 6738576]
52. Stal P, Eriksson PO, Schiaffino S, Butler-Browne GS, Thornell LE. Differences in myosin composition between human oro-facial, masticatory and limb muscles: enzyme-, immunohisto- and biochemical studies. J Muscle Res Cell Motil. 1994; 15(5):517–534. [PubMed: 7860700]
53. Mu L, Su H, Wang J, Sanders I. Myosin heavy chain-based fiber types in the adult human cricopharyngeus muscle. Muscle Nerve. 2007; 35(5):637–648. [PubMed: 17294438]
54. Mu L, Wang J, Su H, Sanders I. Adult human upper esophageal sphincter contains specialized muscle fibers expressing unusual myosin heavy chain isoforms. J Histochem Cytochem. 2007; 55(3):199–207. [PubMed: 17074861]
55. Mu L, Sanders I. Newly revealed cricothyropharyngeus muscle in the human laryngopharynx. Anat Rec (Hoboken). 2008; 291(8):927–938. [PubMed: 18521893]
56. Mu L, Sanders I. Neuromuscular specializations within human pharyngeal constrictor muscles. Ann Otol Rhinol Laryngol. 2007; 116(8):604–617. [PubMed: 17847729]
57. Horton MJ, Rosen C, Close JM, Sciote JJ. Quantification of myosin heavy chain RNA in human laryngeal muscles: differential expression in the vertical and horizontal posterior cricoarytenoid and thyroarytenoid. Laryngoscope. 2008; 118(3):472–477. [PubMed: 18091331]
58. Sciote JJ, Morris TJ, Brandon CA, Horton MJ, Rosen C. Unloaded shortening velocity and myosin heavy chain variations in human laryngeal muscle fibers. Ann Otol Rhinol Laryngol. 2002; 111(2):120–127. [PubMed: 11860063]

59. Toniolo L, Macchi V, Porzionato A, Paoli A, Marchese-Ragona R, De Caro R, Reggiani C. Myosin heavy chain isoforms in human laryngeal muscles: an expression study based on gel electrophoresis. Int J Mol Med. 2008; 22(3):375–379. [PubMed: 18698498]
60. Gunawan AM, Park SK, Pleitner JM, Feliciano L, Grant AL, Gerrard DE. Contractile protein content reflects myosin heavy-chain isoform gene expression. J Anim Sci. 2007; 85(5):1247–1256. [PubMed: 17145975]
61. Wimmers K, Ngu NT, Jennen DG, Tesfaye D, Murani E, Schellander K, Ponsuksili S. Relationship between myosin heavy chain isoform expression and muscling in several diverse pig breeds. J Anim Sci. 2008; 86(4):795–803. [PubMed: 18156349]
62. Zhang MY, Zhang WJ, Medler S. The continuum of hybrid IIX/IIB fibers in normal mouse muscles: MHC isoform proportions and spatial distribution within single fibers. Am J Physiol Regul Integr Comp Physiol. 2010; 299(6):R1582–1591. [PubMed: 20861278]
63. Miyata S, Minobe W, Bristow MR, Leinwand LA. Myosin heavy chain isoform expression in the failing and nonfailing human heart. Circ Res. 2000; 86(4):386–390. [PubMed: 10700442]
64. Porter JD. Extraocular muscle: cellular adaptations for a diverse functional repertoire. Ann N Y Acad Sci. 2002; 956:7–16. [PubMed: 11960789]
65. Eberhorn AC, Ardeleanu P, Buttner-Ennever JA, Horn AK. Histochemical differences between motoneurons supplying multiply and singly innervated extraocular muscle fibers. J Comp Neurol. 2005; 491(4):352–366. [PubMed: 16175553]
66. Lindman R, Stal PS. Abnormal palatopharyngeal muscle morphology in sleep-disordered breathing. J Neurol Sci. 2002; 195(1):11–23. [PubMed: 11867069]
67. Sokoloff AJ, Daugherty M, Li H. Myosin heavy-chain composition of the human hyoglossus muscle. Dysphagia. 2010; 25(2):81–93. [PubMed: 19526266]
68. Doty RW, Bosma JF. An electromyographic analysis of reflex deglutition. J Neurophysiol. 1956; 19(1):44–60. [PubMed: 13286721]
69. Palmer PM, Jaffe DM, McCulloch TM, Finnegan EM, Van Daele DJ, Luschei ES. Quantitative contributions of the muscles of the tongue, floor-of-mouth, jaw, and velum to tongue-to-palate pressure generation. J Speech Lang Hear Res. 2008; 51(4):828–835. [PubMed: 18658054]
70. Feng X, Todd T, Lintzenich CR, Ding J, Carr JJ, Ge Y, Browne JD, Kritchevsky SB, Butler SG. Aging-Related Geniohyoid Muscle Atrophy Is Related to Aspiration Status in Healthy Older Adults. J Gerontol A Biol Sci Med Sci. 2012
71. Molfenter SM, Steele CM. Physiological variability in the deglutition literature: hyoid and laryngeal kinematics. Dysphagia. 2011; 26(1):67–74. [PubMed: 20927634]
72. van der Kruis JG, Baijens LW, Speyer R, Zwijnenberg I. Biomechanical analysis of hyoid bone displacement in videofluoroscopy: a systematic review of intervention effects. Dysphagia. 2011; 26(2):171–182. [PubMed: 21165750]
73. Pearson WG Jr, Hindson DF, Langmore SE, Zumwalt AC. Evaluating Swallowing Muscles Essential for Hyolaryngeal Elevation by Using Muscle Functional Magnetic Resonance Imaging. Int J Radiat Oncol Biol Phys. 2012
74. Kim SJ, Han TR. Effect of surface electrical stimulation of suprahyoid muscles on hyolaryngeal movement. Neuromodulation. 2009; 12(2):134–140. [PubMed: 22151287]

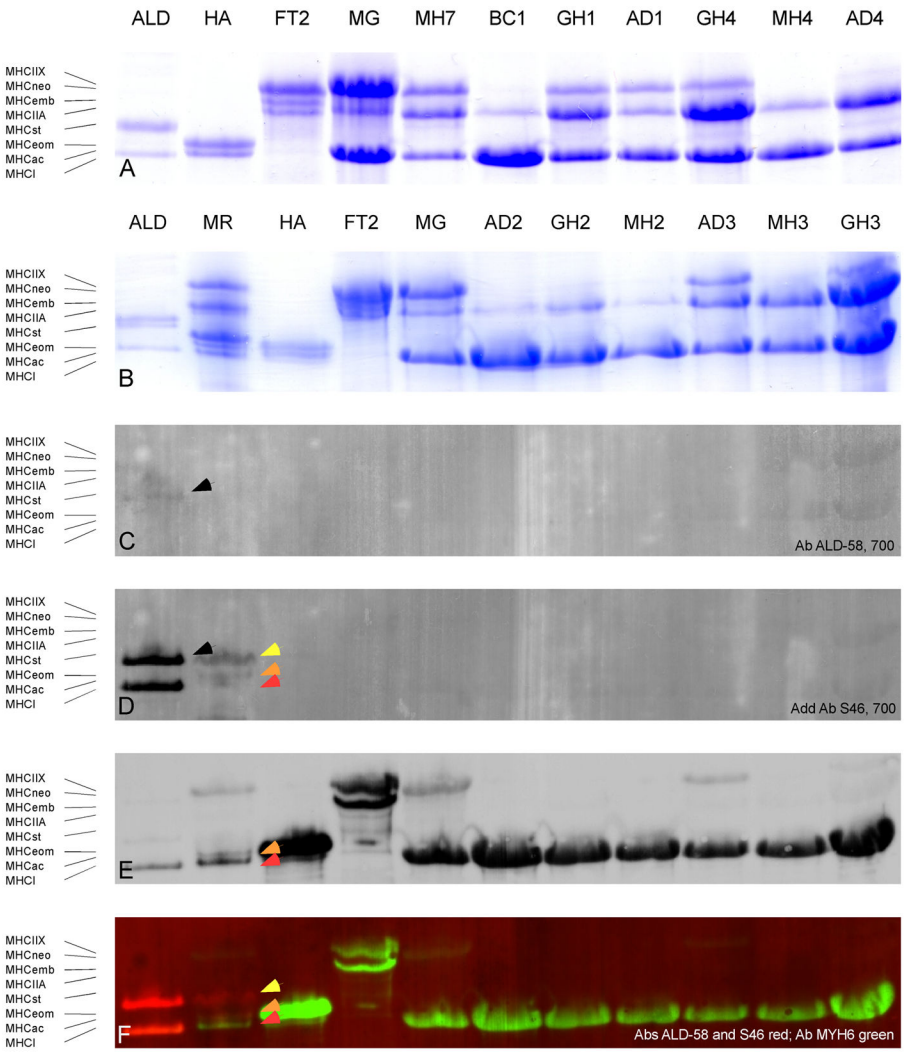

Figure 1.

Separation SDS-PAGE/Coomassie Stain and Western blots of control and suprahyoid muscles. (A). Separation SDS-PAGE/Coomassie of human suprahyoid muscles anterior digastric (AD1, AD4), geniohyoid (GH1, GH4) and mylohyoid (MH4, MH7) and control tissue (chick anterior latissimus dorsi muscle, ALD; human biceps brachii, BC; human late fetal tongue, FT2; human atrium, HA; human medial gastrocnemius, MG). Only MHCI, MHCIIA, and MHCIIX are present in suprahyoid muscles by Coomassie stain. (B-F). Separation SDS-PAGE/Coomassie Stain and Westerns demonstrate absence of developmental and unconventional myosin heavy chain (MHC) in human suprahyoid muscles anterior digastric (AD2, AD3), geniohyoid (GH2, GH3), and mylohyoid (MH2, MH3). By Coomassie stain only MHCI, MHCIIA, and MHCIIX are present in suprahyoid muscles (control tissue ALD, FT2, HA, MG, and human medial rectus, MR). (C, D). Antibodies (Abs) ALD-58 and Ab S46 do not label MHC in suprahyoid muscles. Black arrow indicates band reacting with Abs ALD-58 and S46 in ALD. Yellow arrow, MHCslow tonic (MHCst). Orange arrow, putative MHCalpha-cardiac (MHCac) in MR. Red arrow, putative MYH15. (E). Ab MYH6 labels MHCI, MHCac, MHCembryonic (MHCemb),

MHCneonatal (MHCneo) and MHCIIX. Note the absence of MHCac and MHCemb by Western blot in suprahyoid muscles. (F) Merge of Abs ALD-58 and S46 (red) and Ab MYH6 (green) showing relative location of bands.

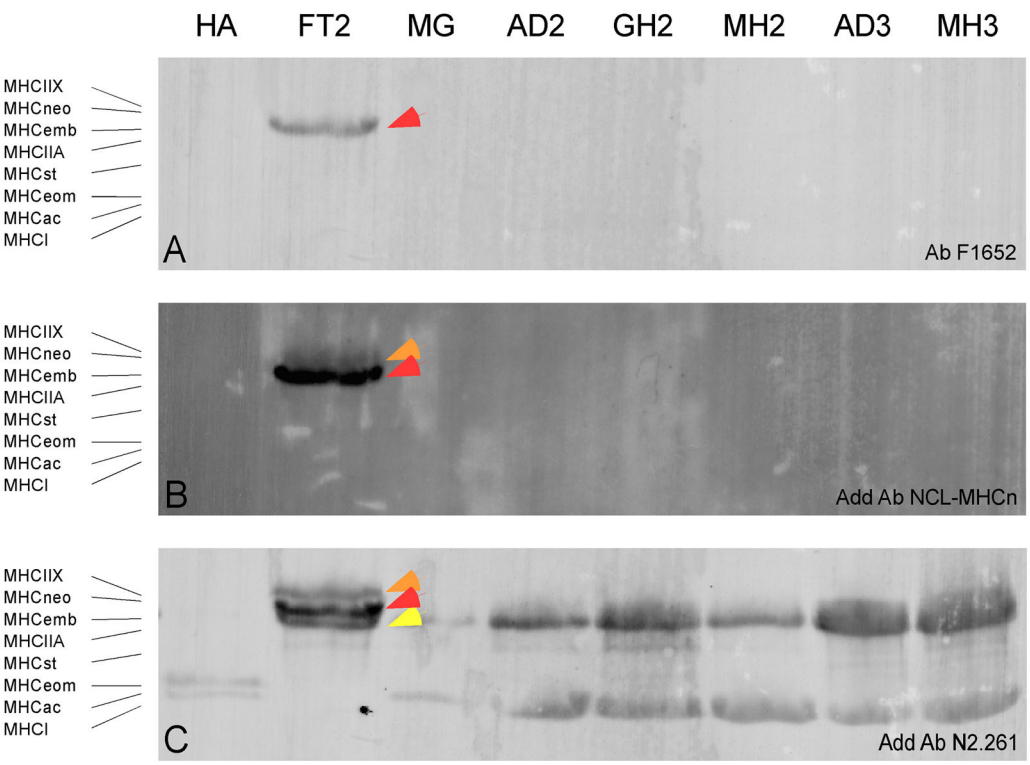

Figure 2.

By Separation SDS-PAGE/Western blot, developmental myosin heavy chain (MHC) is not identified in human suprahyoid muscles anterior digastric (AD2, AD3), geniohyoid (GH2), or mylohyoid (MH2, MH3). (A). Ab F1.652 labels MHCembryonic (MHCemb) in fetal tongue only (FT2, black arrow). (B). Subsequent reaction with Ab NCL-MHCn additionally labels MHCneonatal (MHCneo, hatched arrow) in fetal tissue only. (C). Subsequent reaction with Ab N2.261 labels MHCI and MHCIIA (open arrow) and MHCalpha-cardiac in human atrium (HA). MG, medial gastrocnemius.

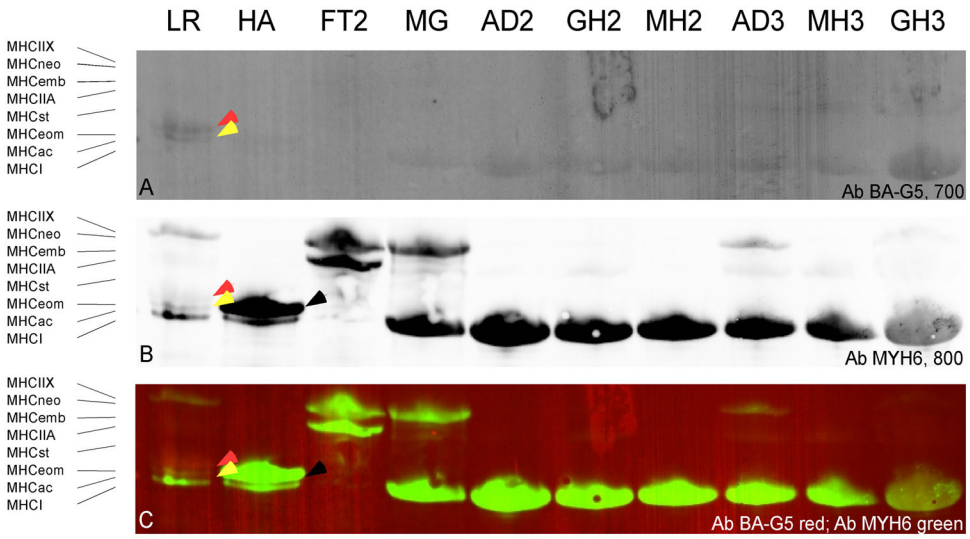

Figure 3.

Separation SDS-PAGE showing absence of myosin heavy chain alpha-cardiac (MHCac) in human suprahyoid muscles anterior digastric (AD2, AD3), geniohyoid (GH2, GH3), and mylohyoid (MH2, MH3). (A). Antibody (Ab) BA-G5 labels MHCextraocular (MHCeom, red arrow) and putative MHCalpha-cardiac (MHCac, yellow arrow) in lateral rectus (LR). Ab BA-G5 does not label MHC in human atrium (HA) or in suprahyoid muscles. (B) Ab MYH6 labels putative MHCac (yellow arrow) in lateral rectus (LR) and MHCac in HA (black arrow). Ab MYH6 does not label MHCac in suprahyoid muscles. (C). Merge of Ab BA-G5 (red) and Ab MYH6 (green). MG, human medial gastrocnemius.

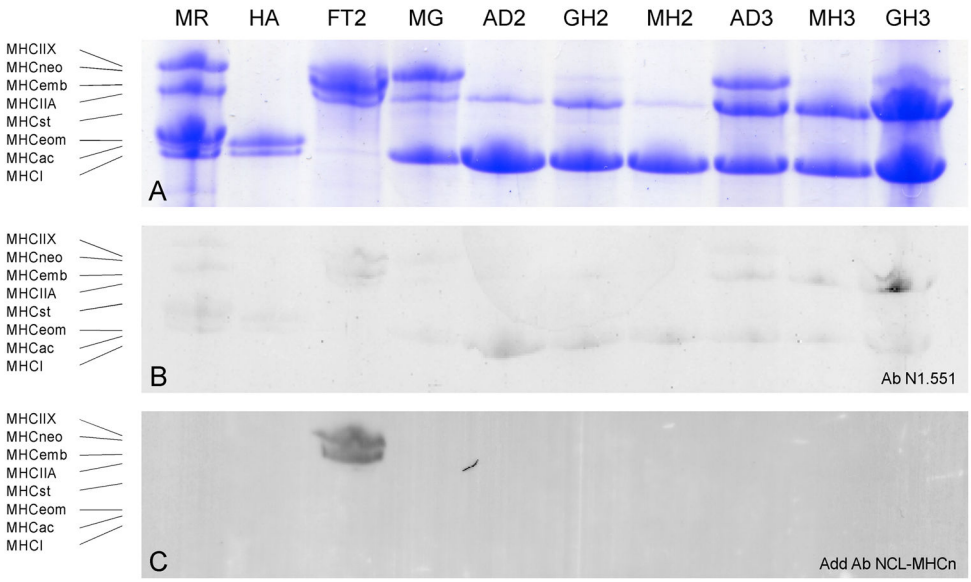

Figure 4.

Separation SDS-PAGE/Western blot showing weak and non-specific staining of myosin heavy chain (MHC) staining with antibody (Ab) N1.551 (A). Coomassie stain. (B) Western blot with Ab N1.551. (C) Subsequent addition of Ab NCL-MHCn labels developmental MHC in fetal tongue (FT2) but not suprahyoid muscles (AD, GH, MH).

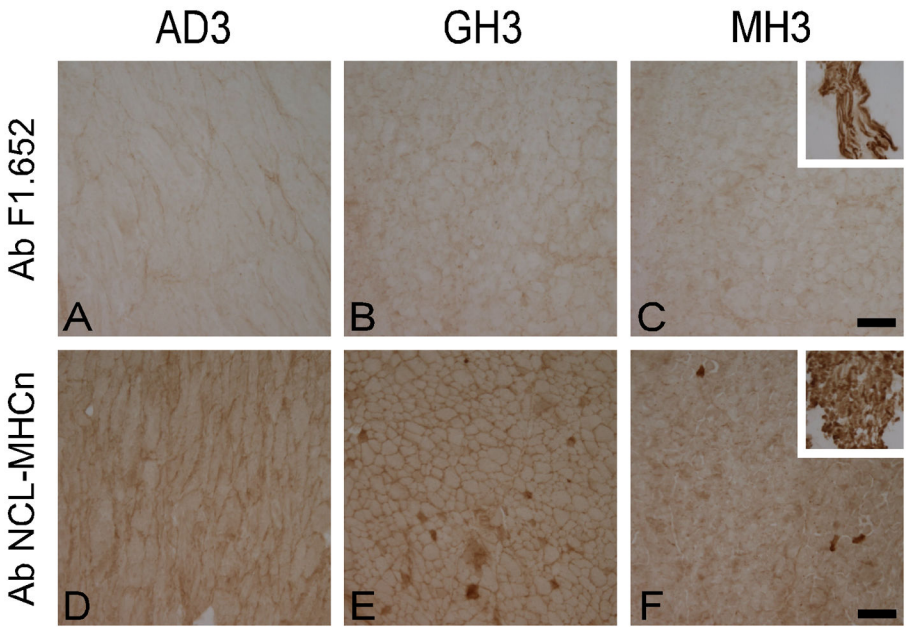

Figure 5.

Immunohistochemical reaction of antibody (Ab) F1.652 (anti-MHCembryonic) and Ab NCL-MHCn (anti-MHCneonatal) on human suprahyoid muscles anterior digastric (AD), geniohyoid (GH) and mylohyoid (MH) (all from Subject 3). No or few fibers are labeled by these Abs (for details see Table 3). Insets show reaction of same Abs with human fetal tongue (FT2) for comparison. For each Ab, all tissue was reacted on the same slide and photographed under identical optical conditions. Calibration bar = 100 microns.

Table 1

Human Subjects for Suprahyoid Muscle Study

Subject	Age	Gender	Muscles Studied by Method and Subject*		
			SDS-PAGE	Immunohistochemistry	PCR
1	86	W	AD, GH, MH	AD, GH	GH
2	63	W	AD, GH, MH	AD, MH	AD, GH, MH
3	82	W	AD, GH, MH	AD, GH, MH	
4†	89	W	AD, GH, MH	AD, GH, MH	
5	74	M		AD, GH, MH	
6	80	M		AD, GH, MH	
7	56	M	MH		MH

*AD = anterior digastric; GH = geniohyoid; MH = mylohyoid

†Parkinson Disease

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Table 2

Prevalence of MHC mRNA by Quantitative PCR

Sample	MHCI	MHCIIA	MHCIIX	MHCac*
GH1	60.5%	3.3%	30.9%	4.9%
GH2	59.7%	22.7%	17.1%	0.4%
MH2	85.7%	10.5%	2.7%	0.9%
MH7	17.5%	22.1%	58.6%	0.9%
AD2	84.0%	6.9%	7.3%	0.7%

*MHCalpha-cardiac

Table 3

Muscle Fibers Per mm^2 Tissue (Average and Range) Positive for Unconventional and Developmental Myosin Heavy Chain (MHC)

Muscle	N	MHCslow tonic	MHCembryonic*	MHCneonatal
Anterior Digastric	5	0.5 (0.1–1.1)	0.7 (0.0–1.8)	0.9 (0.1–2.1)
Geniohyoid	6	0.5 (0.0–0.9)	0.3 (0.0–0.8)	3.2 (0.5–11.7)
Mylohyoid	5	0.4 (0.1–1.0)	0.4 (0.0–1.1)	2.9 (0.2–9.8)

* Characterized with antibody NCL-MHCd.