doi:10.1093/brain/awl095 Brain (2006), **129**, 1481–1492

MRI and clinical studies of facial and bulbar muscle involvement in MuSK antibody-associated myasthenia gravis

Maria Elena Farrugia,[1,2] Matthew D. Robson,[4] Linda Clover,[2] Phil Anslow,[5] John Newsom-Davis,[1] Robin Kennett,[1] David Hilton-Jones,[1] Paul M. Matthews[1,3] and Angela Vincent[1,2]

[1]Department of Clinical Neurology and [2]Neurosciences Group, Weatherall Institute of Molecular Medicine, [3]Centre for Functional Magnetic Resonance Imaging of the Brain and [4]Oxford University Centre for Clinical Magnetic Resonance Research, University of Oxford and [5]Department of Neuroradiology, Radcliffe Infirmary, Oxford, UK

Correspondence to: Prof. Angela Vincent, Neurosciences Group, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK
E-mail: angela.vincent@imm.ox.ac.uk

A proportion of patients with myasthenia gravis (MG) without acetylcholine receptor (AChR) antibodies have antibodies to muscle-specific kinase (MuSK). MG with MuSK antibodies (MuSK-MG) is often associated with persistent bulbar involvement, including marked facial weakness and tongue muscle wasting. The extent of muscle wasting in MuSK-MG, and whether it is also found in the few acetylcholine receptor (AChR-MG) patients who have persistent bulbar involvement, is not clear. We studied 12 MuSK-MG patients and recruited 14 AChR-MG patients matched broadly for age, sex ratio, duration of disease and degree of ocular, bulbar and facial weakness. We used coronal and sagittal T_1-weighted (T_1W) and T_2-weighted (T_2W) magnetic resonance imaging (MRI) to assess muscle wasting in facial and tongue muscles. Hyperintense signal on T_1W MRI and comparison of axial T_1W sequences with cUTE sequences were used to assess fibrous/fatty tissue in the tongue. We compared the results with those of four patients with myotonic dystrophy and 12 healthy individuals. We correlated the changes with clinical and treatment histories, and established a new ocular-bulbar-facial-respiratory (OBFR) score. At the time of study, none of the clinical measures, including the OBFR score, differed between the two MG groups. MRI demonstrated thinning of the buccinator, orbicularis oris (O.oris) and orbicularis oculi (O.oculi) muscles in MuSK-MG patients compared with healthy controls, whereas thinning of these muscles was not significant in AChR-MG. Tongue areas with T_1W high signal were increased in MuSK-MG patients and the intensity of the signal on axial T_1W sequences was greater in MuSK-MG than in controls. To look for possible correlations between imaging and clinical findings, we pooled results from all MG patients. The duration of treatment with prednisolone at >40 mg on alternate days (AD) correlated positively with the percentage of tongue area with high signal ($P = 0.006$) and negatively with MRI measurements of individual muscles and with the mean muscle dimensions ($P = 0.001$). The new OBFR score correlated positively with current Myasthenia Gravis Foundation of America grades and with the percentage of high signal ($P = 0.004$) and negatively with the mean muscle dimensions ($P < 0.001$). The results show that bulbar and facial muscle weakness and wasting are associated with significant muscle atrophy and fatty replacement in MuSK-MG, which was not found in the AChR-MG patients. MuSK antibodies *per se* may predispose to muscle thinning, but the difficulties in obtaining clinical remission under steroid therapy in some patients, resulting in long duration of treatment with higher doses (>40 mg AD), may be an additional factor.

Keywords: myasthenia gravis; seronegative myasthenia gravis; muscle-specific kinase; magnetic resonance imaging; ultra-short echo time

Abbreviations: AChR = acetylcholine receptor; AD = alternate day; cUTE = conventional ultra-short echo time; MD=myotonic dystrophy; MG = myasthenia gravis; MGFA = Myasthenia Gravis Foundation of America; MRI = magnetic resonance imaging; MUAP = motor unit action potential; MuSK = muscle-specific tyrosine kinase; O.oculi and O.oris = orbicularis oculi and orbicularis oris; OBFR = oculobulbar facial respiratory score; AChR-MG = seropositive (acetylcholine receptor antibody positive) MG; T_1W and T_2W = T_1 and T_2 weighted

Received December 6, 2005. Revised March 16, 2006. Accepted March 22, 2006. Advance Access publication May 3, 2006

Introduction

Myasthenia gravis (MG) is characterized by failure of neuromuscular transmission. In ~80% of the cases with generalized MG there are antibodies to the nicotinic acetylcholine receptor (AChR; AChR-MG). Of the remaining so-called seronegative MG patients, 3–70% have antibodies to muscle-specific tyrosine kinase (MuSK; MuSK-MG; Hoch *et al.*, 2001; Scuderi *et al.*, 2002; Sanders *et al.*, 2003*a*; McConville *et al.*, 2004; Yeh *et al.*, 2004; Zhou *et al.*, 2004; Vincent and Leite 2005). MuSK is a receptor tyrosine kinase, which is essential for the agrin-mediated clustering of AChRs during development (DeChiara *et al.*, 1996). However, AChR levels are not reduced at the neuromuscular junctions in MuSK-MG patients (Shiraishi *et al.*, 2005), and the disease mechanisms in these patients are not yet clear.

MuSK-MG patients usually have generalized weakness at or shortly after onset, but often develop particular involvement of the bulbar and facial muscles, and may first present with isolated weakness of the neck, shoulder or respiratory muscles (Scuderi *et al.*, 2002; Evoli *et al.*, 2003; Sanders *et al.*, 2003*a*; Zhou *et al.*, 2004). Neurophysiological studies of proximal muscles in five patients demonstrated short-duration motor unit action potentials (MUAPs), and deltoid muscle biopsy showed muscle fibre atrophy in four patients (Sanders *et al.*, 2003*a*). Some of these patients responded poorly to conventional steroid treatments (see also Evoli *et al.*, 2003), although some did well on newer treatments such as mycophenolate mofetil (Zhou *et al.*, 2004). Interestingly, generalized muscle weakness in these patients frequently improves with treatment, whereas bulbar and facial weakness may persist despite years of steroid treatment (Evoli *et al.*, 2003; Newsom-Davis, unpublished observations). Indeed, in contrast to typical AChR-MG patients, there may be little evidence of impaired neuromuscular transmission in limb muscles following treatments (Nemoto *et al.*, 2005; Stickler *et al.*, 2005; Farrugia et al., 2006). Marked facial weakness and wasting of the tongue has also been reported in a few cases of AChR-MG (De Assis *et al.*, 1994; Oosterhuis, 1997) but there has been little characterization of the atrophy or study of its frequency.

Collectively, these observations suggest that facial and bulbar involvement is particularly common in MuSK-MG, and we hypothesized that it could be compounded by use of long-term treatment with steroids. We, therefore, performed a comprehensive magnetic resonance imaging (MRI) study in MuSK-MG and selected AChR-MG patients, and related the findings to clinical and treatment features.

Material and methods

Subjects

All examinations were carried out with the approval of the Oxfordshire Research Ethics Committee (OxREC 01.194, 02.256 and 02.224). The records of the 15 available MuSK-MG patients who were regularly attending the Oxford myasthenia gravis centre were documented for clinical features. These records were compared with >190 case notes of AChR-MG patients, making it possible to select 15 subjects who were approximately matched with the MuSK-MG cohort for sex, age at onset, duration of disease and pattern of weakness. Since only 12 MuSK-MG patients and 14 AChR-MG patients consented to the MRI studies, the data are presented only for these patients (Table 1). A total of 12 healthy volunteers were recruited (mean age 35 years; age range 21–60; 8 females, 4 males) as healthy controls, and four patients with myotonic dystrophy were recruited as positive controls. AChR and MuSK antibody status was confirmed using MuSK and AChR antibody tests (RSR Ltd, UK).

Clinical assessment

Patient records for MuSK-MG and AChR-MG patients were assessed and an MGFA Class (Jaretzki *et al.*, 2000*a*, *b*) assigned for maximum severity and for their status at the time of the study. Their current disabilities were also measured with the quantitative MG score (QMG; Barohn *et al.*, 1998), the MG activities of daily living score (ADL; Barohn *et al.*, 1998) and the manual muscle test (MMT) (Sanders *et al.*, 2003*b*).

In order to provide a scoring system that reflected the muscles most involved in these patients, which could be used to correlate with other clinical or experimental measurements, we developed a scoring system to quantify ocular, bulbar, facial and respiratory function further (OBFR score). Details are given in the legend to Table 7.

Magnetic resonance imaging

MRI images were acquired on a 1.5 tesla Siemens Sonata (Siemens Medical Solutions, Erlangen, Germany) using the head coil and the front element of the neck coil, which boosts the signal from around the mouth. After initial localization a T_1 weighted (T_1W) axial dataset was acquired (spin-echo, TR = 500, TE = 7.7 ms, 1.2 × 0.9 × 5 mm^3, resolution over 19 slices with 2 averages requiring

Table 1 Summary of clinical presentation and progression of disease in MuSK-MG and AChR-MG patients

Identity	Sex	Age at onset	Presenting symptoms	MuSK or AChR antibody (nM)	MGFA at max. severity	Duration of MG at time of study (years)	MGFA at time of study
MuSK01	F	3	Facial, oculobulbar	6.9	4b	21	3b
MuSK02	F	6	Ocular	12.9	4	13	2a
MuSK03	F	14	Oculobulbar	14.8	4b	7	0
MuSK04	F	14	Oculobulbar	17.5	4b	4	3a
MuSK05	F	17	Bulbar, neck	21.7	3b	9	3b
MuSK08	F	26	Bulbar, respiratory	9	4b	21	3b
MuSK10	F	27	Ocular	19.5	3	12	0
MuSK11	M	27	Oculobulbar, neck	2.1	3b	20	0
MuSK12	F	41	Oculobulbar	8.4	4b	4	3b
MuSK13	F	42	Ocular	28.1	5	2.75	4b
MuSK14	M	45	Oculobulbar	13.4	4b	22	2a
MuSK15	F	57	Oculobulbar	0.7	2b	1	2b
AChR01	F	12	Bulbar, respiratory limbs, neck	1.2	5	17	4b
AChR02	F	22	Facial, bulbar	43.7	5	16.5	2b
AChR03	F	22	Facial, oculobulbar	0.6	2a	10	2a
AChR04	F	22	Facial	8.5	2b	5	3b
AChR05	M	24	Ocular	1.3	3a	30	2a
AChR06	F	27	Ocular	5.4	4	5	4b
AChR07	F	32	Oculobulbar	16.9	3b	5	3a
AChR09	F	41	Oculobulbar	23.1	2b	30	2a
AChR10	M	50	Oculobulbar	21.2	3b	19	0
AChR11	F	51	Limbs, neck, ptosis	1.3	2	15	2b
AChR12	F	52	Ocular	1.2	3	8	3a
AChR13	F	54	Oculobulbar	11.7	3	3	0
AChR14	M	67	Bulbar	18.2	3b	2.5	0
AChR15	M	69	Oculobulbar	32.4	4	0.5	2b
MuSK-MG, median (range)		26 (3–57)			4 (2–5)	10.5 (1–22)	2.5 (0–4)
AChR-MG, median (range)		36 (12–69)			3 (2–5)	9 (0.5–30)	2 (0–4)
Mann–Whitney *P*		0.14			0.14	0.94	0.86

For calculation of the median and range, the MGFA grades (I–V) were first converted to a numerical scale (1–5).

3 min and 16 s). Slices were positioned to include the level just above the nose and just below the tongue. T_2 weighted (T_2W) images were acquired with and without fat saturation (TR = 4010 ms, TE = 99 ms, acceleration factor of 11, $1.0 \times 1.0 \times 3.0$ mm^3 resolution over 22 slices with 4 averages requiring 2 min and 52 s in each case). The plane of these T_2W images was oriented to be parallel to the plane of the face with the first image through the skin (ignoring the nose) and including slices with the posterior aspect of the constrictor of the pharynx. Additional T_1W images were acquired with a sagittal orientation centred on the midline of the head. Finally, ultra-short TE (UTE) weighted images were acquired in axial planes matching the first of the T_1W datasets (TR = 400 ms, TE = 0.070, 4.76, 9.53, 14.3 ms, a flip angle of 30°, and 8 ms/points sampling, and $1.3 \times 1.3 \times 5$ mm^3 resolution with 4 averages requiring 10 min and 26 s) yielding conventional UTE (cUTE) images (Gatehouse and Bydder, 2003; Robson *et al.*, 2003). An additional three sets of images were calculated from the UTE dataset by subtracting each of the later echo images (TE = 4.76, 9.53, and 14.3 ms) from the first echo image (TE = 0.070 ms) yielding difference UTE (dUTE) images (Gatehouse and Bydder, 2003). This subtraction removes the contribution of long T_2 components while retaining the effects of ultra-short T_2 components.

Quantitative image analysis was performed on a personal computer running CMR Tools (Imperial College, London), a package allowing manual segmentation of images and measurement of local signal intensity. The cross-sectional areas of selected muscles were measured in triplicate by a single observer (M.E.F.) and values were averaged. In an initial evaluation phase, scans from 12 healthy subjects were reanalysed, at least 2 weeks later, to test for reproducibility of measurements of tongue areas and masseter volumes. Results on two serial measures of the same muscle were highly correlated ($r^2 = 0.98$, $P < 0.0001$) and the coefficient of variation was calculated as 3.7%.

Measurements of different muscles and signal intensity

The intrinsic tongue areas were measured by outlining on the midline slices of sagittal T_1W sequences. The tongue cross-sectional area was related to the length of the oral cavity, defined as the distance between symphysis menti and the anterior wall of the first cervical vertebra. Facial muscles were identified on different imaging sequences. For the masseter, insertions of the muscle were identified and the muscle outlined in each axial slice. The volume of the masseter was estimated by summing the product of the area and thickness across all slices. The volumes were normalized to the lean body mass for each subject. The medial and lateral pterygoids were assessed on axial views at the level of their maximal areas. The

maximal width of the temporalis muscle was assessed only from coronal views. The widths of the O.oculi on coronal views, O.oris on coronal and axial views, and buccinator on coronal views were measured on both sides on several consecutive slices, and the slice in which cross-sectional dimensions were largest was selected for triplicate measurements. However, the analysis was not formally blinded and it was relatively easy for M.E.F. to identify individual patients from the MRI features. In order to confirm the results, therefore, two further investigators (L.C. and A.V.) remeasured all O.oculi, O.oris and buccinator muscles, blind to the identity of the patient. The results presented for these muscles, therefore, are the means of 2–5 independent analyses for each muscle.

The percentage of tongue replacement with high signal was measured on sagittal T_1W sequences by defining the area of high signal and expressing this as a percentage of the total sagittal-section area of the intrinsic muscles of the tongue. Measurements were performed three times and the mean taken. To measure the relative signal intensity, the cursor was placed over different regions on sagittal T_1W, axial T_1W and axial cUTE sequences and the signal intensity related to that of an uninvolved muscle, in which the signal appeared on visual inspection to be homogeneous. For the sagittal midline T_1W sequences, the tongue was divided into five segments (superior anterior, superior posterior, inferior anterior and inferior posterior intrinsic muscle and extrinsic muscle regions) and signal intensities were averaged from three different voxels within each region and expressed relative to that of the signal in the trapezius. If an abnormally high signal was noted within any of these regions, then the cursor was placed so as to obtain measurements over this specific region. On axial T_1W and axial cUTE sequences, signal intensities were obtained from within the central axial slice of the tongue muscle thickness. Measurements were obtained from across the anterior half of the tongue (because in the cUTE sequences artefactual high signal tended to be present in the posterior half), at seven points from left to right, and were expressed relative to the signal intensities measured in the masseter, from each subject, which radiologically did not demonstrate any abnormal signal within the muscle bulk.

Statistics

One way ANOVA with multiple Bonferroni post-tests was used for comparison of results for each muscle. Spearman rank correlations were used for correlation between imaging results and clinical scores or treatments. All statistical analyses were performed using GraphPad PRISM 4.0. Only P values < 0.05 are shown.

Results

Clinical features in MuSK-MG and AChR-MG groups

The MuSK-MG patients were unselected but the AChR-MG patients were chosen from clinical records to match the MuSK-MG patients with respect to clinical presentation and current disability. The presenting features, maximum severity and current disease status are shown in Table 1. There were no significant differences with respect to sex ratio, age at onset or presenting symptoms. The MuSK antibody titres were typical of the range found in other series (e.g. McConville *et al.*, 2004), but it was notable that five of the selected AChR-MG patients had very low AChR antibody titres (<1.5 nM); four of these had tested negative (<0.5 nM) on some occasions during their histories. None of the AChR-MG patients had MuSK antibodies, and none of the MuSK-MG patients had AChR antibodies.

There were no significant differences between the MGFA grades at maximum severity or at the time of study, and the MuSK-MG and AChR-MG patients had similar disease durations (Table 1); three patients in each group were in clinical remission. Wasting of the tongue was frequent in MuSK-MG, with lateral thinning in three, central furrowing in two and triple furrowing in two (e.g. Fig. 1a), compared with central furrowing in two and triple furrowing in one of the AChR-MG patients; but the proportion of patients with visible tongue wasting did not differ between the two groups.

Of the 11 MuSK-MG patients, 5 had a poor clinical response to acetylcholinesterase inhibitors and one had an adverse effect to the drug, whereas only 2 of 14 AChR-MG patients had an unsatisfactory response (not significant). Two MuSK-MG patients had received a thymectomy with no obvious clinical benefit. All had received steroids, but four were off steroids at the time of study. Of the 15 AChR-MG patients, 8 had received a thymectomy; 3 had not received steroids at any time and 1 additional patient was off steroids and managing currently with regular plasma exchange.

Imaging of masticatory and facial muscles

Muscle dimensions

In order to assess muscle wasting in the facial and bulbar muscles, we performed T_1W and T_2W MRI, comparing the MG patients with healthy individuals and with myotonic dystrophy patients. Examples of MRI scans are shown in Fig. 1B–I and the results are summarized in Table 2. We first measured differences in the dimensions of the tongue on midline sagittal T_1W images as illustrated in Fig. 2A. There were no differences in the extrinsic muscle compartment in the MuSK-MG or the AChR-MG patients compared with the healthy controls (data not shown). The area of the intrinsic tongue muscle was somewhat lower in the MuSK-MG patients (Fig. 2B), although this was not significant by ANOVA. To take account of individual variation, we normalized the intrinsic tongue area to the length of the oral cavity in each individual. The normalized tongue areas were not different in the MuSK-MG, AChR-MG patients or the four myotonic dystrophy patients (Table 2).

Because of the variability in masseter volumes, we normalized values to the lean body mass for each subject. Masseter volumes for the MuSK-MG and AChR-MG patients were different from those of the healthy controls ($P = 0.03$) but neither was significant on Bonferroni post-tests (Table 2). However, the facial muscles, O.oris (Fig. 2C, D), O.oculi and buccinator, measured on coronal T_2W images, and O.oris on axial T_1W images, were all significantly lower in MuSK-MG (Table 2). In contrast, the maximal areas for

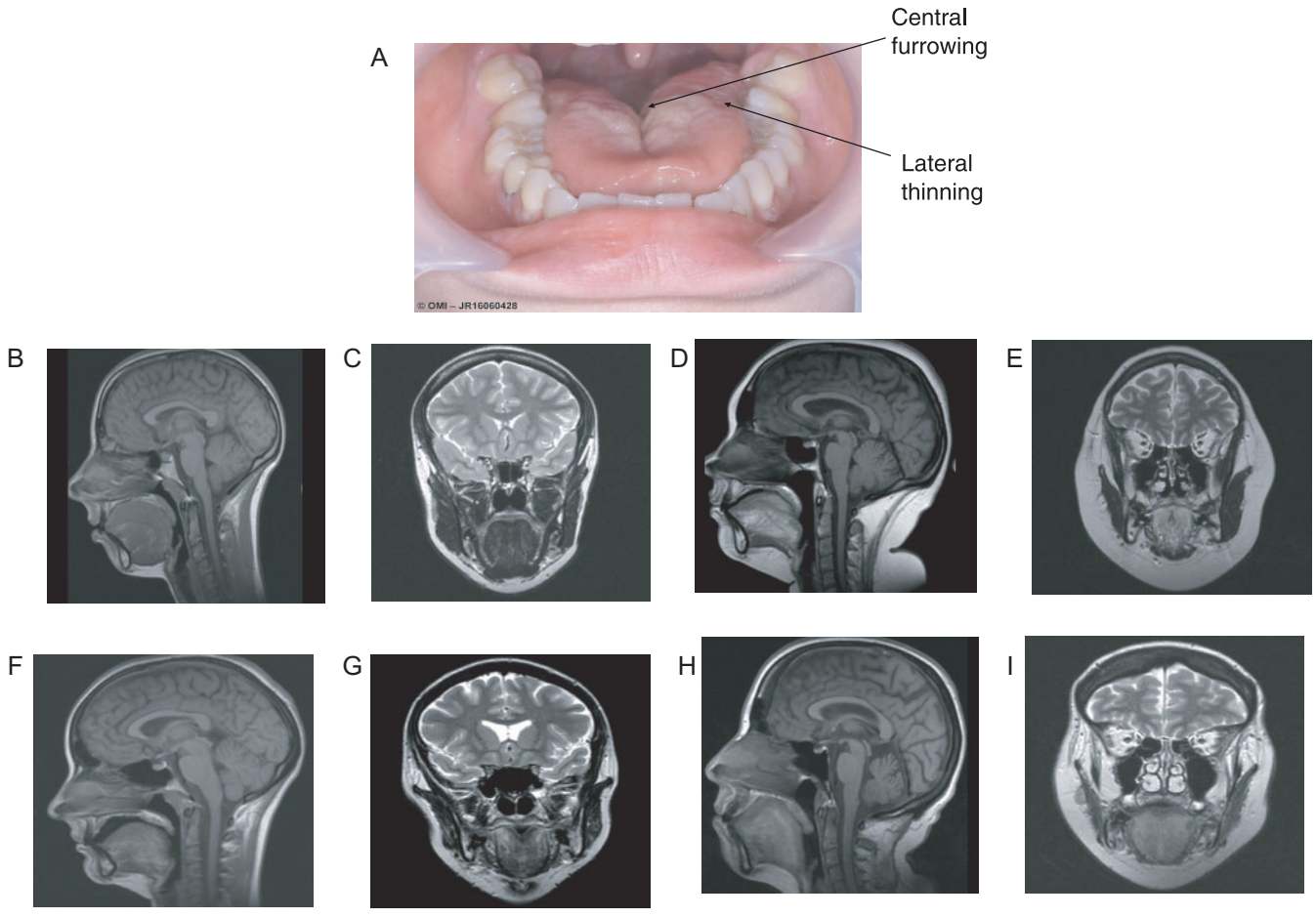

Fig. 1 (**A**) Photograph demonstrating central tongue wasting (central furrowing) with some lateral thinning giving a 'triple furrowed' tongue in a MuSK antibody positive patient (MuSK01). T_1W sagittal midline and coronal T_2W images of a healthy individual (**B** and **C**); MuSK01 (**D** and **E**); AChR01 (**F** and **G**); and a myopathic control subject with myotonic dystrophy (**H** and **I**). The axial images are at the level of the O.oris (anteriorly).

Table 2 Mean results of muscle dimensions

Patient or control group (no. of individuals)	Tongue intrinsic area/ length of oral cavity	Masseter volume/ lean body mass	Orbicularis oculi, coronal width (n = 2)	Orbicularis oris, coronal width (n = 2)	Orbicularis oris, axial width including pouch (n = 3)	Buccinator, coronal width (n = 5)	Mean of muscle dimensions normalized to healthy controls (%)
Healthy (12)	31.63 ± 2.30	217.9 ± 57.2	6.78 ± 1.09	5.82 ± 0.86	10.21 ± 1.26	11.93 ± 1.5	100.0 ± 12.5
MuSK-MG (12)	29.32 ± 2.80	179.1 ± 52.7	5.5 ± 0.88	3.93 ± 1.18	8.04 ± 2.05	9.67 ± 1.52	78.4 ± 13.8
AChR-MG (14)	31.30 ± 4.5	171.0 ± 47.7	6.09 ± 0.85	4.82 ± 1.1	9.24 ± 1.91	10.84 ± 1.8	89.1 ± 14.3
Myotonic dystrophy (4)	34.33 ± 2.36	132.3 ± 37.82	5.99 ± 0.26	4.55 ± 1.15	8.4 ± 2.02	10.01 ± 1.1	84.2 ± 12.1
ANOVA	ns	0.03	0.025	0.001	0.03	0.02	0.004
Post test MuSK versus healthy		ns	0.05	0.001	0.05	0.05	0.01

One way ANOVA was used with multiple Bonferroni post-tests to look for differences between MuSK-MG, AChR-MG and control values. All measurements are given as mm, mean ± SD. n values denote number of independent measurements made. ns = not significant.

medial and lateral pterygoids measured on axial T_1W images, and the temporalis muscle on coronal T_2W images, showed no differences between the groups (data not shown).

In order to obtain an individual score for muscle thinning, we normalized the tongue, masseter, O.oculi, O.oris (coronal) and buccinator measurements to the means of the control data and obtained a mean percentage value for each patient. The values in MuSK-MG patients were significantly lower than the values in healthy controls (P = 0.01; Table 2). Although the values in the AChR-MG group were lower than those of the healthy controls, this was not significant on post-test.

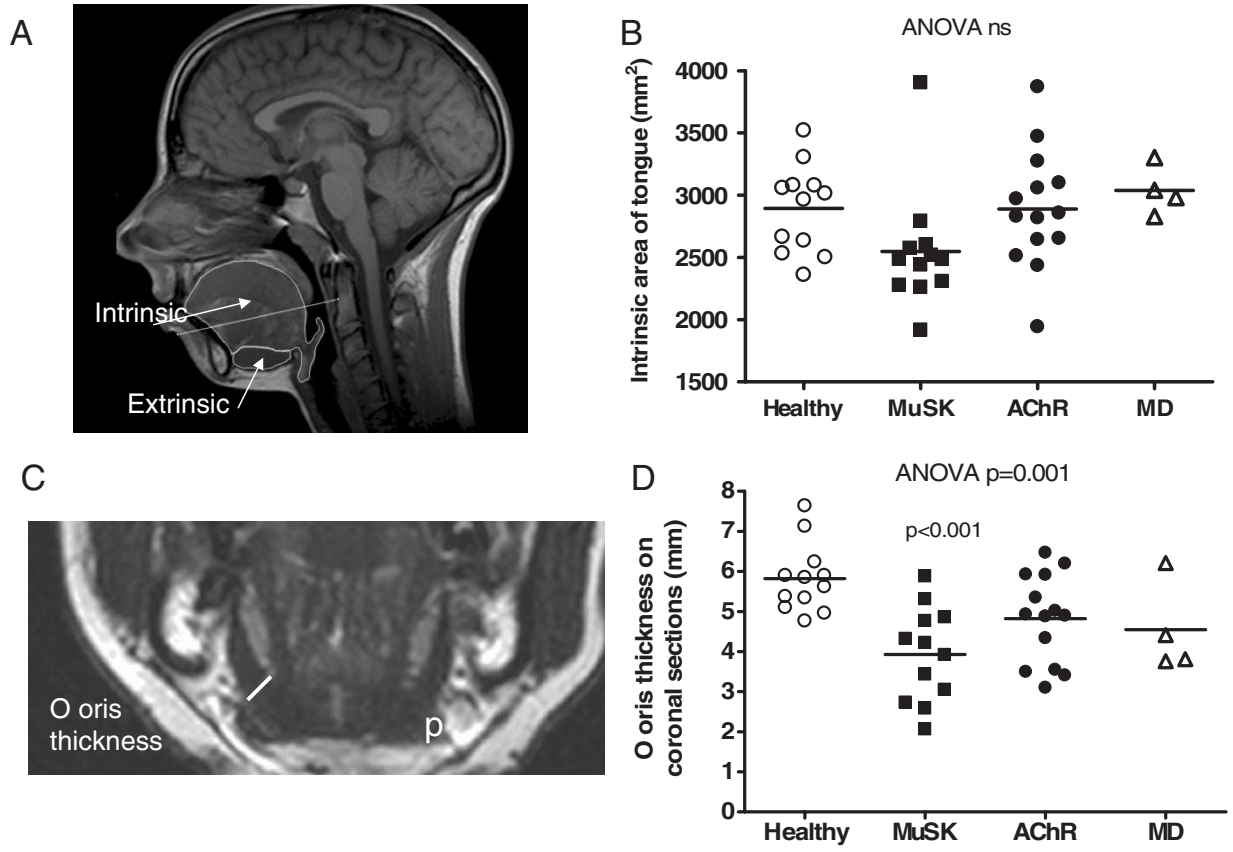

Fig. 2 (**A**) The intrinsic area of the tongue was measured from midline sagittal T_1W sequences and related to the length of the oral cavity, which was taken as the distance from the symphysis menti to the anterior wall of the first cervical vertebra, represented here as a straight line. (**B**) Measurements of intrinsic tongue areas plotted for different patient groups; (**C**) O.oris muscle thickness was measured on coronal T_2W images; p denotes the O.oris pouch, which was included in the axial measurements (Table 2). (**D**) Results of O.oris coronal thickness plotted for different patient groups.

High signal in intrinsic muscles of the tongue

Figure 1B–I shows typical sagittal and coronal images from a healthy individual and three patients. Compared with a small amount of high signal in the healthy individual (Fig. 1B and C), there was increased high signal noted in some MuSK-MG and AChR-MG patients, (Fig. 1D–G), with increased signal noted to be uniformly distributed throughout the tongue in the myotonic dystrophy (MD) patient (Fig. 1H and I).

To quantify the extent of high signal replacement, we first expressed the area of high signal as a percentage of the intrinsic tongue muscle area (as in Fig. 3A). The mean values for the percentage of high signal replacement in the MuSK-MG and AChR-MG groups, and in the myotonic dystrophy patients, were increased but one-way ANOVA was significant only for MuSK-MG (Fig. 3B, Table 3). No high signal was noted in the extrinsic muscles of the tongue.

We then measured the intensity of the signal from each of four regions on sagittal views of the intrinsic muscles (as in Fig. 3A), relating the measurements to those of the trapezius. The increased signal in the MuSK-MG patients was predominantly localized to the anterior inferior region (Fig. 3C).

cUTE sequences to analyse the nature of the high intensity signal

High intensity signal on T_1W images in the tongue muscles could reflect either fatty replacement or fibrosis. When we performed T_2W coronal sequences, with and without fat saturation, the abnormal high signal in the tongue was suppressed on sequences with fat saturation, suggesting that most of the contribution to this signal change was from fat. However, in order to test for the presence of fibrous tissue such as collagen, which cannot be visualized on these sequences, we compared the findings from conventional axial T_1W sequences with cUTE axial sequences (Gatehouse and Bydder, 2003; Robson *et al.*, 2003). We first analysed the signal at seven points from left to right across the bulk of the intrinsic tongue muscles (Fig. 4A). Varying extents of high signal were noted on the axial T_1W sequences in the MuSK-MG patients (Fig. 4C and D), and the mean values of MuSK-MG, AChR-MG and healthy individuals showed some variation across the tongue (Fig. 4E). However, the MuSK-MG patients, and the MD patients, showed significantly higher signal intensity in the central tongue region compared with healthy controls (Fig. 4F; Table 3).

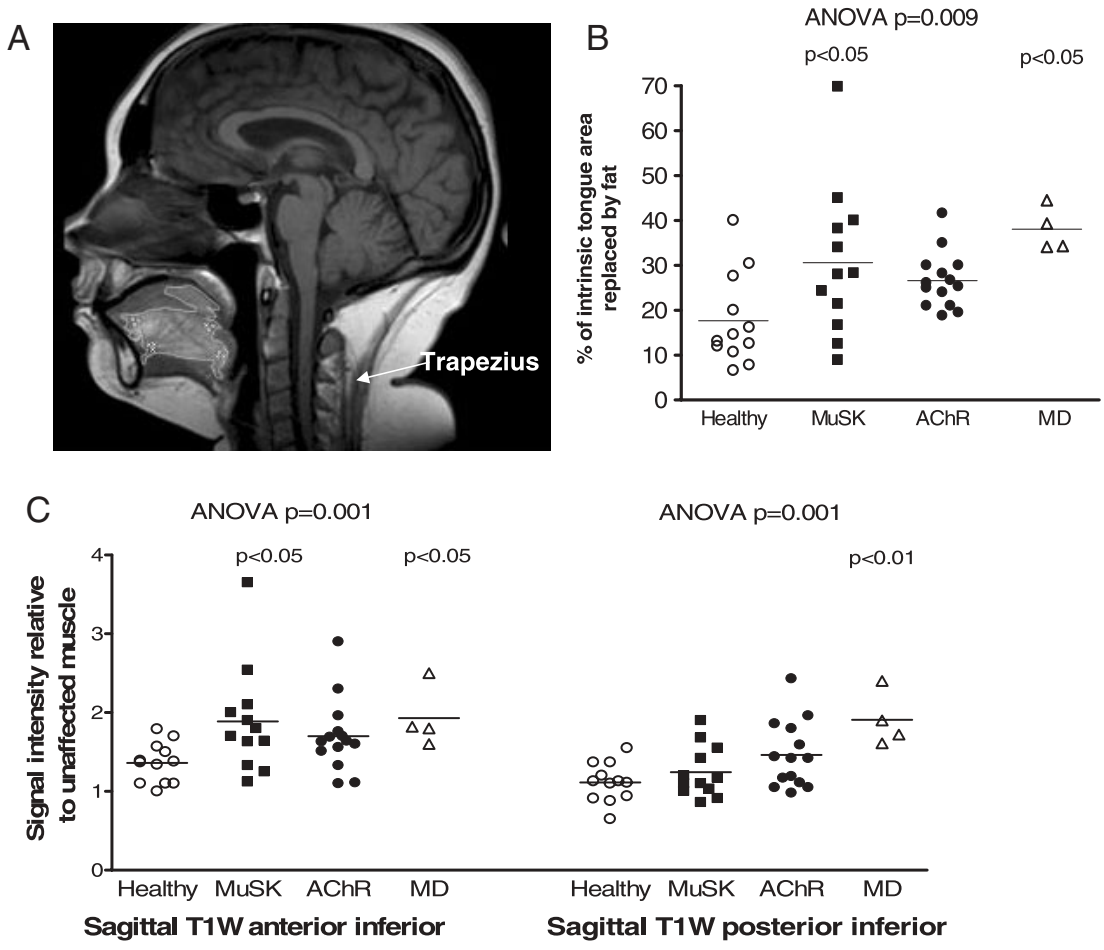

Fig. 3 (**A**) The percentage of tongue containing high signal was outlined on the midline T_1W sagittal views. The cursors denote sites in the tongue (superior anterior and posterior and inferior anterior and posterior) where signal intensities where measured. In each subject, signal intensities in the tongue were related to those obtained from the trapezius muscle. (**B**) Results of percentage tongue replacement with high signal. (**C**) Relative signal intensities on sagittal T_1W sequences for the anterior and posterior inferior regions of the tongue.

Table 3 Mean results of signal intensity measurements

	Percentage of tongue with high signal	Signal intensity at midline on axial scans (relative to masseter)
Healthy (12)	17.63 ± 10.11	1.46 ± 0.13
MuSK-MG (12)	30.59 ± 16.6	2.12 ± 0.18
AChR-MG (14)	26.15 ± 6.25	1.52 ± 0.16
Myotonic dystrophy (4)	38.05 ± 4.927	2.53 ± 0.25
One way ANOVA	0.008	0.001
MuSK-MG versus controls, *P* value	<0.05	<0.05

One way ANOVA with multiple Bonferroni post-tests was used to test for differences between MuSK-MG and control values. All measurements are given as mean ± SD.

The axial dUTE sequences are illustrated in Fig. 4B and D and the mean values of all patients in Fig. 4G. Signal intensities from these derivative subtraction images were very similar between each of the groups, and did not vary appreciably across the tongue, suggesting that the high signal on T_1W sequences is likely to be due to fatty replacement rather than fibrous tissue.

Relationship between muscle atrophy, previous treatments and clinical features

We asked whether the patients' clinical or treatment histories correlated with the MRI findings. There was no apparent relationship with use of cholinesterase inhibitors or thymectomy. Steroids (prednisolone except in one patient who had methylprednisolone) were given to all of the MuSK-MG patients and 12 of the AChR-MG patients. Previous use of steroids was defined in three ways: the maximum dose (mg/kg/day) given; the total amount of steroids given (mg/kg × months); and the duration of time (months) for which an arbitrarily defined dose of 40 mg or greater on alternate days (AD) was given. These, and current doses, did not differ significantly between the two groups (Table 4).

Because of the small numbers and the need to use non-parametric ranking, we pooled data from both AChR-MG and MuSK-MG groups. Table 5 shows the relationships

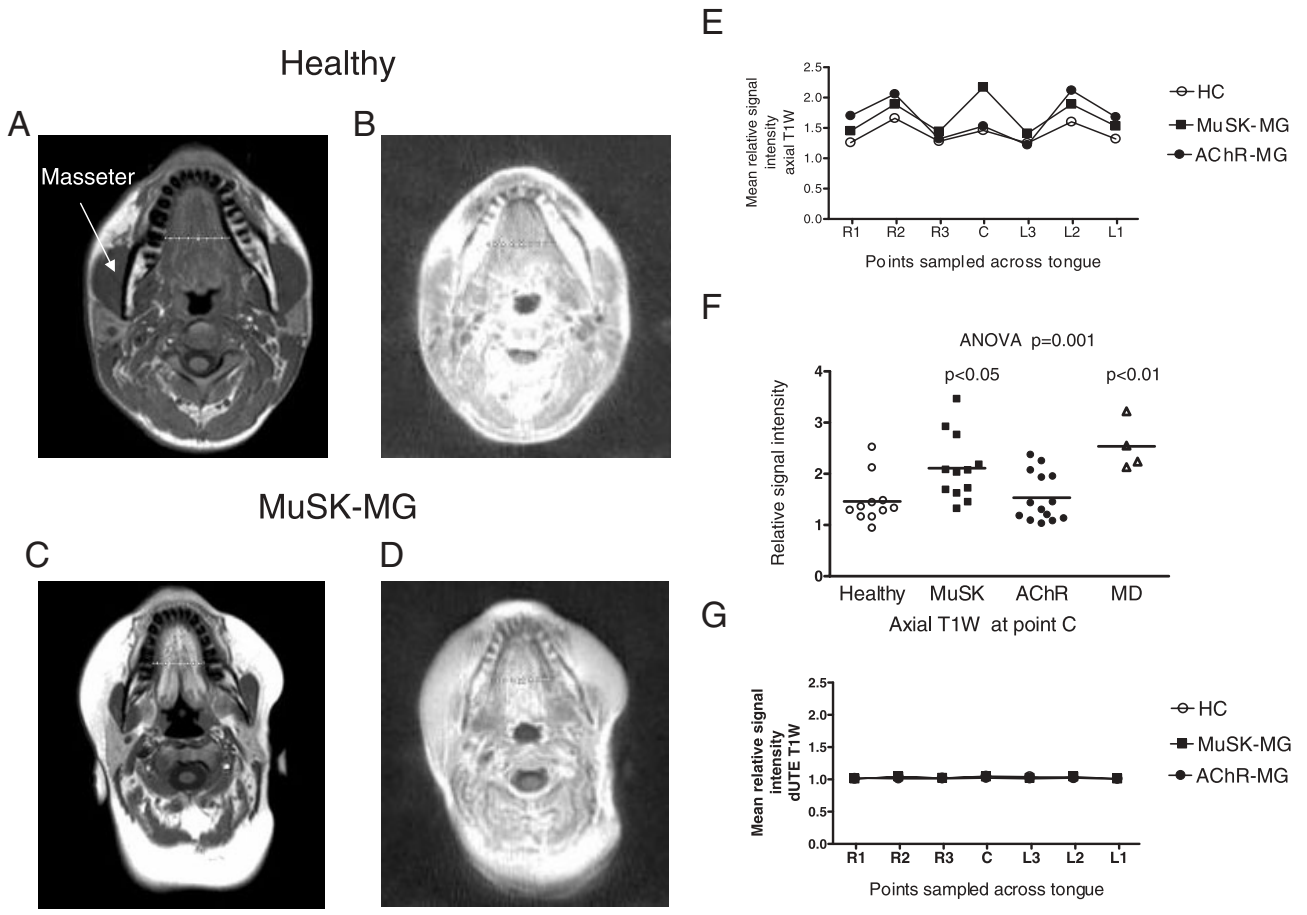

Fig. 4 (**A** and **B**) Axial T_1W and dUTE sequences of the tongue in a healthy control individual. (**C** and **D**) Axial T_1W and dUTE sequences of the tongue in a MuSK-MG patient. The line drawn over the midline of the tongue, in all four images, represents the region over which the signal intensities were measured: 'x' denotes the central region of the tongue while the other cursors are the three points at which measurements where taken to the left and to the right of the central point of the tongue. (**E**) Results for the mean signal intensities of the tongue at the seven points in the three groups for axial T_1W sequences. (**F**) The relative signal intensities, from axial T_1W sequences, are shown for the central point of the tongue. (**G**) Relative signal intensities of the central region of the tongue plotted for dUTE sequences. The latter results confirmed that the central high signal in MuSK-MG patients on axial T_1W sequences was due to fatty replacement and not fibrotic tissue.

Table 4 Summary of steroid treatment at time of study

	Years to starting steroids	Maximum AD dose (mg/kg)	Duration maximum dose (months)	Cumulative dose (months × mg AD/kg)	Duration of >40 mg AD (months)	Current steroids (mg AD)
MuSK-MG, median (range)	2 (0.5–9)	1 (0.3–2)	11.5 (1–68)	24.5 (3.5–115)	17.5 (0–120)	12.5 (0–60)
AChR-MG, median (range)	1.5 (0–20)	1 (0–1.6)	8 (0–84)	11.5 (0–98)	9.5 (0–108)	16.0 0–100)
Mann–Whitney *P* value	0.89	0.54	0.32	0.28	0.16	0.55

Table 5 Correlations between the percentage of tongue with high signal and clinical and treatment factors

	Age at onset	Duration of MG	MGFA at maximum severity	MGFA at time of study	Time to starting prednisolone (years)	Maximum prednisolone dose (mg/day)	Cumulative prednisolone (mg AD/kg × months)	Duration of >40 mg AD (months)
Spearman rank coefficient, *r*	−0.2761	0.2479	0.2649	0.3998	0.2071	0.1640	0.3924	0.5229
P value	0.1721	0.2221	0.2007	0.0430	0.3102	0.4233	0.0474	0.0061

MGFA grades (I-V) were converted to a numerical scale (1-5)

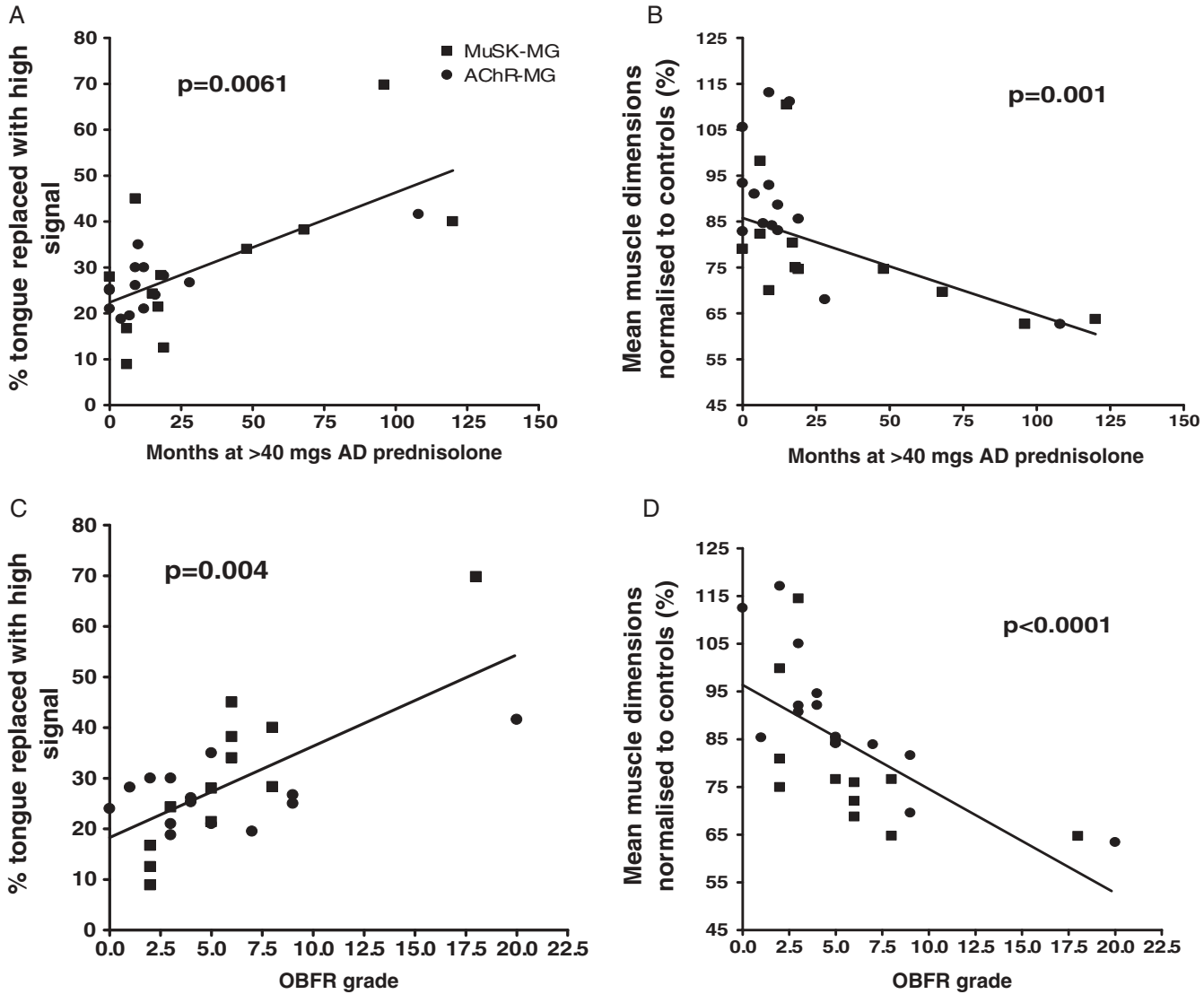

Fig. 5 (**A**) Relationship between duration of moderate/high steroid treatment and percentage of tongue replacement with high signal, and (**B**) with mean muscle dimensions. (**C**) The new OBFR score significantly correlates with the percentage of tongue replacement with high signal, and (**D**) with mean muscle dimensions. *P* values are for Spearman rank correlations.

between the clinical features and the percentage of high signal replacement in the tongue. There was no correlation with age at onset, disease duration, MGFA grade at maximum severity, duration of disease before starting steroid treatment, maximum steroid dose used or current steroid dose. There was a weak correlation with current MGFA grade ($P = 0.043$) and total amount of prednisolone given ($P = 0.047$) and a stronger correlation with duration of treatment with prednisolone at >40 mg AD ($P = 0.006$; Fig. 5A). Prednisolone treatment at >40 mg AD also correlated inversely with the dimensions of the tongue, O.oculi and O.oris muscles (Table 6), and the mean muscle dimensions ($P = 0.001$; Fig. 5B).

Clinical assessment of MG

Patients were first graded using the well-established QMG, MG-ADL and MMT scores. The results are shown in Table 7 and did not differ significantly between MuSK-MG and AChR-MG patients. To establish a scoring system that reflected better the disability of patients with predominantly bulbar and facial involvement, we used the bulbar components of the QMG, and added additional features to develop a new OBFR scoring system (see footnotes to Table 7). These scores also did not differ significantly between the two patient groups. They correlated strongly with the current MGFA and QMG scores (Table 8) and the percentage of high signal replacement of the tongue and inversely with the mean muscle dimensions (Fig. 5B).

Discussion

We sought to define the incidence and nature of persistent muscle atrophy in MuSK-MG patients. We found that some

Table 6 Correlations between duration of treatment with >40 mg AD steroids and MG muscle measurements

	Tongue intrinsic area/length of oral cavity	Masseter volume/ lean body mass	O.oculi, coronal	O.oris, coronal	O.oris, axial	Buccinator, axial	Mean of muscle dimensions normalized to healthy controls (%)
Spearman rank coefficient, *r*	−0.44	−0.34	−0.62	−0.69	−0.43	−0.434	−0.61
P value	0.024	0.09	0.001	0.001	0.027	0.027	0.001

Table 7 Summary of clinical scores at time of study

	MMT	MG-ADL	QMG	New OBFR
MuSK-MG median (range)	6 (2–16)	5 (0–9)	11.5 (3–17)	5.5 (2–18)
AChR-MG median (range)	4 (2–48)	3 (0–15)	9.5 (3–27)	4 (0–20)
Mann–Whitney *P* value	0.96	0.29	0.94	0.59

In order to provide a scoring system that reflected the muscles most involved in these patients, and that could be used to correlate with other clinical or experimental measurements, we developed a scoring system to quantify ocular, bulbar, facial and respiratory function further. We performed the QMG, ADL and MMT on all patients according to previously published methods. For the new OBFR score, we selected the bulbar and respiratory scores of the QMG and added the following: The strength of five facial muscles, frontalis, O.oculi, corrugator supercilii, O.oris and buccinator, was assessed and scored as 0–2 for each muscle, with 0 for normal strength, 1 for weakness and 2 for no apparent movement, with a maximum score of 10. The appearance of the tongue was scored as 0 if normal, 1 if lateral thinning, 2 if central furrowing or 3 if triple furrowing was present. The function of the soft palate was scored as 0 for normal, 1 for sluggish movement or 2 if not reactive. Based on swallow times from the healthy controls, the upper limit of normal for swallowing 100 ml of water was 8 s, and swallow times were scored as 0 if below 8 s, 1 if between 8 and 15 s, 2 if between 16 and 30 s, and 3 if above 30 s. Forced vital capacity (FVC) measurements in these patients were graded according to the QMG score (see above).

patients in both MuSK-MG and AChR-MG groups had visible tongue atrophy but MRI evidence of atrophy of individual facial muscles was only significant in MuSK-MG patients. There were some patients with high signal intensity on T_1W MRI of the tongue, which we showed, by using a novel dUTE sequence, was probably due to fatty rather than fibrous tissue. Overall muscle atrophy and the area of tongue with high signal correlated with the duration of moderate/high dose steroids (>40 mg AD) and with a new OBFR clinical score.

We included all available MuSK-MG patients, and also tested a group of AChR-MG patients, but in order to match these two groups for disease course and persistent facial weakness, we had to search through a large number of records (>190) of AChR-MG patients. The AChR-MG patient with most marked atrophy had childhood onset and low AChR antibodies and her bulbar involvement and muscle atrophy were very similar to that in MuSK01 (Table 1). Despite some evidence for atrophy and high signal in the other AChR-MG cases, however, only MuSK-MG patients demonstrated significant reduction in the average muscle dimensions. Thus our results confirm that MuSK antibodies associate with a form of MG that frequently involves wasting of the facial and tongue muscles.

In a proportion of the MG cases high signal was identified in the muscles on T_2W sequences, and suppression on T_2W sequences with fat saturation suggested that this was due to fatty replacement. To test whether there was a significant component of fibrosis, we used cUTE, a relatively novel sequence, which has not been previously applied to study the facial muscles (Chappell *et al.*, 2003; Gatehouse and Bydder, 2003; Gatehouse *et al.*, 2004; Waldman *et al.*, 2003). UTE sequences allow the selective detection of signal contributions from short T_2 components (Gatehouse and Bydder, 2003; Robson *et al.*, 2003). Diseases associated with chronic fibrosis (as well as calcification and haemorrhage) increase the signal from short T_2 components. The negative findings on the cUTE studies suggest that fibrosis does not make a substantial contribution to the pathologically high signal changes on the conventional T_2W scans of the MG patients and, therefore, indicates that this high signal arises principally from fat.

Neurophysiological studies on the same patients (Farrugia *et al.*, submitted for publication) found no evidence for denervation. Collectively, therefore, these findings indicate that the muscle atrophy in MG patients is probably myopathic in nature. Both Sanders *et al.* (2003*a*) and Evoli *et al.* (2003) have pathological confirmation of a myopathic process in biopsies from individual MuSK-MG patients and the latter authors have also reported similar changes in some AChR-MG patients. 'Myopathic' findings were previously reported on the basis of MUAP analysis and muscle biopsy in a few patients with MG (Humphrey *et al.*, 1962; Somnier *et al.*, 1993). Thus, on the basis of these reports and the current results, the differences between MuSK-MG and AChR-MG appear to be mainly quantitative.

The lack of emphasis on bulbar function assessment in the QMG scoring systems means that MuSK-MG patients tend to do well on this score and explains the poor correlation with the imaging measurements (data not shown). We, therefore,

Table 8 Correlations between new OBFR score and other clinical scores and muscle measurements

	MGFA at time of study	MMT	MG-ADL	QMG	Mean of muscle dimensions normalized to healthy controls (%)	MRI % high signal
Spearman rank correlation	0.7246	0.3491	0.1982	0.5581	−0.7285	0.5448
P value	**<0.0001**	**0.0805**	0.3317	**0.0030**	**<0.0001**	**0.0040**

devised a new OBFR score that reflects better the facial and bulbar involvement and correlates better with several of the muscle measurements (Table 8). Nevertheless, there were some patients who did not score highly because of the very restricted nature of their disease. This emphasizes the difficulties in establishing a scoring system that reflects adequately the severity of the disease in patients with relatively restricted muscle involvement. Patient-specific scoring systems should be considered when assessing treatment responses in MuSK-MG.

It is not clear why the facial and tongue muscles should be selectively involved in MuSK-MG. These muscles are complex in fibre-type composition with varied metabolic requirements and contractile demands. O.oculi and O.oris are mainly composed of Type II fibres (Stal *et al.*, 1994; Goodmurphy *et al.*, 1999), whereas buccinator consists mainly of Type I fibres (Stal *et al.*, 1994; Goodmurphy *et al.*, 1999) and the muscles of mastication (masseter, medial and lateral pterygoids and temporalis) are mainly Type I fibres with unusual motor units that contain large and fast-twitch fibres with varying degrees of fatigability (McComas *et al.*, 1998). The tongue is spatially heterogeneous; the anterior aspects consist mainly of Type II fibres and are adapted for rapid movements important for phonetics and articulation, whereas the posterior aspects are adapted for tonic phases related to deglutition and respiration and consist mainly of Type I and Type IM/IIC fibres (Stal *et al.*, 2003). Our results show a tendency towards more marked involvement of Type II muscles in MuSK-MG. However, curiously, the extrinsic muscles of the tongue, which consist of both Type I and II fibres in a similar distribution to that of the intrinsic muscles of the tongue (Saigusa *et al.*, 2001), were spared.

We found that the only strong correlate of muscle thinning and high signal was the duration of moderate/high dose steroid treatment of the patients; the total cumulative dose showed only a weak correlation. In contrast, and perhaps surprisingly, there was no correlation with the maximum dose or duration of symptoms although there was a weak correlation with the current MGFA grade. Since Type II fibre atrophy is one of the adverse effects associated with high-dose corticosteroid treatment (Mastaglia, 1982), it may be that steroids themselves are contributing to the muscle atrophy. Alternatively, the relationship with duration of moderate-to-high steroid dosage may merely reflect the resistance of these patients to immunosuppressive therapy and the duration of time before they achieve adequate clinical benefit. In either case, steroid treatment would only be one factor in predisposing to muscle atrophy, since some patients with short duration of steroid treatments also had relatively marked atrophy in some muscles or high intensity signal (Fig. 5). It will be interesting to see whether treatment with newer drugs, such as mycophenolate mofetil, which appear to be clinically more effective (Sanders *et al.*, 2003*a*; Zhou *et al.*, 2004), will prevent development of muscle wasting. Preliminary experimental work suggests that MuSK-MG plasma and IgG upregulate expression of muscle ring finger protein 1 (*MURF-1*), which is an atrophy-related gene, both in a muscle cell culture system and in mouse masseter muscle (a Type II muscle in rodents; Benveniste *et al.*, 2005). Animal models and further *in vitro* work are needed to determine how these antibodies predispose to muscle atrophy and whether these effects are compounded by a long duration of steroid treatment.

Acknowledgements

M.E.F. was a clinical research fellow with the Muscular Dystrophy Campaign/Myasthenia Gravis Association and is grateful to the Oxford Health Services Research Committee for additional support. We would like to thank Jane Francis for help with the scanning, Greame Bydder for general comments on MRI study design, and the patients and healthy volunteers for their time and patience. P.M.M. thanks the Medical Research Council for personal support and for support of imaging facilities.

Conflict of interest statement: A.V. and the Department of Clinical Neurology in Oxford received royalties and payments for the MuSK antibody analyses. Funding to pay the Open Access publication charges for this article was provided by Clinical Neurology Departmental funds.

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