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Myosin Heavy Chain Composition of the Human Genioglossus Muscle

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Abstract

Background—The human tongue muscle genioglossus (GG) is active in speech, swallowing, respiration and oral transport, behaviors encompassing a wide range of tongue shapes and movement speeds. Studies demonstrate substantial diversity in patterns of human GG motor unit activation but whether this is accompanied by complex expression of muscle contractile proteins is not known.

Purpose—We tested for conventional myosin heavy chain MHCI, MHCIIA, MHCIIIX, developmental MHCembryonic and MHCneonatal and unconventional MHC cardiac, MHCextraocular and MHCslow tonic in antero-superior (GG-A) and posterior (GG-P) adult human GG.

Method—SDS-PAGE, Western blot and immunohistochemistry were used to describe MHC composition of GG-A and GG-P and the prevalence of muscle fiber MHC phenotypes in GG-A. Results: By SDS-PAGE, only conventional MHC are present with ranking from most to least prevalent MHCIIA>MHCI>MHCIIIX in GG-A and MHCI>MHCIIA>MHCIIIX in GG-P. By immunohistochemistry many muscle fibers contain MHCI, MHCIIA and MHCIIIX but few contain developmental or unconventional MHC. GG-A is composed of five phenotypes (MHCIIA>MHCI-IIX>MHCI>MHCI-IIA>MHCIIIX). Phenotypes MHCI, MHCIIA and MHCIIIX account for 96% of muscle fibers.

Conclusions—Despite activation of GG during kinematically diverse behaviors and complex patterns of GG motor unit activity, the human GG is composed of conventional MHC isoforms and three primary MHC phenotypes.

Keywords

tongue; swallowing; speech; musculoskeletal system; normal respiration

Introduction

The human tongue muscle genioglossus (GG) is active in speech, swallowing, respiration and oral transport, behaviors that encompass a wide range of tongue shapes and movement speeds (Cheng, Peng, Chiou, & Tsai, 2002; Hirose & Kirtani, 1979; Napadow, Chen, Wedeen, & Gilbert, 1999; Shcherbaty & Liu, 2007; Tasko, Kent, & Westbury, 2002). Recent studies also demonstrate that motor units in the human GG exhibit a wide diversity of activation patterns, including differential modulation during inspiration and expiration (e.g., Bailey, Fridel, & Rice, 2007a; Saboisky et al., 2006; Wilkinson et al., 2010). Muscle fiber contractile properties are related to myosin heavy chain (MHC) composition (Bottinelli & Reggiani, 2000; D'Antona et al., 2002; Galler, Hilber, & Pette, 1997; Reiser, Moss, Giulian, & Greaser, 1985; Schiaffino & Reggiani, 1996) and it has been suggested that muscles with complex functional demands might have complex patterns of MHC expression (Butler-Browne, Eriksson, Laurent, & Thornell, 1998; Hoh, 2005). In human appendicular muscles, muscle fiber contractile diversity is typically achieved by homogeneous expression

of conventional MHCI, MHCIIA or MHCIIIX in individual muscle fibers and only limited hybridization of these isoforms (primarily MHCIIA-MHCIIIX hybridization; Andersen, Gruschy-Knudsen, Sandri, Larsson, & Schiaffino, 1999a; Canepari, Pellegrino, D'Antona, & Botinelli, 2010; Williamson, Gallagher, Carroll, Raue, & Trappe, 2001). In some human head and neck muscles, however, increased fiber contractile diversity is achieved by the expression of developmental and unconventional MHC (MHC cardiac, MHCembryonic, MHCextraocular, MHCneonatal, MHCslow tonic) and the hybridization of developmental, unconventional and conventional MHC in single fibers. Human extraocular muscles, for example, are composed of MHCI, MHCIIA, MHCIIIX, MHC cardiac (MHCac), MHCextraocular (MHCeom) and MHCslow tonic (MHCst) with as many as five MHC isoforms expressed in individual muscle fibers (Bormioli, Torresan, Sartore, Moschini, & Schiaffino, 1979; Kjellgren, Thornell, Andersen, & Pedrosa-Domellof, 2003; Wieczorek, Periasamy, Butler-Brown, Whalen, & Nadal-Ginard, 1985). MHCneonatal (MHCneo) and MHCac are expressed in the human masseter and pterygoid muscles and are hybridized with conventional MHC (Monemi, Liu, Thornell, & Eriksson, 2000; Yu, Stal, Thornell, & Larsson, 2002). Expression of unconventional MHC and conventional-unconventional MHC hybridization in single fibers may extend the range and fineness of gradation of muscle fiber contractile properties (D'Antona et al., 2002; Li, Rossmannith, & Hoh, 2000).

By virtue of activity during kinematically diverse behaviors and complex motor unit activation patterns, the human GG might be expected to exhibit a complex MHC organization. Previous studies reported primarily Type I and Type IIA fiber types in adult human GG by histochemical staining for myosin adenosinetriphosphatase (ATPase) (Carrera et al., 2004; Saigusa, Niimi, Yamashita, Gotoh, & Kumada, 2001; Sériès, Simoneau, St Pierre, & Marc, 1996) and predominantly MHCI, MHCIIA and developmental MHC in the neonate by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blot (Lloyd, Brozanski, Daood, & Watchko, 1996) but did not directly address the presence of MHC hybrid fibers or of developmental and unconventional MHC in adult human GG. Recently, the presence of MHCst in human GG has been proposed based on motor endplate (MEP) morphology (Mu & Sanders, 2010). We previously found minimal MHCst in two adult GG by IHC (Sokoloff, Yang, Li, & Burkholder, 2007b), but did not test for MHCst by region or by separation SDS-PAGE. To our knowledge the presence of other unconventional and developmental MHC has not been studied in the adult human GG. To address these issues we describe the MHC composition of the human GG by separation SDS-PAGE and immunohistochemistry (IHC).

Materials and Methods

Subjects and Tissue Preparation

Muscle tissue was taken from the left or right genioglossus within 9 hours post-mortem from ten adult human subjects (GG1-GG10) with no known neuromuscular disease (Table 1). Muscle was sampled from two architecturally-discrete regions of the GG (Figure 1): the (1) anterior and superior GG prior to entry into the tongue body (here designated GG-A, corresponding to anterior GG *sensu* Doran and Baggett (1972) and oblique GG *sensu* Mu and Sanders (2010) and (2) posterior and inferior GG (here designated GG-P, likely corresponding to the inferior portion of the oblique GG and horizontal GG *sensu* Doran and Baggett (1972) and the horizontal GG *sensu* Mu and Sanders (2010)). Additional tissue for immunohistochemical and electrophoresis control was obtained within 12 hours post-mortem from a human fetal tongue (FT), the medial gastrocnemius of an 80 year old male (MG), the extraocular inferior oblique muscle of a 63 year old female (IO), the atrium of a 62 year old female (HA) and the anterior latissimus dorsus muscle of the chick (ALD).

Tissue samples were immediately mounted onto tongue depressors with OCT tissue tek, quick-frozen in isopentane cooled by liquid nitrogen, and stored at -80°C. Tissue was obtained from the Emory University School of Medicine Body Donor Program (EUSMBDP) and from the National Disease Research Interchange (NDRI); all tissue used in this study is IRB-exempt.

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

Tissue preparation—Samples of GG-A, GG-P, and control FT, HA, IO, MG and chick ALD were prepared for electrophoretic identification of MHC (see Table 1 for age and sex of subjects used for GG SDS-PAGE). Control tissue was selected to enable identification of conventional, unconventional and developmental MHC bands. By SDS-PAGE and Western blot, human atrium contains MHCac and MHCI (Reiser, Portman, Ning, & Schomisch-Moravec, 2001), human gastrocnemius contains MHCI, MHCIIA and MHCIIIX (Widrick et al., 2001; see McGuigan et al., 2001 for MG), human fetal tongue muscle (GG) contains MHCemb, MHCneo, MHCI and MHCIIA (Lloyd et al., 1996) and human extraocular muscle contains MHCEom, MHCst, MHCI, MHCIIA and MHCIIIX (Liu, Eriksson, Thornell, & Pedrosa-Domellof, 2002; Kjellgren et al., 2003; Rossi, Mammucari, Argentini, Reggiani, & Schiaffino, 2010).

Approximately 40-50 mgs of muscle tissue was homogenized in 200µl of 0.1M potassium phosphate (PBS) buffer (pH 7.3) and 5% protease inhibitor cocktail (Sigma, Aldrich) with a Tissuemiser 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 (1993), 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 6× 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 (2.0 µg/lane; except for chick ALD 0.25 µg/lane) were loaded (following Bamman et al., 1998). 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 (HP scanjet 5400C).

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 0.5× blocking buffer (USB Corporation) at room temperature for 40 minutes, membranes were incubated overnight with primary antibody in

0.5× blocking buffer with 0.1% Tween 20 at 4°C (for Antibody (Ab) concentrations see Table 2). Following PBS wash (4×5 minutes each), membranes were incubated with appropriate secondary IRDye 700D× goat anti-mouse IgG (Rockland, Gilbertsville, PA), IRDye 800CW goat anti-mouse IgM (Rockland, Gilbertsville, PA), or with Daylight 800 goat anti-rabbit IgG (Thermoscientific, Rockford, IL) and visualized by Odyssey Infrared Imaging System (LI-COR). On additional membranes, different Abs were reacted sequentially to assist in identification of MHC bands.

Immunohistochemical Methods

Serial 12-μm thick cross-sections were cut on a cryostat at −23°C and mounted onto gelatin-subbed slides. For analysis of muscle fiber phenotype, sections of GG-A (GG1, GG2, GG3, GG4, GG5 and GG9) were reacted with a battery of Abs to identify developmental (MHCemb, MHCneo), conventional (MHCI, MHCIIA, MHCIIX) and unconventional (MHCac, MHCeom, MHCst) MHC.

IHC can resolve MHC identity at the single fiber level but may result in false-positive assignment of MHC due to Ab cross-reactivity. We sought to minimize false-positive assignment by (1) using a battery of Abs commonly used to identify human MHC and (2) testing reactivity of the recently developed Ab MYH6 at multiple Ab concentrations.

To determine expression of developmental MHC we reacted tissue with Ab NCL-MHCd (Vector Laboratories, Burlingame, CA), which in mammals is likely specific for MHCembryonic (MHCemb) (Brueckner, Itkis, & Porter, 1996) and Ab NCL-MHCn (Vector Laboratories), which in mammals is considered specific for MHCneo (Ecob-Prince, Hill, & Brown, 1989; Yu et al., 2002).

To determine expression of unconventional MHC we reacted tissue with Ab 4A6 (Developmental Studies Hybridoma Bank, Iowa, DSHB), which in rabbits and likely baboons and humans is specific for MHCeom (Kjellgren et al., 2003; Lucas & Hoh, 1997; Sokoloff, Daugherty, & Li, 2010), Ab MYH6 (Sigma-Aldrich), which in humans is reported to be specific for MHCac (Stirn Kranjc, Smerdu & Erzen, 2009) and Ab S46 (DSHB), which reacts with putative MHCst in adult human muscle spindles and human extraocular muscles but does not cross-react with MHCac or MHCI in adult catarrhine primate muscle (Sokoloff, Yang, Li, & Burkholder, 2007b).

To determine expression of conventional MHC we reacted tissue with Ab A4.84 (DSHB), which in humans is reported to be specific for MHCI(cardiac) (Hughes et al., 1993; Li, Lehar, Nakagawa, Hoh, & Flint, 2004), Ab MY-32 (Sigma-Aldrich, St Louis, MO), which recognizes MHCII isoforms in mammals and MHCneo in rats (Harris, Fitzsimons, & McEwan, 1989), Ab S5-8H2 (Agro-Bio, La Ferté Saint Aubin, France) which in humans reacts with MHCI and MHCIIX (Serrano et al., 2001) and Ab SC-71 (American Type Culture Collection, ATCC) which in humans is reported to be specific for MHCIIA (Li et al., 2004; Serrano et al., 2001), to label MHCIIA>MHCIIX (Smerdu & Soukup, 2008; Yu et al., 2002) or to label MHCIIA and MHCIIX (Bamman et al., 1998).

In three additional tongues (GG5, GG6, GG8) sections of GG-A and GG-P were reacted with Ab S46, Ab NCL-MHCn and Ab F1.652 (DSHB, specific for MHCemb, Cho et al., 1994) to enable analysis of MHCst, MHCneo and MHCemb by GG region. All MHC Abs were raised in mouse with the exception of Ab MYH6 (rabbit host).

Ab MY-32 was reacted at dilution of 1:400, Abs SC-71 and S46 were reacted at dilution of 1:25, Ab S5-8H2 was reacted as supernatant, Abs 4A6, A4.84, F1.652, NCL-MHCd, NCL-MHCn were reacted at dilution of 1:5 and Ab MYH6 was reacted at dilutions of 1:5, 1:25

and 1:50. On additional serial sections Abs NCL-MHCd, NCL-MHCn and S46 were co-reacted with the anti-laminin Ab D-18 (DSHB, 1:25 or 1:100 dilution) to aid in muscle fiber visualization.

Tissue was reacted following the protocol of Eason, Schwartz, Shirley, and English (2000). 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 secondary antibody (for Ab 4A6, peroxidase-conjugated goat IgM fraction to mouse immunoglobulins, Capel dilution 1:100; for Ab MYH6 peroxidase-conjugated goat IgM fraction to rabbit immunoglobulins, Capel dilution 1:100; for all other Abs, peroxidase-conjugated goat IgG fraction to mouse immunoglobulins, Capel dilution 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% H₂O₂). Slides were then washed with water for 1 minute, dehydrated, and coverslipped in permount.

Tissue sections were viewed on an Olympus B×51 microscope at 100×, 200× and 400× magnification. Images were collected with Neurolucida software (Microbrightfield, Burlington, VT) and a MicroFire digital microscope camera (Optronics, Goleta, CA) and stored onto computer (Dell Optiplex GX270, 1280 × 1024 pixel resolution).

Control Experiments

Specificity of Ab MYH6 by IHC—By IHC we previously demonstrated specificity of Ab S46 for putative MHCst in adult human extraocular muscle and muscle spindles (MS), of Ab 4A6 for putative MHCeom in adult human extraocular muscle, and of Abs NCL-MHCd and NCL-MHCn for developmental isoforms (Sokoloff et al., 2007b, Sokoloff et al., 2010). Here we tested the putative anti-MHCac Ab MYH6 (Stirn Kranjc et al., 2009) by IHC. Ab MYH6 at dilution of 1:5 and 1:25 weakly-to-strongly labeled control MS (for presence of MHCac in MS see Liu et al., 2003) in GG and weakly to moderately labeled extrafusal fibers positive for Ab A4.84 (Figures 2D, 2E). Ab MYH6 at 1:50 dilution weakly labeled MS and did not label extrafusal fibers (data not shown). We propose that Ab MYH6 reacts with MHCac more strongly than MHCI by IHC and ascribe strong label at 1:25 and weak label at 1:50 to the presence of MHCac.

Putative Mobility of MHC by SDS-PAGE of Control Tissue—SDS-PAGE of control tissue of known composition enabled identification of MHC bands in our hands. By coomassie staining, three bands were present in MG (putative MHCI, MHCIIA, MHCIIIX; McGuigan et al., 2001; see Figures 5, 6), two bands were present in HA (putative MHCI and MHCac; Reiser et al., 2001; see Figures 3, 5), four bands were present in IO (putative MHCI, MHCeom, MHCIIA, MHCIIIX; Liu et al, 2002; Kjellgren et al., 2003; see Figure 5) and four bands were present in FT (putative MHCI [weakly], MHCIIA, MHCemb, and MHCneo, see Lloyd et al., 1996; Figure 5). Putative MHCI, MHCIIA and MHCemb occupied unique positions. Putative MHCac migrated slightly faster or overlapped with putative MHCeom (Figure 3). Putative MHCneo co-migrated with putative MHCIIIX (Figure 3B).

Confirmation of MHC Mobilities by Western Blot—The identity of putative MHC bands was confirmed by Western blot (Figures 3-6). Ab A4.84 strongly labeled the fastest migrating MHC band, present in IO, HA and MG, i.e., MHCI (see Figure 5B; e.g., Li et al., 2004). Ab A4.84 also labeled a second discrete band of fast mobility in HA only, i.e., MHCac. Ab A4.84 did not label a band with similar mobility in extraocular muscles

(putative MHCeom). The MHCac band was also labeled by Ab MYH6 and Ab N2.261 (see below).

Ab NCL-MHCd labeled a single band in FT, i.e., MHCemb (Figure 4D). Ab NCL-MHCn labeled MHCemb and also the band with slowest migration in FT, i.e., MHCneo (Figures 3D, 4B). Abs NCL-MHCn and NCL-MHCd did not label any bands in any other muscles. Ab SC-71 moderately labeled the putative MHCIIA band in MG, extraocular muscle and FT and weakly and variably labeled the band of slowest mobility in MG (i.e., MHCIIIX, Figure 6C) but not in FT (i.e., MHCneo, Figure 4E). Ab S5-8H2 labeled MHCI and the slowest band in MG and extraocular muscle, i.e., MHCIIIX, but did not label a band with similar mobility in FT (i.e., MHCneo) (Figure 5D). Ab S46 labeled a band between MHCeom and MHCIIA in extraocular muscle not visible by coomassie, i.e., MHCst (Figure 3C). A similar migration for putative MHCst (MHCintrafusil) in human muscle spindles was noted by Liu et al. (2002). Ab MY-32 labeled MHCeom, MHCIIA, MHCneo and MHCIIIX bands (Figure 3E). Ab N2.261 labeled MHCI, MHCac, MHCeom and MHCIIA (see also Kjellgren et al., 2003; Liu et al., 2002) (Figure 5C). Ab MYH6, a putative anti-MHCac Ab (Stirn Kranjc et al., 2009) strongly labeled MHCac and moderately labeled MHCI, MHCemb, MHCneo and MHCIIIX bands (Figure 4C).

Table 2 summarizes Ab specificities on Western blots. Western blots confirm that MHC mobilities by SDS-PAGE are similar to previous human studies (e.g., D'Antona et al., 2002; Li et al., 2004; Lloyd et al., 1996; Yu et al., 2002) although in our hands MHCIIA migrates faster than developmental MHC (see Figure 4). Because of co-migration of MHCneo and MHCIIIX and overlapping migration of MHCeom and MHCac, confident identification of these MHC requires Western blot.

IHC Methods for GG MHC Phenotype Classification

Fascicles selected for MHC phenotype analysis (GG1-GG5, GG9) were negative for Ab MYH6 (dilution 1:50) and for Abs NCL-MHCn and 4A6 eliminating false-positive attribution of MHCIIIX by Ab MY-32 due to cross-reaction with MHCneo and MHCeom. We therefore determined MHC phenotype with Abs A4.84, MY-32 and SC-71. Fibers reacted strongly or moderately with Ab A4.84 and strongly, moderately or weakly with Abs SC-71 and MY-32. In the light of cross-reaction of Ab SC-71 with MHCIIIX (e.g., Smerdu & Soukup, 2008) we assign weak label with Ab SC-71 to reaction with MHCIIIX. Criteria for phenotype assignment based on reaction profiles are defined in Table 3. Assignment of phenotype was tested in a subset of fibers from GG2, GG3 and GG4 reacted with Ab S5-8H2 (anti-MHCI-MHCIIIX, Serrano et al., 2001). Ab S5-8H2 reacted with 306/306 MHCI fibers, 449/449 MHCI-IIX fibers, 17/19 MHCI-IIA fibers and 0/589 MHCIIA fibers (Figure 2F).

Most Abs either labeled many or no muscle fibers, but with Abs F1.652, NCL-MHCd, NCL-MHCn and S46 occasional fibers were labeled. For reactions with these Abs we counted every labeled muscle fiber and expressed this number relative to cross-sectional area of GG tissue analyzed (Stereo Investigator, MBF Bioscience, Burlington, VT).

Analysis of MHC Phenotype by IHC—Antibody reaction profiles and fiber MHC phenotype were determined for GG-A from six individuals. Fiber perimeters were traced and fiber cross-sectional area, perimeter and diameter were calculated in Neurolucida software. Fiber counts per 1000 were calculated for each phenotype. Ninety-five percent confidence intervals were estimated for the prevalence rates using exact methods based on the Poisson distribution. Repeated-measures analyses using mixed linear models were performed for perimeter, diameter and area utilizing SAS Proc Mixed (version 9, SAS Institute, Cary, NC). These models provide separate estimates of the means by phenotype. A

compound symmetry variance-covariance form in repeated measurements was assumed for each outcome, and robust estimates of the standard errors of parameters were used to do statistical tests and construct 95% confidence intervals. The model-based means are unbiased with unbalanced and missing data so long as the missing data are non-informative (missing at random). Statistical tests were 2-sided. A P-value less than 0.05 was considered statistically significant for the overall test comparing the 5 phenotypes for each outcome (diameter, cross-sectional area and perimeter). Based on post-hoc visual inspection of the estimated means we compared MHCIIA/MHCIIX to the other 3 phenotypes using a t-test for this contrast for each outcome and a P-value less than 0.05 was considered statistically significant. No other statistical comparisons were performed between the phenotypes for each outcome.

Analysis of MHC Composition by SDS-PAGE

MHC Composition of GG-A versus GG-P—Coomassie-stained bands in GG were quantified by reference to MHCI, MHCIIA and MHCIIIX bands in MG (IMAGE J software, NIH) and results from two gels averaged. Repeated-measures analyses using mixed linear models were performed for the relative percentages of the three primary MHC isoforms (MHCI, MHCIIA, MHCIIIX) of the human GG using SAS Proc Mixed (version 9, SAS Institute, Cary NC). The models provided separate estimates of the anterior (GG-A) and posterior (GG-P) region means for each of the three isoforms. A compound symmetry variance-covariance form in repeated measurements was assumed for the outcome, and robust estimates of the standard errors of parameters were used to do statistical tests and calculate the standard error of the mean.

MHC Composition of GG-A by Age—Similar repeated-measures analyses were performed for the anterior samples only to compare MHC prevalence between the three younger subjects (< 60 years) and the five older subjects (≥ 60). The model-based means (least-squares means) are unbiased with unbalanced and missing data so long as the missing data are non-informative (missing at random). Statistical tests were 2-sided. A P-value less than 0.05 was considered statistically significant. Caution is necessary in the interpretation due to the small sample sizes.

Results

Electrophoretic Identification of MHC Isoforms

Developmental and unconventional MHC was not identified in Western blots of GG-A or GG-P (reaction in GG-A shown in Figure 4). Western blots of GG-A and GG-P demonstrated MHCI, MHCIIA and MHCIIIX (weakly). MHCI, MHCIIA and MHCIIIX were quantified in coomassie-stained gels of GG-A and GG-P (Figure 5A). Compared to GG-A, GG-P contained less MHCIIIX (5.3% versus 7.7%, P value 0.02), less MHCIIA (41.7% versus 58.0%, P value 0.04) and more MHCI (52.0% versus 34.4%, P value 0.04) (Table 4; Figure 7). Although the sample size was small, comparison of GG-A MHC prevalence between “young” (<60 years of age) and old subjects (>60 years of age) revealed a trend to less IIA in old subjects (P=0.52) (Table 5).

Immunohistochemical Identification of MHC Isoforms

MHCI, MHCIIA, MHCII—Abs A4.84, MY-32 and SC-71 each labeled many fibers in all GG-A samples (Figure 2). Of 2671 fibers, 1330 (50%) were positive for Ab A4.84, 2203 (82%) were positive for Ab MY-32 and 1408 (53%) were positive (moderately or strongly labeled) for Ab SC-71.

MHCac, MHCemb, MHCeom, MHCneo and MHCst—Abs F1.652, MYH6, 4A6, NCL-MHCd, NCL-MHCn and S46 labeled few or no fibers in GG-A and GG-P sections (Figure 2). No fibers were positive for Ab 4A6 (anti-MHCeom; data not shown) and few were positive for Ab NCL-MHCn (anti-MHCneo), Abs NCL-MHCd or F1.652 (anti-MHCemb), Ab S46 (anti-MHCst) or Ab MYH6 (anti-MHCac>MHCI) (Table 6).

Muscle Fiber Phenotype Classification and Size Measures—The prevalence and morphometry of muscle fiber phenotypes was determined from GG-A muscle fibers from 6 subjects. Table 7 provides a summary of the fiber counts/1000 for each phenotype by individual. The most frequent fiber type was MHCIIA, followed by MHCI-IIX, MHCI, MHCI-IIA and MHCIIIX (Figure 8).

Overall mean differences between phenotypes were detected for the diameter ($P = 0.01$), cross-sectional area ($P = 0.0017$) and perimeter ($P = 0.004$) (Figure 9). Mean morphometric measures were highest for MHCIIA and MHCIIIX compared to the other 3 phenotypes ($P = 0.001$, $P = 0.008$, and $P = 0.0015$ for the diameter, cross-sectional area and perimeter).

Discussion

Summary

The primary finding of this study is that the adult human GG is composed primarily of MHCI and MHCIIA with limited MHCIIIX and no appreciable developmental and unconventional MHC. A secondary finding is that, by our criteria, MHCIIA, MHCI-IIX and MHCI comprise 96% of MHC phenotypes in GG-A. Thus despite a complex activation of GG motor units in kinematically diverse oromotor behaviors, the GG is similar to most human appendicular muscles in conventional MHC composition and limited MHC phenotype diversity.

Comparisons to Other Studies of Human Tongue Muscles

Myosin Heavy Chain Composition—In a study of human fetal and neonatal GG Lloyd et al. (1996) described MHCI and MHCIIA at gestational age 31-42 weeks with limited developmental MHC (<10%) and MHCIIIX (<2%). Thus with the exception of developmental MHC, MHC composition of the neonatal and adult human GG is similar. By SDS-PAGE of human intrinsic tongue muscles, Granberg et al. (2010) reported MHCIIA>MHCI, limited MHCIIIX and no developmental and unconventional MHC, a composition similar to our determination by MHC mRNA Polymerase Chain Reaction (PCR) of MHCIIA>MHCI=MHCIIIX and limited developmental and unconventional MHC in human anterior tongue body muscles (Rahnert, Sokoloff, & Burkholder, 2010). Previously we were unable to identify developmental MHC in adult human tongue muscles styloglossus and hyoglossus by immunoblot and IHC (Sokoloff et al., 2007a; Sokoloff et al., 2010) or MHCst in adult human extrinsic and intrinsic tongue muscles by IHC (Sokoloff et al., 2007b). In concert these studies demonstrate that human tongue muscles are comprised of conventional MHC with minimal developmental and unconventional MHC. In this respect human tongue muscles are similar to human appendicular muscles and many human head and neck muscles (e.g., Andersen et al., 1999a; Toniolo et al., 2008; Williamson et al., 2001) but differ from human eye and masticatory muscles which express appreciable unconventional and developmental MHC (Kjellgren et al., 2003; Korfage, Brugman, & Van Eijden, 2000; Yu et al., 2002).

In our limited sample there was significantly less MHCIIA and MHCIIIX and significantly more MHCI in GG-P compared to GG-A (Table 4). A “slower” profile of MHC in posterior regions thus appears to be a general feature of human tongue muscles GG, T, V, SL (see

Granberg et al., 2010; Stal, Marklund, Thornell, De Paul, & Eriksson, 2003). By ATPase, a “slower” Fiber Type profile was reported in the horizontal versus anterior-oblique GG of the dog (Mu & Sanders, 1999). In contrast, a faster MHC profile was reported in the posterior versus anterior rat GG by SDS-PAGE (Volz et al., 2007). We also noted a trend toward less MHCIIA in the GG-A with aging. A shift to a slower profile of MHC occurs in the rat anterior GG with aging (Schaser, Wang, Volz, & Connor, 2010).

Myosin Heavy Chain Phenotype—By ATPase, approximately 30% Type I and 70% Type II fibers were reported in the human anterior GG in normal, snorer and sleep-apnea hypopnea subjects (Saigusa et al., 2001; Sériès et al., 1996). In contrast, a later study reported 64% Type I and 36% Type II fibers in the human GG (Carrera et al., 2004, Control data from their Table 3). By IHC we found 17.6% phenotype MHCI, 49.6% phenotype MHCIIA and 29.3% MHCI-IIX hybrid fibers (29.3%) in the GG-A. By IHC, MHC composition of the GG-A is thus similar to human extrinsic tongue muscles hyoglossus and styloglossus with >94% phenotypes MHCIIA, MHCI and MHCI-IIX (Sokoloff et al., 2007a; Sokoloff et al., 2010).

In human anterior intrinsic tongue muscles, Granberg et al. (2010) reported 21.4% MHCI, 58.5% MHCIIA, 7.9% MHCI-IIA and 1% MHCIIIX fiber phenotypes by IHC, a distribution similar to our findings in the GG-A (see Stal et al., 2003 for Type distribution by ATPase). In contrast to the GG-A, Granberg et al. (2010) reported 11.3% MHCIIA-IIX and 0% MHCI-IIX phenotypes. Methodological differences may in part explain disparate findings of MHCIIA-IIX and MHCI-IIX in anterior intrinsic and anterior GG tongue muscles. We may have overestimated the prevalence of MHCI-IIX by categorization of 127 fibers with Ab profile A4.84-positive/MY-32-positive/SC-71-weak as MHCI-IIX. Antibody A4.74, used as a marker of MHCIIA by Granberg et al. (2010), may weakly react with MHCIIIX (Smerdu & Soukup, 2008), and it is possible that some fibers identified as MHCI-IIA by Granberg et al. (2010) are phenotype MHCI-IIX (or phenotype MHCI-IIA-IIX). However, we can assign the 654 GG-A fibers with profile A4.84-positive/MY-32-positive/SC-71-negative to MHCI-IIX with confidence. Further, we could not identify phenotype MHCIIA-MHCIIIX with the anti-MHCI-IIX Ab S5-8H2 in GG-A. It thus appears that anterior intrinsic tongue muscles and GG-A differ in the presence of phenotype MHCIIA-MHCIIIX and possibly MHCI-IIX. MHC expression is related to the pattern of nerve activation (Ausoni, Gorza, Schiaffino, Gundersen, & Lomo, 1990; Pette, 2001a; Schiaffino, Sandri, & Murgia, 2007) and MHC phenotype disparity might reflect differential patterns of motor unit recruitment between anterior intrinsic and GG-A (and other extrinsic) tongue muscles.

By IHC, developmental and unconventional MHC were present in no or few fibers in GG-A and GG-P. Recent studies with sensitive IHC and PCR methods also document little or no developmental and unconventional MHC in adult human hyoglossus and styloglossus (Sokoloff et al., 2007a, 2007b, 2010) and adult human intrinsic tongue muscles (Granberg et al., 2010; Rahnert et al., 2010; Sokoloff et al., 2007b). A limited expression of developmental MHC is characteristic of many human head and neck muscles and may relate to fiber remodeling due to disease, injury, aging or normal muscle use (e.g., St Pierre & Tidball, 1994; Rosser, Waldbillig, Lovo, Armstrong, & Bandman, 1995; Snow, McLoon, & Thompson, 2005). Absence of appreciable developmental MHC in human tongue muscles suggests these muscles are adapted to normal mechanical requirements without persistent remodeling, even in the elderly.

Among adult human extrafusal muscle fibers appreciable MHCst is documented in extraocular muscle fibers (Bormioli et al., 1979; Kjellgren et al., 2003, Rahnert et al., 2010; Rossi et al., 2010; Sokoloff et al., 2007b) in association with novel neuromuscular features including multiple motor endplate (MEP) morphotypes, multiple MEPs/muscle fiber and

likely polyneuronal innervation (Bormioli et al., 1979; Fujii, Abe, Nunomura, Moriuchi, & Hozawa, 1990; Oda, 1986). Due to the presence of “en grappe” MEP morphology and dual MEPs on human tongue muscle fibers (Alvarez et al., 1989; Slaughter, Li, & Sokoloff, 2005; Mu & Sanders, 2010), Mu and Sanders (2010) suggested that human tongue muscles including posterior GG contain MHCst, but the absence of appreciable MHCst in extrinsic and intrinsic human tongue muscles by IHC, SDS-PAGE and PCR (Sokoloff et al., 2007b; Granberg et al., 2010; Rahnert et al., 2010, present study) precludes this possibility. En grappe MEP morphology and multiple MEP innervation are also present in human laryngeal muscles that lack appreciable MHCst (Brandon et al., 2003; Périè et al., 1997; Sokoloff et al., 2007b; Toniolo et al., 2008). Whether MEP morphotypes are related to conventional MHC phenotypes in human tongue muscles is not known.

Muscle Fiber Hybridization of MHC in the GG-A

Intra-fiber hybridization of MHCIIA-MHCIIX and of MHCI-MHCIIA is common in human muscles, including intrinsic tongue muscles (Canepari et al., 2010; Kohn, Essen-Gustavsson, & Myburgh, 2007; Granberg et al., 2010). Intra-fiber hybridization of MHCI-MHCIIX however is rare in human appendicular muscles (Andersen et al., 1999a; Andersen, Terzis, & Kryger, 1999b; Canepari et al., 2010; Larsson & Moss, 1993), but is present in some human head and neck muscles including extrinsic tongue muscles hyoglossus and styloglossus (e.g., Korfage & Van Eijden, 2003; Monemi et al., 2000; Sokoloff et al., 2007a; Sokoloff et al., 2010). Because contractile properties of MHC-hybrid fibers are related to the prevalence of constituent MHC (Larsson & Moss, 1993; Andruchov, Wang, Andruchova, & Galler, 2004), variation in relative MHC by fiber may increase gradation of fiber contractile properties. The extent to which MHCI-IIX hybridization promotes fiber contractile diversity in GG-A fibers is however unclear; although we documented 29.3% fibers of phenotype MHCI-IIX in GG-A, we found limited MHCIIX by SDS-PAGE suggesting a strong bias toward MHCI in MHCI-IIX hybrid fibers and consequently limited contractile diversity.

In some human muscles the prevalence of MHC hybrid fibers, including MHCI-IIX, increases with age (e.g., Andersen et al., 1999b). In our limited GG-A sample, prevalence of MHCI-IIX fibers was similar in old and young subjects suggesting normative and not age-related expression. Muscle fiber MHC is modulated by nerve activity in a muscle-dependent manner with MHCI expression generally associated with persistent low-frequency nerve activation and MHCIIX expression with high-frequency activation and also muscle disuse (Ausoni et al., 1990; Pette & Staron, 2001b; Talmadge, 2000). Studies of human GG motor unit activity during respiration identify multiple motor unit groups based on activity profile, although composition of these groups is not fixed (e.g., Saboisky et al., 2006; Bailey et al., 2007a; Wilkinson et al., 2010). This raises the possibility that MHCI-IIX fibers may belong to motor units with varying activation in different behaviors, for example, predominantly low frequency activation in one behavior (e.g., respiration) and high frequency activation in another (e.g., swallowing, rapid tongue movement; Bailey, Rice, & Fuglevand, 2007b; Miller & Bowman, 1974). Additionally, expression of MHCIIX mRNA increases with high eccentric load (Friedmann-Bette, et al., 2010). Co-activation of human tongue muscle anatomical “antagonists” in oromotor behaviors (e.g., Miyawaki, Hirose, Ushijima, & Sawashima, 1975; Mateika, Millrood, Kim, Rodriguez, & Samara, 1999) is compatible with the eccentric activation of tongue muscle fibers.

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Figure 1.

Diagrammatic illustration of the human genioglossus indicating the anterior (GG-A) and posterior (GG-P) regions studied. See text for further description.

Figure 2.

Conventional myosin heavy chain (MHC) isoforms are predominant in the human genioglossus. Serial sections from the human anterior genioglossus (Subject GG2) reacted with (a) Antibody (Ab) NCL-MHCn (anti-MHCneonatal), (b) Ab NCL-MHCd (anti-MHCembryonic), (c) Ab S46 (anti-MHCslow tonic), (d) Ab MYH6 (putative anti-MHC cardiac, dilution 1:5), (e) Ab MYH6, (putative anti-MHC cardiac, dilution 1:25), (f) Ab S5-8H2 (anti-MHCI-IIX), (g) Ab A4.84 (anti-MHCI), (h) Ab MY-32 (anti-MHCII-MHCneo-MHCeom) and (i) Ab SC-71 (anti-MHCIIa>IIx). Tissue in Figures b, c, g, h, and i also reacted with Ab D-18 (anti-laminin) to assist in fiber identification. Whereas Abs to conventional MHCI, MHCII and MHCIIA react with many extrafusal muscle fibers, Abs to unconventional and developmental MHC react with few or no fibers. Note reaction of intrafusal muscle spindle (MS) fibers with Abs to developmental and unconventional MHC. *, ^, and + denote three fibers with phenotype MHCIIA, MHCI and MHCI-IIX respectively. Calibration bar = 50 μ m.

Figure 3.

Electrophoretic separation of myosin heavy chain (MHC) in control muscle tissue demonstrates MHC mobilities. Separation gel electrophoresis and Western blot of myosin fraction in human inferior oblique (IO), human atrium (HA), human fetal tongue (FT) and chick anterior latissimus dorsi (ALD) showing migration of human myosin heavy chain (MHC). (b) Note similar migration of MHCIX and MHCneonatal (MHCneo) and of MHCextraocular (MHCEom) and MHC cardiac (MHCac). (c) Note reaction of antibody (Ab) S46 with putative MHCslow tonic (MHCst) in human IO despite absence of corresponding band in coomassie stain (a, b). (d) Ab NCL-MHCn labels MHCembryonic (MHCemb) and MHCneo bands in FT. (e) Ab MY-32 labels MHCi, MHCeom, MHCiia, MHCneo and MHCiix bands.

Figure 4.

Developmental and unconventional myosin heavy chain isoforms are not present in Western blots of the anterior human genioglossus. Separation gel electrophoresis of myosin fraction in control muscles chick anterior latissimus dorsi (ALD), human inferior oblique (IO), human fetal tongue (FT) and human atrium (HA) and in the human anterior genioglossus (subjects GG1, GG2, GG3) reacted for antibodies (Abs) to developmental and unconventional MHC. Genioglossus is negative for antibodies to MHCslow tonic (MHCst) (a) and developmental MHC (b, d). (c) Putative anti-MHC cardiac antibody MYH6 reacts strongly with MHC cardiac (MHCac) in atrium but also reacts with MHCi, MHCneonatal (MHCneo), MHC embryonic (MHCemb) and MHCIIIX. In genioglossus Ab MYH6 labels only the MHCi band. Sequential application of antibodies to MHCemb (d), MHCIIA (e) and developmental MHC (f) identifies fast-to-slow migration of MHCIIA, MHCemb and MHCneo.

Figure 5.

Developmental and unconventional myosin heavy chain (MHC) isoforms are not present in Western blots of the human anterior (GG5-A, GG6-A) and posterior (GG5-P, GG6-P) genioglossus. (a) Coomassie-stained gel of myosin fraction of human anterior and posterior GG showing presence of MHCI, MHCIIA and minimal MHCIIIX. (b) Antibody A4.84 labels MHCI in GG and both MHCI and MHC cardiac (MHCac; arrow) in human atrium (HA). (c) Antibody N2.261 labels MHCI and MHCIIA (dotted line) in human medial gastrocnemius (MG) and genioglossus, MHCI and MHCac in HA, and MHCI, MHCIIA and MHCextraocular (MHCeom, white arrow) in inferior oblique (IO). (d) Additional application of antibody S5-8H2 demonstrates reaction with MHCI and MHCIIIX (black arrows). Note slight upward curvature of left side of membranes in (c) and (d).

Figure 6.

Conventional myosin heavy chain (MHC) isoforms MHCi and MHCIIA are predominant in the human anterior genioglossus. Coomassie-stained gel (a) and Western blots (b-d) of myosin fraction of human medial gastrocnemius (MG) and anterior genioglossus (subjects GG1-GG4). (b) The anti-MHCi antibody (Ab) A4.84 strongly labels MHCi in all samples. (c) The anti-MHCIIA>IIX antibody SC-71 strongly labels MHCIIA in genioglossus and labels MHCIIA and MHCIIIX (weakly) in MG. (d) MHCIIIX in genioglossus is below visual resolution in coomassie but is weakly labeled with antibody MY-32.

Figure 7.

Regional differences in myosin heavy chain composition of the human genioglossus. Quantification of MHCI, MHCIIA and MHCIIIX in anterior genioglossus (GG-A; N=8) versus posterior genioglossus (GG-P, n=5) by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and coomassie stain. Compared to GG-A, GG-P contains relatively less MHCIIIX (P value 0.02), less MHCIIA (P value 0.04) and more MHCI (P value 0.04).

Figure 8.

Five muscle fiber phenotypes are defined in the human anterior genioglossus by myosin heavy chain (MHC) composition with average ranking from most to least prevalent, MHCIIA>MHCI-IIX>MHCI>MHCI-IIA>MHCIIX. Subjects are identified under each pie chart (e.g., GG1).

Figure 9.

Mean diameter (μm), mean cross-sectional area (CSA, μm^2) and mean perimeter (μm) of muscle fibers in the human anterior genioglossus grouped by myosin heavy chain (MHC) phenotype (same sample as in Figure 8 and Table 7). Lines represent the model-based means and the vertical bars are the 95% confidence intervals. Overall mean differences between phenotypes were detected for the diameter ($P = 0.01$), cross-sectional area ($P = 0.0017$) and perimeter ($P = 0.004$). Mean morphometric measures were highest for the MHCIIA/MHCIX compared to the other 3 phenotypes ($P = 0.001$, $P = 0.008$, and $P = 0.0015$ for the diameter, cross-sectional area and perimeter).

Table 1
Human Subjects for Genioglossus Study

Subject	Age	Sex	Muscle Region(s) Studied ^a	Method of Investigation
GG1	80	M	A	Electrophoresis, Immunohistochemistry, Phenotype Analysis
GG2	19	M	A	Electrophoresis, Immunohistochemistry, Phenotype Analysis
GG3	46	F	A	Electrophoresis, Immunohistochemistry, Phenotype Analysis
GG4	80	M	A	Immunohistochemistry, Phenotype Analysis
			A, P	Electrophoresis
GG5	56	M	A	Phenotype Analysis
			A, P	Electrophoresis, Immunohistochemistry ^b
GG6	86	F	A, P	Electrophoresis, Immunohistochemistry ^b
GG7	82	F	A, P	Electrophoresis
GG8	66	F	A, P	Immunohistochemistry ^b
GG9	47	F	A	Phenotype Analysis
GG10	87	F	A, P	Electrophoresis

^a Anterior (A) and Posterior (P) regions of the genioglossus defined in text.

^b Tested for developmental and unconventional myosin heavy chain (MHC) with Abs S46 (anti-MHCslow tonic), F1.652 (anti-MHCembryonic) and NCL-MHCn (anti-MHCneonatal).

Table 2

Specificity of Antibodies on Western Blots of Human Muscle

Myosin heavy chain isoform	S5-8H2 (1:5)	A4.84 (1:500)	MYH6 ^a (1:1000)	MY-32 (1:1×10 ⁶)	NCL-MHCd (1:1000)	NCL-MHCn (1:1000)	N2.261 ^b (1:1000)	S46 (1:500)	SC-71 ^c (1:25)
MHCIIIX	X		X	X					X
MHCneonatal			X	X		X			
MHCembryonic			X		X	X			
MHCIIA				X			X		X
MHCslow tonic								X	
MHCextraocular				X			X		
MHC cardiac		X	X				X		
MHCI	X	X	X				X		

^aReacts strongly with MHC cardiac and MHCI, moderately with MHCembryonic and MHCneonatal and weakly with MHCXII.

^bReacts strongly with MHCIIA and MHCextraocular and weakly with MHCI and MHC cardiac.

^cReacts strongly with MHCIIA and weakly with MHCIIIX.

Table 3

Muscle Fiber Antibody (Ab) Reaction Profiles and Myosin Heavy Chain Phenotype Classification.

Myosin Heavy Chain (MHC) Isoform	Ab A4.84	Ab MY-32	Ab SC-71
MHCI	+++ / ++	-	-
MHCIIA ^a	-	+++ / ++ / +	+++ / ++
MHCIIIX	-	+++ / ++ / +	+ / -
MHCI-IIA ^b	+++ / ++	+++ / ++ / +	+++ / ++
MHCI-IIIX ^c	+++ / ++	+++ / ++ / +	+ / -

^a Assignment of MHCIIA supported by lack of reaction in 589/589 fibers tested with Ab S5-8H2 (anti-MHCI-IIIX).^b Assigned to MHCI-IIA but cannot rule out co-expression of MHCIIIX.^c Antibody SC-71 negative in 654/781 MHCI-IIIX fibers.

+++ = strong reaction

++ = moderate reaction

+ = weak reaction

- = no reaction

Table 4

Regional Differences in Myosin Heavy Chain Composition of Human Genioglossus by Electrophoresis.

Myosin Heavy Chain (MHC) Isoform	Muscle Region ^a	Sample Size	Percent of Total MHC Mean $\pm$ SEM	P Value
MHCIX	GG-A	8	7.7 $\pm$ 1.0	0.02
	GG-P	5	5.3 $\pm$ 1.0	
MHCIIA	GG-A	8	58.0 $\pm$ 3.4	0.04
	GG-P	5	41.7 $\pm$ 3.6	
MHCI	GG-A	8	34.4 $\pm$ 3.8	0.04
	GG-P	5	52.0 $\pm$ 4.2	

^aDefinition of anterior (GG-A) and posterior (GG-P) genioglossus regions provided in the text.

Table 5
Prevalence of Myosin Heavy Chain Isoforms in Anterior Genioglossus (GG-A) by Age

Myosin Heavy Chain (MHC) Isoform	Age Group ^a	Sample Size	Percent of Total MHC Mean $\pm$ SEM	P Value
MHCIX	Young	3	7.7 $\pm$ 2.1	0.99
	Old	5	7.7 $\pm$ 0.9	
MHCIIA	Young	3	65.8 $\pm$ 4.8	0.052
	Old	5	53.3 $\pm$ 3.1	
MHCI	Young	3	26.6 $\pm$ 6.2	0.11
	Old	5	39.0 $\pm$ 3.5	

^aYoung subjects = less than 60 years of age. Old subjects = 60 years of age.

Number of Muscle Fibers in Human Genioglossus Labeled by Antibodies to Developmental and Unconventional Myosin Heavy Chain^a.

Table 6

Subject	Muscle Region ^b	MHCneonatal ^c	MHCembryonic	MHCslow tonic	MHC cardiac
GG1	GG-A	3 (0.19)	2 (0.13)	21 (1.36)	2 (0.13)
	GG-A	4 (0.29)	1 (0.07)	0	0
GG2	GG-A	6 (0.51)	2 (0.17)	7 (0.59)	0
GG3	GG-A	5 (0.32)	0	6 (0.39)	0
GG4	GG-A	8 (0.34)	3 (0.13)	1 (0.04)	-
	GG-P	13 (0.55)	4 (0.17)	0	-
GG5	GG-A	85 (9.88)	4 (0.47)	0	-
	GG-P	9 (1.05)	1 (0.12)	0	-
GG6	GG-A	50 (2.17)	8 (0.35)	1 (0.04)	-
	GG-P	5 (0.22)	5 (0.22)	1 (0.04)	-

^aMHCneonatal determined by reaction with antibody NCL-MHCn; MHCembryonic determined by reaction with antibody NCL-MHCd (GG1-GG4) or antibody F1.652 (GG5, GG6, GG8); MHCslow-tonic determined by reaction with antibody S46; MHC cardiac determined for GG1-GG4 by moderate or strong reaction with antibody MYH6 at 1:25 dilution.

^bAnterior (GG-A) and posterior (GG-P) regions defined in the text.

^cNumbers in parentheses indicate the number of positive fibers per mm² tissue.

Table 7
Prevalence of Muscle Fiber Phenotypes in the Human Anterior Genioglossus (Counts/1000 Fibers).

Phenotype ^a	All Subjects n=6	GG1	GG2	GG3	GG4	GG5	GG9
MHCI	176 (159-191) ^b	130 (100-166)	149 (116-187)	411 (353-476)	141 (109-179)	53 (34-78)	191 (145-248)
MHCI-IIA	31 (25-39)	57 (38-82)	6 (1-18)	39 (23-62)	4 (1-15)	27 (15-47)	66 (40-102)
MHCI-III	293 (273-314)	337 (287-392)	279 (234-330)	388 (332-451)	332 (282-388)	101 (75-134)	343 (280-416)
MHCIIA	496 (469-523)	465 (406-529)	566 (501-637)	162 (127-205)	523 (460-592)	815 (736-900)	373 (307-448)
MHCIIIX	6 (3-10)	12 (5-27)	0 (0-8)	0 (0-8)	0 (0-8)	4 (0-15)	26 (11-52)

^aPhenotype determined by reaction of antibodies to myosin heavy chain (MHC) defined in Table 3.

^bNumbers in parentheses are 95% confidence intervals.